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# Chronic Cassava Toxicity

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

**Editors: Barry Nestel and Reginald MacIntyre**



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## Biosynthesis of Cyanogenic Glucosides in Cassava (*Manihot* spp.)

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**Abstract** Cyanogenic materials could not be detected in seeds of sweet cassava (*Manihot* spp.) cultivars, whereas low levels of these materials were found in seeds of bitter cultivars. However, both types of seeds synthesised high levels of cyanogens during germination and growth. Linamarin, 2( $\beta$ -D-glucopyranosyloxy)isobutyronitrile, accounted for 93%, while lotaustralin, 2( $\beta$ -D-glucopyranosyloxy)2-methylbutyronitrile, accounted for 7% of the total cyanogenic glucosides in cassava. Seedlings efficiently incorporated L-valine- $^{14}\text{C}$  (U) and L-isoleucine- $^{14}\text{C}$  (U) into the aglycone moieties of linamarin and lotaustralin, respectively. Appreciable radioactivity from these amino acids were also incorporated into asparagine.

Linamarase, the  $\beta$ -glucosidase which catalyses the hydrolysis of linamarin and lotaustralin, was identified and isolated in crude form from seedlings and leaves of sweet and bitter cultivars. Thus both cultivars contained the enzymes which catalyse the biosynthesis and degradation of the glucosides. The free amino acid profiles of seeds and seedlings indicated that during germination, the action of proteolytic enzymes on seed storage proteins resulted in the rapid accumulation of valine and isoleucine, from which the glucosides were rapidly synthesised. During the growth of seedlings, the concentration of cyanogenic glucosides increased and then fluctuated, without the release of hydrogen cyanide (HCN). Studies with  $\text{H}^{14}\text{CN}$  showed that hydrogen cyanide released intracellularly from the glucosides was rapidly incorporated in asparagine, and subsequently into metabolic pools involved with respiration and protein and carbohydrate synthesis.

Cassava plants assimilated  $\text{H}^{14}\text{CN}$  as efficiently as  $^{14}\text{CO}_2$  in the light. The pathway of  $\text{H}^{14}\text{CN}$  assimilation was found to proceed by the reaction of cyanide with serine and cysteine, which resulted in the formation of asparagine. Seedling homogenates showed the presence of equally high activities of  $\beta$ -cyanoalanine synthase and rhodanese, the enzymes which catalyse cyanide detoxification. Both enzyme activities were found to be localised in cassava mitochondria, which showed very low sensitivity toward cyanide during respiration.

Electronmicroscopic studies on cassava seed tissues showed the presence of large amounts of fat and protein bodies in all cells. Organelles were little differentiated. At the onset of active cyanogen synthesis, the cytoplasmic organelles were well developed, especially in the roots.

**Résumé** Nous n'avons pu déceler de matériaux cyanogènes dans les graines de cultures douces de manioc (*Manihot* spp.), alors que nous en avons trouvé de faibles quantités dans les graines de cultures amères. Par ailleurs, les deux types de graines synthétisent de fortes quantités de cyanogènes au cours de la germination et de la croissance. La linamarine, 2( $\beta$ -D-glucopyranosyloxy)isobutyronitrile, est responsable de 93%, alors que la lotaustraline, 2( $\beta$ -D-glucopyranosyloxy)2-méthylbutyronitrile, est responsable de 7% des glucosides cyanogènes totaux du manioc. Les jeunes plants incorporent efficacement la L-valine- $^{14}\text{C}$  (U) et la L-isoleucine- $^{14}\text{C}$  (U) dans la portion

aglycone de la linamarine et de la lotaustraline, respectivement. Une quantité appréciable de radioactivité provenant de ces acides aminés s'incorpore également à l'asparagine.

La linamarase, la  $\beta$ -glucosidase qui catalyse l'hydrolyse de la linamarine et de la lotaustraline, a été identifiée et isolée dans sa forme brute sur de jeunes plants et des feuilles des cultures douces et amères. Les deux types contiennent donc les enzymes qui catalysent la biosynthèse et la dégradation des glucosides. Les profils d'acides aminés libres des graines et des jeunes plants indiquent qu'au cours de la germination, l'action des enzymes protéolytiques sur les protéines de réserve des graines produit une accumulation rapide de valine et d'isoleucine, à partir desquelles les glucosides sont rapidement synthétisés. Lors de la croissance des jeunes plants, la concentration des glucosides cyanogènes augmente et ensuite varie, sans qu'il y ait libération d'acide cyanhydrique (HCN). Des études avec  $H^{14}CN$  démontrent que l'acide cyanhydrique libéré intracellulairement à partir des glucosides est rapidement incorporé dans l'asparagine et par la suite, dans les pools métaboliques impliqués dans la respiration et la synthèse des protéines et des hydrates de carbone.

Les plants de manioc assimilent  $H^{14}CN$  aussi efficacement que  $^{14}CO_2$  à la lumière. On a découvert que la voie métabolique d'assimilation de  $H^{14}CN$  procède par réaction du cyanure avec la sérine et la cystéine, résultant dans la formation d'asparagine. Des homogénats de jeunes plants indiquent la présence d'activités également élevées de  $\beta$ -cyanoalanine synthase et rhodanèse, deux enzymes qui catalysent la désintoxication du cyanure. On a découvert que l'activité de ces deux enzymes est localisée dans les mitochondries du manioc, qui démontrent une très basse sensibilité au cyanure au cours de la respiration.

L'examen au microscope électronique de tissus de graines de manioc révèle la présence, dans toutes les cellules, de fortes quantités de corps contenant des lipides et des protéines. Les organites sont peu différenciés. Au début de la synthèse active du cyanogène, les organites cytoplasmiques sont bien développés, surtout dans les racines.

CASSAVA, manioc, or tapioca (*Manihot esculenta* Crantz, *M. utilissima* Pohl) is one of the most extensively cultivated food plants in the developing countries of the tropics, where its starchy root tubers form a major source of industrial and dietary carbohydrates (and often proteins). The utilisation of cassava root tuber and products as a major staple food presents a variety of health problems. Because of the low protein content, cassava food products have been implicated in the high incidence of Kwashiorkor, the common protein-deficiency syndrome of the developing countries (Jones 1959). The high moisture content and the high ratio of carbohydrates to nitrogen make cassava tubers an excellent substrate for microbial growth and production of high levels of toxic metabolites. It has been shown that *Aspergillus flavus* thrives on cassava meal substrate and produces relatively high levels of aflatoxins (Nartey 1966). These heat-stable carcinogenic metabolites inhibit protein synthesis, and cause liver damage and hepatoma in animals (Butler and Barnes 1963). Thus food products derived from field-dried tubers may represent another health hazard involving protein metabolism in man in the tropics.

A common biochemical feature of the cassava plant is that it synthesises and accumulates cyanogenic materials in its vegetative tissues, especially

the edible leaves and tubers. These materials, on hydrolysis, give rise to moderate to lethal concentrations of prussic acid or hydrogen cyanide (HCN), a powerful specific and nonspecific inhibitor of several essential enzyme-catalysed processes, notably the cytochrome oxidase system in respiration (Dixon and Webb 1965). The consumption of cassava products containing high levels of cyanogenic glucosides therefore constitutes a serious health hazard since cyanide released from these food products will act as enzyme poisons. Indeed, cyanide poisoning and death have resulted from the consumption of poorly prepared diets of cassava tubers and products containing lethal amounts of cyanogenic glucosides (Sreeramamurthy 1945; Jones 1959). Furthermore, ataxic neuropathy is endemic in developing countries where cassava products form the major staple food; chronic ingestion of cyanide (cyanogenic glucosides) may contribute to the high incidence of this neuropathological syndrome (Osuntokun 1968).

### Cyanogenesis in Cassava

Tissue of all cassava cultivars so far examined contains cyanogenic glucosides, although in varying concentrations. Variations in the HCN con-

TABLE 1. Concentration of cyanogenic glycosides in tissues of "sweet" and "bitter" cultivars of cassava (data from Nartey 1968).

Cultivar	Tissue	Cyanogenic glucosides (mg HCN/kg fresh wt tissue)
Sweet (3 varieties)	Seeds	0.00
	Seedlings (10-day-old)	285.00
	Leaves (mature)	468.00
	Roots	126.50
	Tubers	402.00
Bitter (3 varieties)	Seeds	7.50
	Seedlings (10-day-old)	245.00
	Leaves (mature)	310.00
	Roots	185.00
	Tubers	395.00

centrations in tubers, as well as the morphological characteristics of the plants, form the basis of a taxonomic differentiation between the bitter (high HCN) and the sweet (low HCN) cultivars (Rogers 1965). This basis for delineation does not appear to offer an adequate means for differentiating cassava cultivars. Since definite metabolite concentrations are not strictly inherited, the variations in the concentrations of cyanogenic glucosides encountered in different cultivars are probably the result of phenotypic differences and factors such as photoperiodism, thermoperiodism, and nutritional status. These factors reflect the capacity of the species to react in a different manner under any given condition. On the basis of present knowledge, it appears that *Manihot* spp. are genetically cyanophoric, with some dominant cyanogenesis-controlling gene being present in successive cultivars and phenotypes. Thus all cassava varieties probably contain all the enzymes which catalyse the biosynthesis of cyanogenic materials and their degradation. Variations in the concentrations of cyanogenic glucosides in cassava tissues and cultivars may therefore determine enzyme activities, substrate concentrations, and availability, as well as rates of transport, storage, and degradation in specific tissues and cultivars.

A comparative study of cyanogenesis in different tissues of sweet and bitter varieties of cassava showed that seeds of the former contained no detectable amounts of cyanogens, whereas seeds of

the latter contained low levels of cyanogens (Nartey 1968). Table 1 shows the variations in the concentrations of HCN evolved from different tissues of six cassava cultivars grown from seed to mature plants. Table 1 also shows that during germination in the dark, seeds of all six cultivars synthesised high levels of cyanogenic materials, and thus provided excellent materials for the study of the biosynthesis of cyanogenic glucosides in cassava. In the light, the concentration of cyanogens increased significantly, but decreased sharply after about 2 days, and then fluctuated without the release of HCN into the closed system. These fluctuations indicated that cassava cyanogens were dynamic metabolites, and that the plants contained the enzymes which catalysed the biosynthesis of the glucosides as well as those which catalysed their degradation. An enzyme with the latter activity was isolated in crude form from various cassava tissues (Nartey 1968). The crude enzyme preparation showed strong activity against linamarin and lotaustralin, mild activity against salicin, and weak activity against  $\beta$ -methyl glucoside.

### Cassava Cyanogenic Glucosides

Until recently, only one cyanogenic glucoside, linamarin, was known to occur in cassava tissues. Linamarin, 2( $\beta$ -D-glucopyranosyloxy)isobutyronitrile, was isolated and characterised by Dunstan et al. (1906) as a glucoside of 2-hydroxy-isobutyronitrile. That is, on enzymic or non-enzymic hydrolysis, linamarin gives rise to glucose, acetone, and HCN. Butler (1965) showed that most plants containing linamarin also contained a higher homologue of this glucoside, methyl-linamarin or lotaustralin, 2( $\beta$ -D-glucopyranosyloxy)2-methylbutyronitrile, a glucoside of 2-hydroxy-2-methylbutyronitrile. On hydrolysis, lotaustralin gives rise to glucose, methylethyl ketone, and HCN. Both linamarin and lotaustralin were identified in the roots of *Manihot carthaginiensis* and shown to constitute 96 and 4%, respectively, of the total cyanogenic materials present. Nartey (1968) showed that linamarin and lotaustralin constitute the cyanogenic materials of cassava (*Manihot utilissima*, *M. esculenta*) in the proportions of 93 and 7%, respectively. The occurrence of lotaustralin (methyl-linamarin) with linamarin in cassava tissues was later confirmed by Bisset et al. (1969).

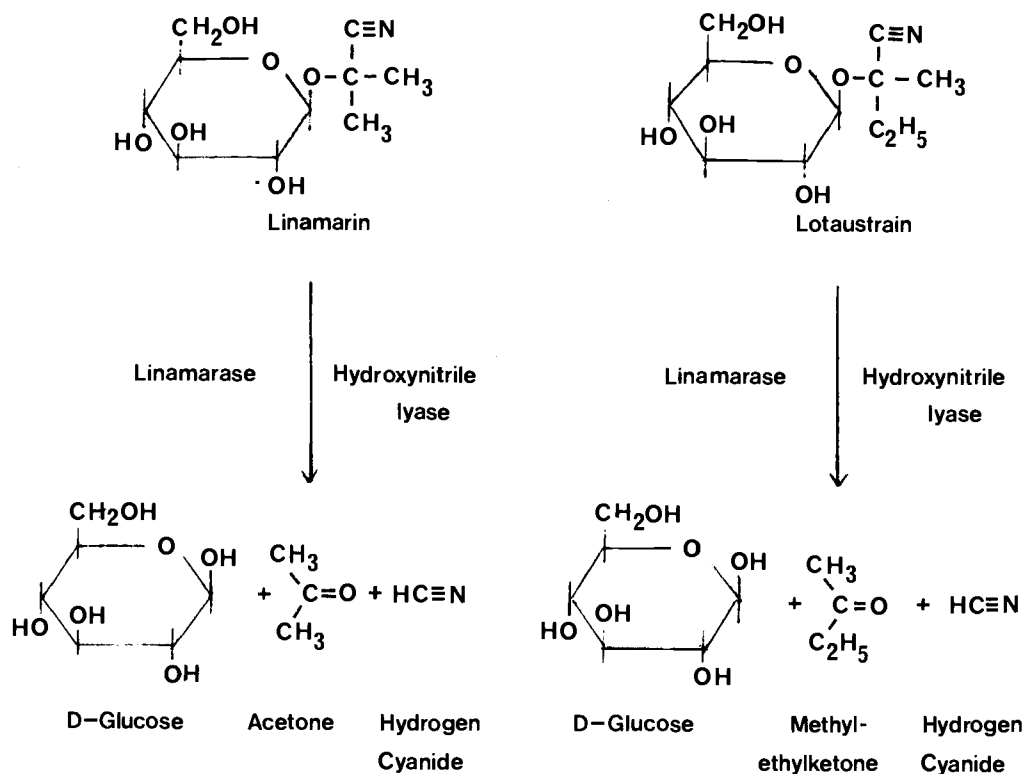


FIG. 1. The structures and hydrolytic products of the cyanogenic glucosides of cassava, linamarin and lotaustralin.

Figure 1 shows the structures and the hydrolytic products of linamarin and lotaustralin.

### Biosynthesis of Linamarin and Lotaustralin

The demonstration in different laboratories that the aglycone moieties of cyanogenic glucosides may be synthesised from structurally related amino acids motivated studies on the effectiveness of valine and isoleucine as precursors of the aglycone moieties of linamarin and lotaustralin, respectively. Results showed that both sweet and bitter cassava seeds contained only trace amounts of valine and isoleucine. However, during germination, large amounts of these amino acids accumulated through the degradative action of proteolytic enzymes on seed storage proteins. Concurrently, the concentrations of linamarin and lotaustralin increased sharply. When uniformly labelled L-valine- $^{14}\text{C}$  and L-isoleucine- $^{14}\text{C}$  were administered to cassava seedlings during the period of active cyanogen synthesis (10–14 days), large

amounts of radioactivity were subsequently found incorporated in the aglycone moieties of linamarin and lotaustralin, respectively. Table 2 shows the amounts of radioactivity from these amino acids incorporated in the aglycone moieties of cassava glucosides. The table incorporates also radioactivity found in asparagine. The latter is significant with respect to the mechanisms for cyanide detoxification in cassava, which will be dealt with later.

TABLE 2. Incorporation of radioactivity from L-valine- $^{14}\text{C}$  and L-isoleucine- $^{14}\text{C}$  into the aglycones of linamarin and lotaustralin, and into asparagine by cassava seedlings (data from Nartey 1969).

Label administered	Incorporation (%)		
	Linamarin	Lotaustralin	Asparagine
L-Valine $^{14}\text{C}$ (U)	13.2	—	1.1
L-isoleucine $^{14}\text{C}$ (U)	—	2.4	0.53



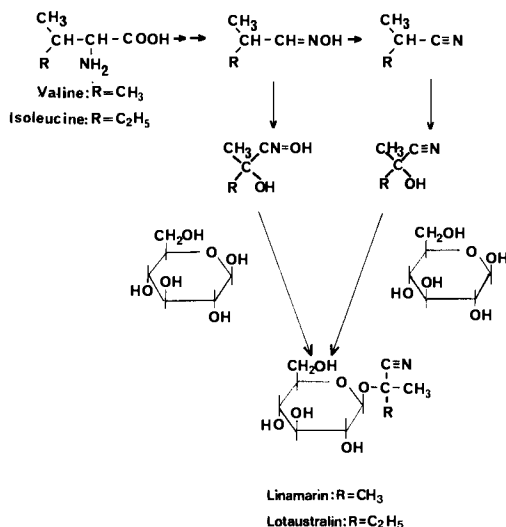


FIG. 2. The biosynthesis of linamarin and lotaustralin in cassava. Pathway incorporates data from Conn and Butler 1969, Nartey 1968 and 1969, and Tapper et al. 1972.

From the foregoing, it is clear that linamarin and lotaustralin are synthesised from valine and isoleucine, respectively, by cassava plants. It is also evident that factors such as the intracellular concentrations of valine and isoleucine, as influenced by protein degradation or synthesis, control the biosynthesis and accumulation of cassava cyanogenic glucosides.

The biosynthesis of linamarin and lotaustralin in cassava proceeds by the pathway illustrated in Fig. 2. Other plant species such as *Trifolium*, *Linum*, and *Lotus* (and *Phaseolus* ?), which accumulate linamarin and lotaustralin during germination and growth, synthesise these cyanogens by the same pathway (Butler and Conn 1964; Abrol and Conn 1966; Tapper et al. 1971).

### Cyanide Detoxification in Cassava

As indicated earlier, the total concentration of cyanogenic glucosides in cassava plants fluctuates with growth period and growth condition. In closed systems, decreases in cyanogen content do not lead to the release of detectable amounts of HCN into the system. Furthermore, cassava plants kept in closed systems containing high levels of HCN appear to grow normally. Under these

circumstances, it would appear that cassava plants possess mechanisms for the detoxification of high levels of HCN. The terminal cytochrome oxidase systems of plants, animals, and microorganisms are particularly susceptible to inhibition by cyanide (Dixon and Webb 1965; Bendall and Bonner 1971). Experiments were therefore designed for the investigation of the possible fate of HCN released intracellularly from cyanogens during the growth of cassava plants, and the possible effects of cyanide on the respiration of isolated cassava mitochondria.

### Detoxification and Metabolism of HCN Catalysed by $\beta$ -Cyanoalanine Synthase

In studies on the fate of HCN in cassava plants, radioactive  $\text{H}^{14}\text{CN}$  was administered to cassava seedlings for various periods of time. In parallel experiments,  $^{14}\text{CO}_2$  and uniformly labelled acetate- $^{14}\text{C}$  were also administered individually to other seedlings. The results of the analysis of extracts from seedlings fed radioactive compounds showed that cassava plants metabolised HCN,  $\text{CO}_2$ , and acetate equally efficiently. However, the patterns of labelling were different. Whereas relatively small fractions of radioactivity from  $^{14}\text{CO}_2$  and acetate- $^{14}\text{C}$  were found in the free amino acid pools, radioactivity from  $\text{H}^{14}\text{CN}$  was predominantly incorporated in the free amino acid pools. A most striking feature of the observed labelling patterns was that although 49% of the total radioactivity from  $\text{H}^{14}\text{CN}$  was located in the free amino acids fraction of seedling extracts, over 95% of the total radioactivity in this fraction was located in asparagine, aspartic acid, glutamine, and glutamic acid. Table 3 shows the amounts of radioactivity from  $\text{H}^{14}\text{CN}$  incorporated into these amino acids by cassava seedlings during various periods of feeding. It is evident from Table 3 that asparagine contained the major fraction of the total radioactivity incorporated into the free amino acid fraction. When labelled asparagine was isolated and degraded, well over 97% of its total radioactivity was located in the amide-carbon atom.

This mechanism for cyanide detoxification operates in a variety of higher plant species (Blumenthal-Goldschmidt et al. 1963). Nartey (1970) also showed that both the carbon and nitrogen atoms of cyanide are specifically incorporated into the amide-carbon and amide-

TABLE 3. Incorporation of radioactivity from  $\text{H}^{14}\text{CN}$  into free amino acids by 10-g cassava seedlings exposed to  $\text{H}^{14}\text{CN}$  released from  $2.3 \mu\text{mole Na}^{14}\text{CN}$ ,  $125 \mu\text{Ci}$ ,  $8.38 \times 10^6 \text{ cpm}$ , in a closed system for the periods specified (data from Narthey 1969).

Period of feeding (min)	Radioactivity ( $\text{cpm} \times 10^{-5}$ ) incorporated in			
	Asparagine	Aspartic acid	Glutamine	Glutamic acid
10	0.86	0.11	0.16	0.07
60	24.91	5.73	0.89	0.18
180	42.03	6.68	0.65	0.43

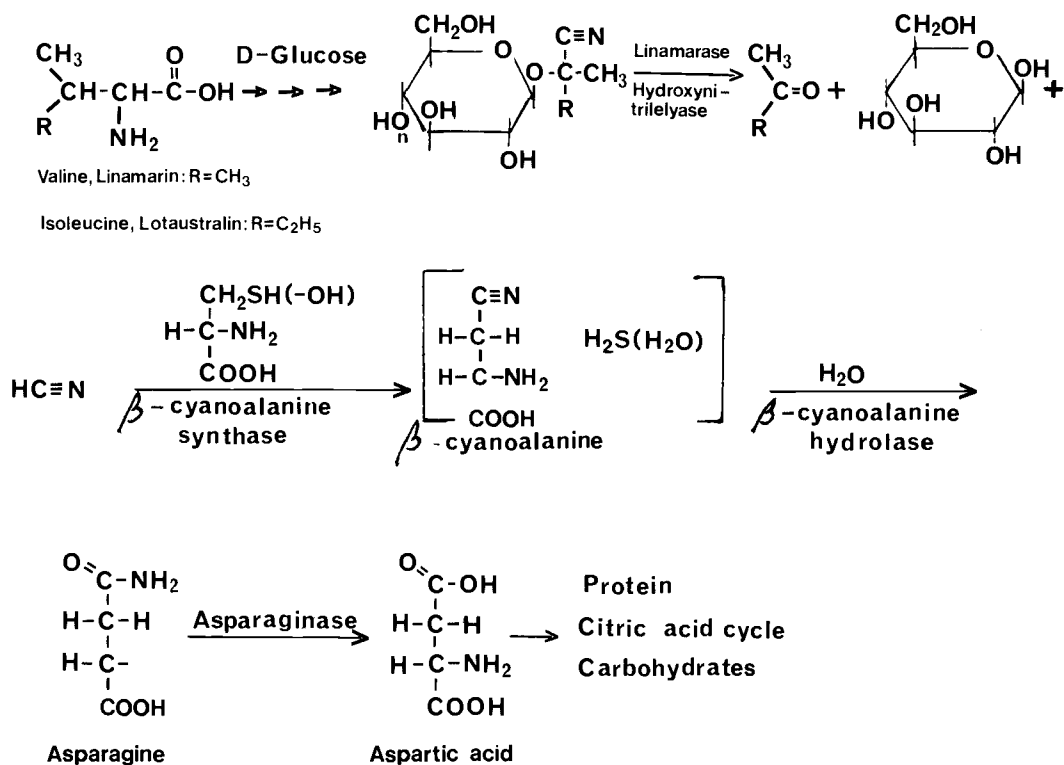


FIG. 3. The detoxification and assimilation of hydrogen cyanide by cassava (Narthey 1969).

nitrogen atoms of asparagine. Both cyanophoric and non-cyanophoric plant species are capable of detoxifying cyanide by this mechanism. Figure 3 illustrates the pathway for the detoxification and assimilation of  $\text{HCN}$  by cassava plants. In this pathway, both serine and cysteine can act as cyanide acceptors, with the resultant formation of  $\beta$ -cyanoalanine as the primary reaction product. Since cassava plants contain only trace amounts of cysteine and relatively higher levels of serine, the quantitative data on cyanide incorporation into useful metabolites indicated that serine was the

natural cyanide acceptor in cassava. In-vitro experiments with seedling extracts confirmed that serine was a more effective cyanide acceptor than cysteine (Narthey 1969).

Although  $\beta$ -cyanoalanine is the primary reaction product in this pathway, the compound could not be detected in cassava plants metabolising  $\text{HCN}$ . Evidently  $\beta$ -cyanoalanine formed during the assimilation of  $\text{HCN}$  by cassava was rapidly converted to asparagine, which in turn was readily converted to aspartic acid. High activities of the enzymes which catalyse the hydrolysis of these two

compounds,  $\beta$ -cyanoalanine synthase and asparaginase, have been detected in cassava seedling extracts (Nartey 1972 unpublished data). The operation of these enzyme systems in cassava therefore ensures that the HCN evolved intracellularly from cyanogenic glucosides by the action of linamarase and 2-hydroxynitrile lyase (or by the non-enzyme catalysed dissociation of the hydroxynitrile moieties) is rapidly converted to amino acids, proteins, carbohydrates, lipids, and other cellular materials (Nartey 1969). While this series of enzyme-catalysed reactions provides an excellent means for the conversion of the toxic HCN into non-toxic and useful cellular materials in plants, it probably reflects the capacity of prebiotic systems to cause the non-enzyme catalysed synthesis of amino acids and proteins from the highly reactive HCN and water (Mathews and Moser 1967; Kliss and Mathews 1962).

### Cyanide Detoxification Catalysed by Rhodanese

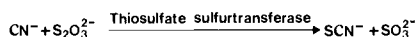
The most extensively studied mechanisms for cyanide detoxification involve reactions in which cyanide accepts sulfur from inorganic and organic sulfur donors with the resultant formation of thiocyanate. These reactions are catalysed by the enzymes rhodanese (thiosulfate sulfurtransferase) and 3-mercaptopyruvate sulfurtransferase, which occur in plants, animals, and microorganisms (Gemeinhardt 1938; Chew and Boey 1972; Sorbo 1953; Fiedler and Wood 1956; Tabita et al. 1969). Figure 4 illustrates thiocyanate formation from cyanide and inorganic and organic sulfur compounds.

Chew and Boey (1972) reported the occurrence of a rhodanese in cassava leaves. Studies with seedling homogenates and mitochondria have confirmed the occurrence of rhodanese activity in cassava, and indicated that both  $\beta$ -cyanoalanine

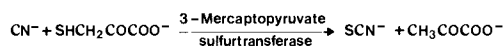
synthase and rhodanese activities were localised in the mitochondrial fractions of tissue homogenates.  $\beta$ -cyanoalanine synthase has been isolated from the mitochondrial fractions of blue lupine (Hendrickson and Conn 1971). Thus, cyanide detoxification in plants proceeds via the  $\beta$ -cyanoalanine and thiocyanate pathways, depending on the presence of the substrate which acts as cyanide acceptor (serine and cysteine) or sulfur donor (thiosulfate, thiosulfonates and 3-mercaptopyruvate). However, the results of the studies with cassava tissue homogenates and isolated mitochondria indicated that cassava rhodanese activity was inhibited by cysteine, while the  $\beta$ -cyanoalanine synthase activity was equally inhibited by thiosulfate (Nartey 1972). Apparently, only one cyanide detoxification mechanism may operate at a time, since the substrate of the one system inhibits the activity of the enzyme which catalyses the other system.

### Cyanogenesis and Ultrastructural Changes in Germinating Cassava Seeds

As indicated earlier, active biosynthesis and degradation of cyanogenic glucosides in cassava seeds occurred after 10–14 days germination. As a preliminary to the localisation of cyanogenesis in specific cell organelles, electronmicroscopic studies were conducted on non-cyanophoric seeds and cyanophoric seedlings. The results of these studies showed that all tissues of dry and imbibed cassava seeds were filled with fat and large protein bodies. Cytoplasmic and organelle membranes were poorly defined, which made the recognition of plastids and mitochondria difficult. Figures 5 and 6 are electronmicrographs of thin sections of the endosperm and radicle of non-cyanophoric cassava seeds. Figures 7 and 9 are electronmicrographs of thin sections of the cotyledon and root tissues of 10-day-old etiolated cassava seedlings. These figures show that while the cotyledonary tissues still contained many lipid and protein bodies, the root tissues had mobilised these reserve substances, and contained scattered large lipid bodies. In the root cells, proplastids and mitochondria were well differentiated, as were the endoplasmic reticulum and the Golgi apparatus. Figures 8, 10a, and 10b are electronmicrographs of thin sections of the green cotyledonary leaf and root tissues of 17-day-old seedlings which had received light over the



Thiosulfate can be replaced by Thiosulfonates



Sulfite, sulfonates and mercaptoethanol can replace cyanide as sulfur acceptors.

FIG. 4. The detoxification of cyanide in plants, animals, and microorganisms by the formation of thiocyanate.

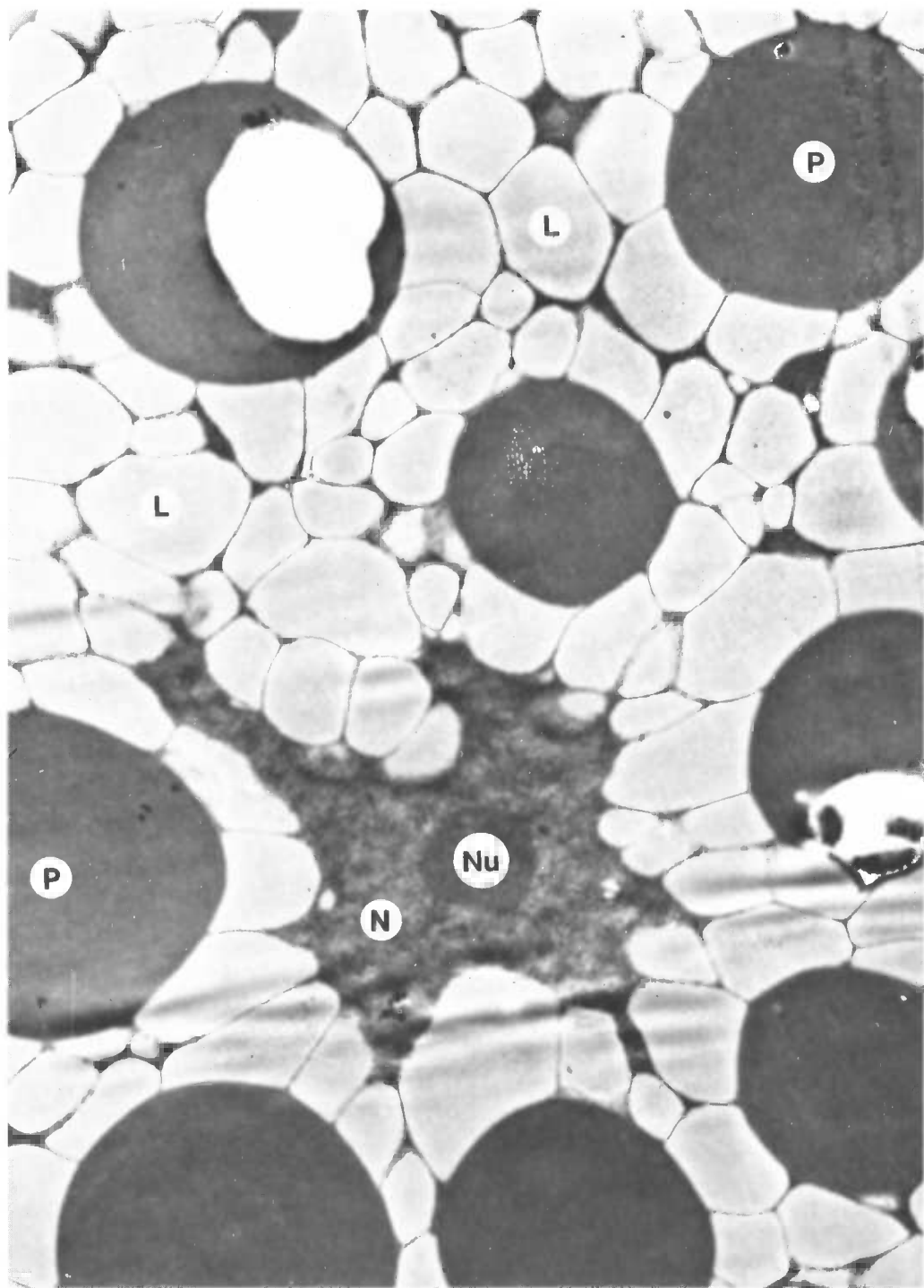


FIG. 5. Electronmicrograph of a thin section through the endosperm of a dry mature cassava seed. The cells are filled lipid bodies (L) and protein bodies (P), Nucleus (N), Nucleolus (Nu). Sections of seed tissues were fixed in 6% glutaraldehyde in phosphate buffer pH 7.2, and post-fixed in 2% osmium tetroxide.  $\times 14,000$ .

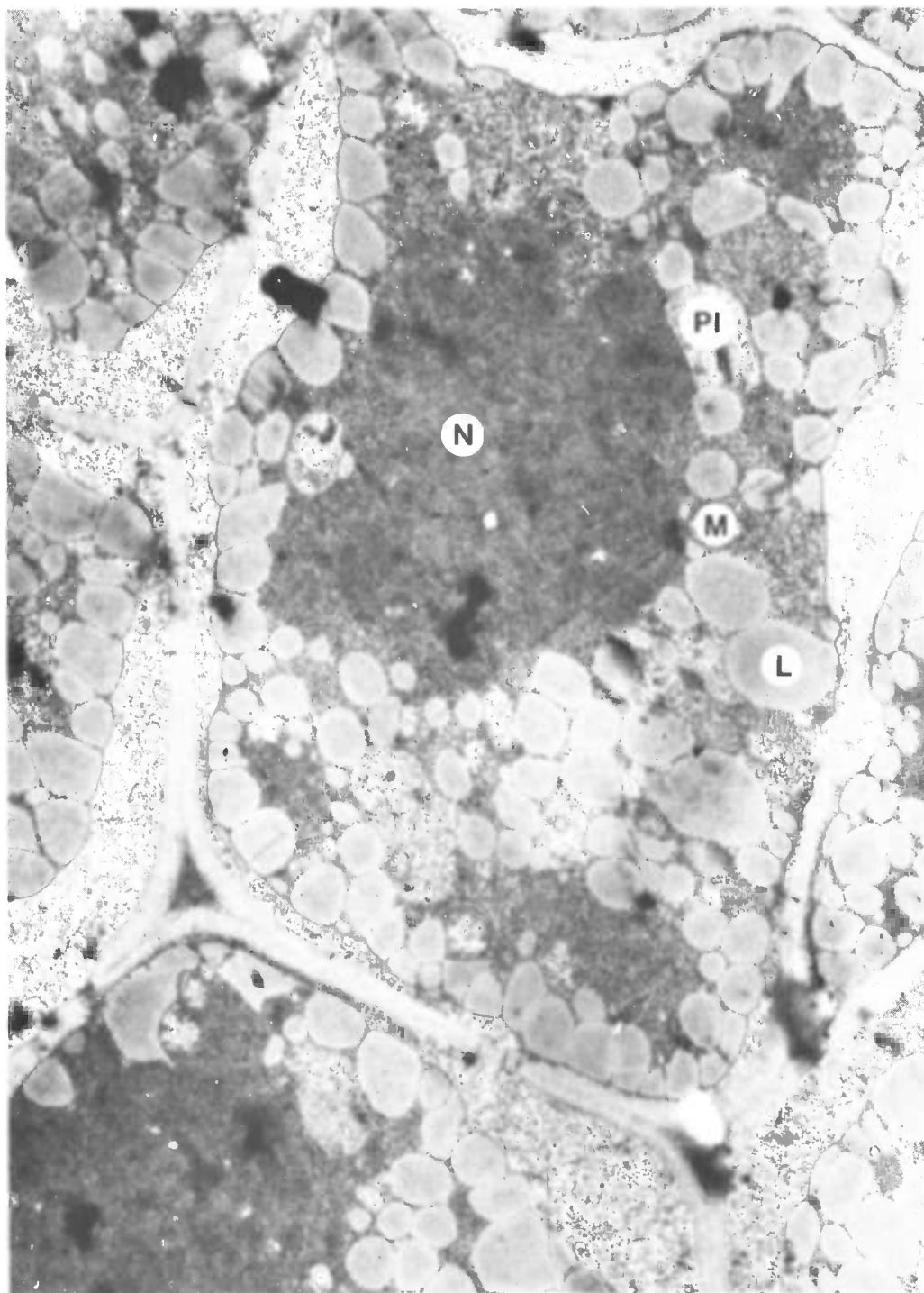


FIG. 6. Electronmicrograph of a thin section through the radicle of a dry mature cassava seed. The cells contain large amounts of lipid bodies (L) in their cytoplasm. Cytoplasmic and organelle membranes are poorly defined. Nucleus (N), Plastid (Pl), Mitochondrion (M).  $\times 14,000$ .

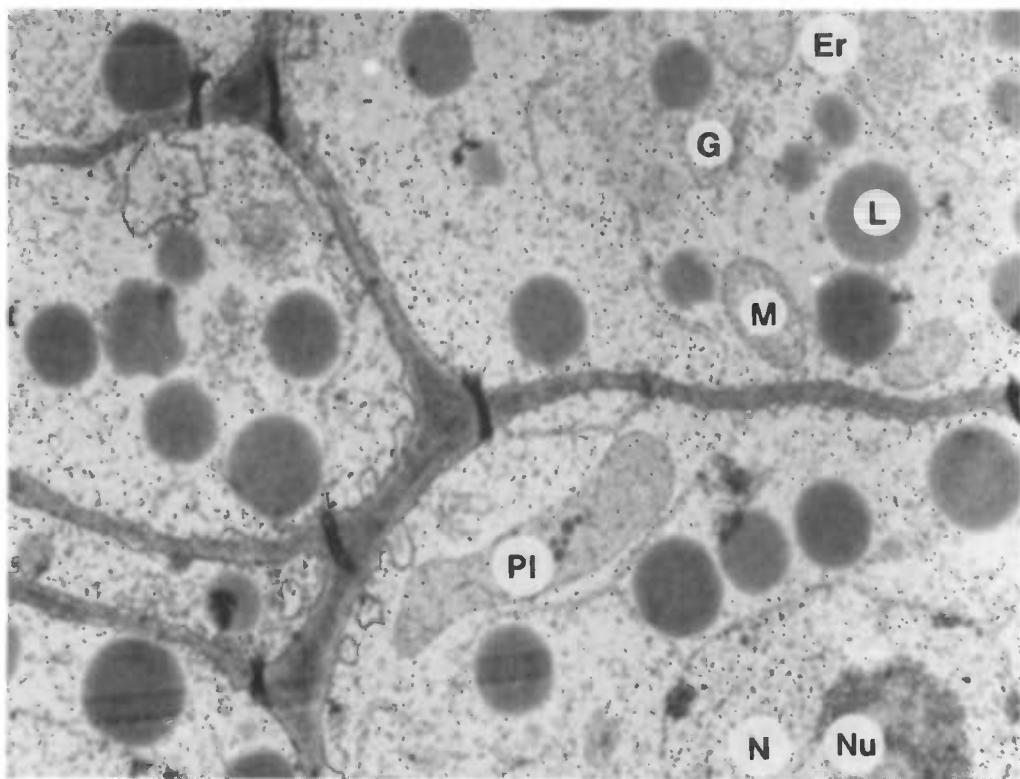


FIG. 7. Electronmicrograph of a thin section through the root of a 10-day-old etiolated cassava seedling. Lipid bodies (L) are scattered through the cytoplasm. Profiles of the endoplasmic reticulum (ER) and Golgi apparatus (G) are well defined. Nucleus (N), Nucleolus (Nu), Plastid (Pl), Mitochondrion (M).  $\times 32,000$ .

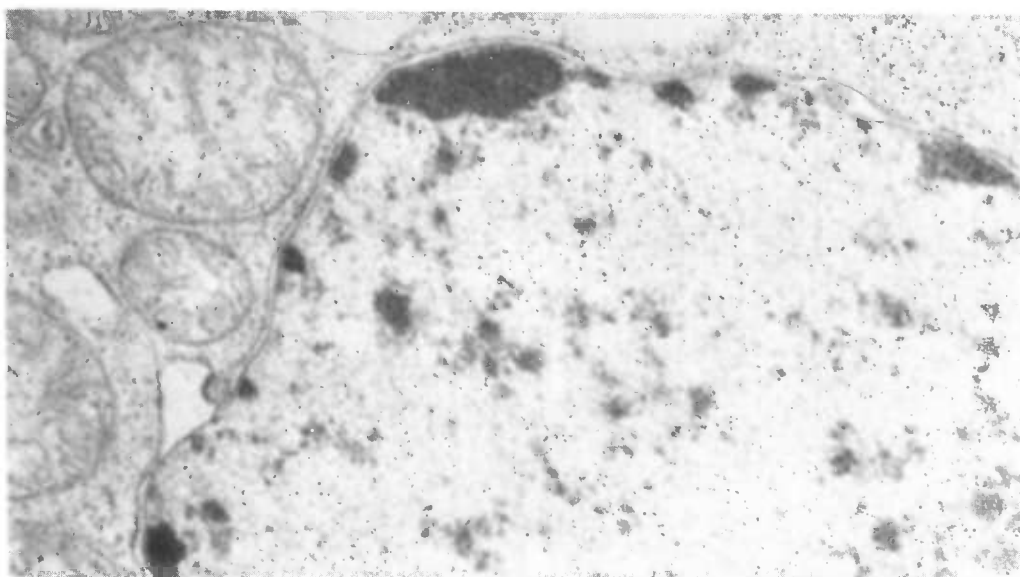


FIG. 8. Electronmicrograph of a thin section through the root of a 17-day-old cassava seedling which had received light over the last 7 days. Cytoplasmic membranes are well defined. Lipid bodies are absent.  $\times 32,000$ .

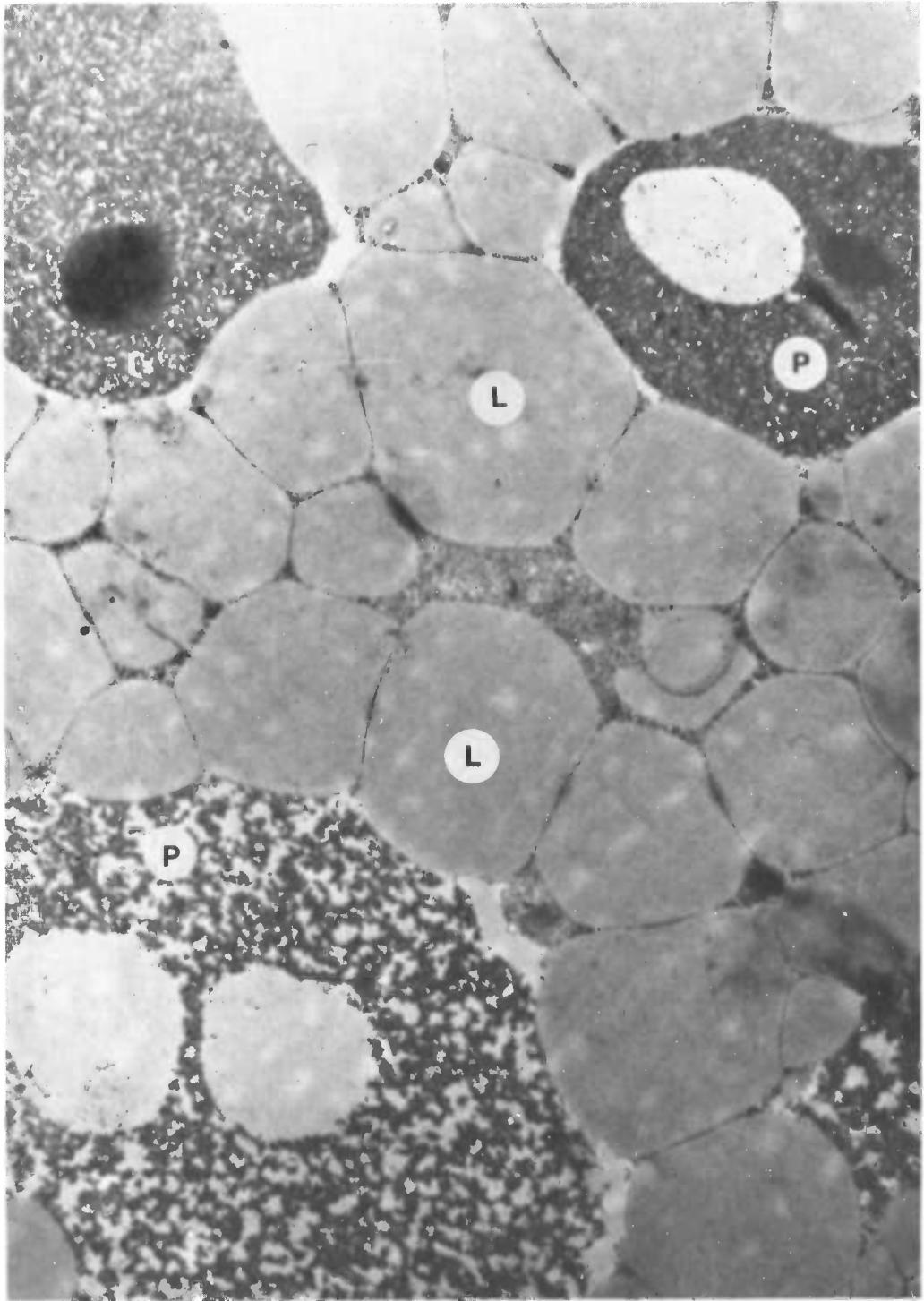


FIG. 9. Electronmicrograph of a thin section through the cotyledon of a 10-day-old etiolated cassava seedling. The cells are packed with lipid (L) and protein (P) bodies.  $\times 14,000$ .

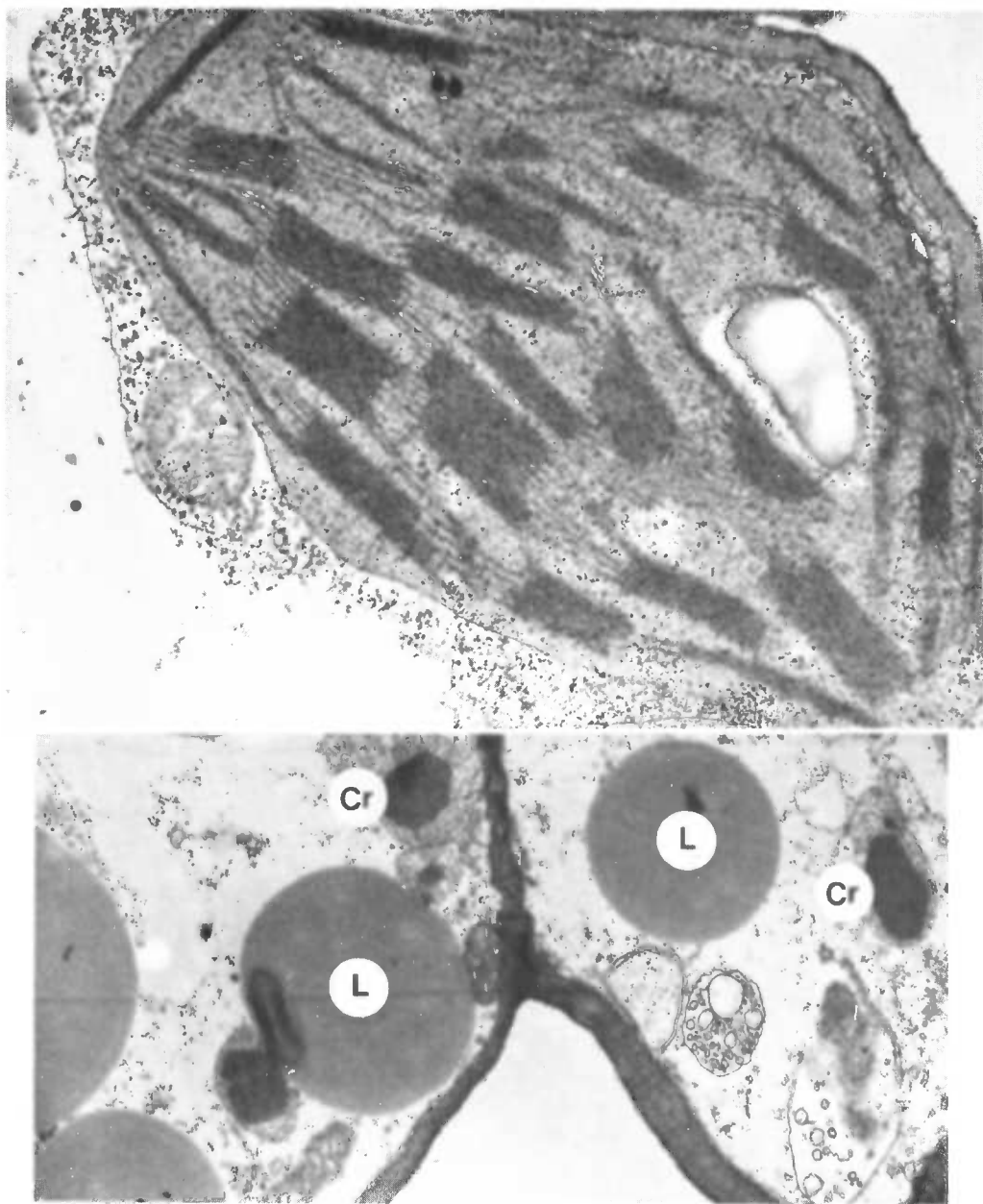


FIG. 10. Electronmicrographs of sections through the green cotyledonary leaf of a 17-day-old cassava seedling which had received light over the last 7 days. The cells contain well-developed chloroplasts (*a*, *top*). Some lipid bodies (L) are still present together with crystalloid containing microbodies (Cr) (*b*, *bottom*).  $\times 28,000$ .

last 7 days. The root cells showed the absence of lipid bodies and the presence of several well-developed organelles. The leaf cells contained well-developed chloroplasts, some lipid bodies, and crystalloid-containing microbodies.

These analyses reveal that the root cells reach ultrastructural organisation, which is characteristic for an active metabolic state, earlier than the cotyledonary cells. This might also suggest that the biosynthesis and degradation of cyanogenic gluco-



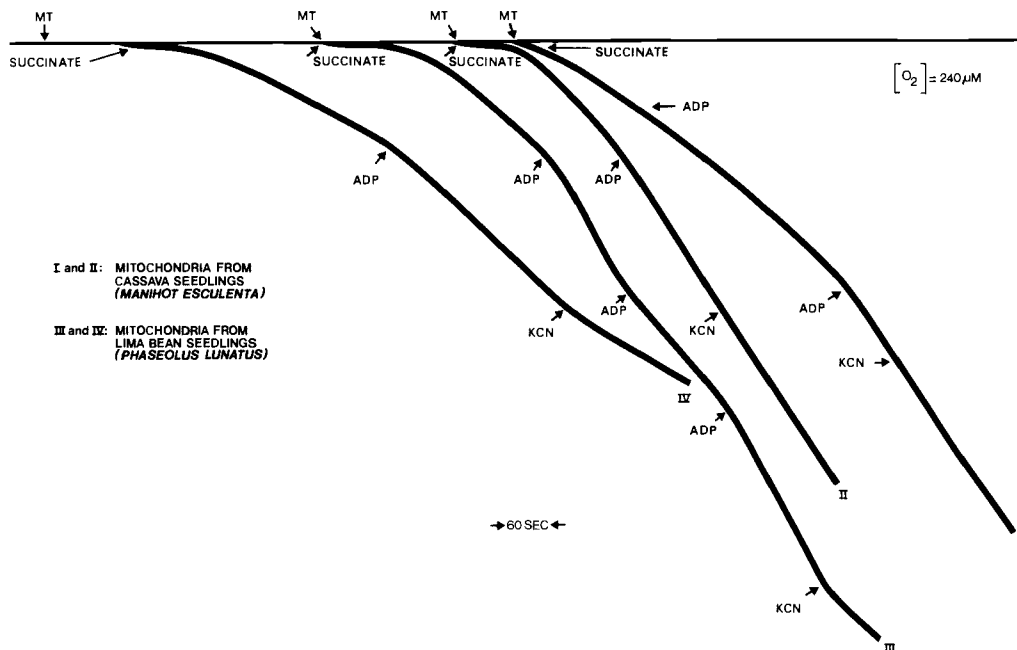


FIG. 11. Typical polarographic traces of the oxidation of succinate by Cassava (A and B) and Lima bean (C and D) mitochondria, showing the effect of neutral KCN on oxygen uptake. Mitochondria were isolated by the methods of Bonner (1967) and Sarkissian and Srivastava (1968). Reaction mixtures contained phosphate buffer pH 7.4, 250  $\mu$ liters mitochondrial suspension (1.2 mg protein), 10 mM succinate, with additions of 225  $\mu$ M ADP and 0.25 mM KCN. Final volume 3 ml. Temperature 25°C. For ADP:O and RC ratios, see text. A Clark-type oxygen electrode (Yellow Springs Instruments Co.) was used in these traces.

sides in actively germinating cassava seeds occur initially in the roots. Autoradiographic studies are planned to further clarify the organelles involved with cyanogenesis in cassava.

### Effect of Cyanide on Respiration of Isolated Mitochondria

Because the observation that the two enzyme systems ( $\beta$ -cyanoalanine synthase and rhodanese) which catalyse cyanide detoxification in cassava were localised in mitochondrial fractions of seedling homogenates, the effect of cyanide on the respiration of isolated mitochondria was studied. Mitochondria isolated from 14-day-old cassava seedlings and 4-day-old lima bean seedlings oxidised succinate, malate, and NADH. Figure 11 is a recording of oxygen uptake by cassava and lima bean mitochondria, measured with a Clark electrode (Yellow Springs). Lima bean mitochondria were more tightly coupled than cassava mitochondria. ADP:O ratios ranged from 1.5 to 2.0 for

cassava mitochondria with succinate as substrate, and from 2.1 to 2.5 for lima bean mitochondria. Respiratory control values ranged from 1.5 to 2.1 for cassava mitochondria, and from 2.0 to 2.6 for lima bean mitochondria. Both types showed a high degree of cyanide-insensitivity during respiration. However, cassava mitochondria were much more insensitive to cyanide than lima bean mitochondria. Oxygen uptake by the former was inhibited by 15.5% in the presence of 0.25 mM KCN (neutralised) while in the latter, the degree of inhibition was 44–50%.

Some plants possess an alternative flavoprotein-mediated cyanide-insensitive respiratory system (Bendall and Bonner 1971). Quite apart from the operation of this system, the activities of  $\beta$ -cyanoalanine synthase and rhodanese in these plants may be involved with the drastic reduction of intracellular cyanide concentrations. Thus, some of the functions of these enzymes may be directly related to the preservation of electron transport via the cytochrome oxidase system, which is highly sensitive to cyanide.

## Conclusions

The available evidence suggests that all cultivars of cassava hitherto studied are cyanophoric, and thus are capable of synthesising and storing lethal concentrations of cyanogenic glucosides in their edible leaves and tubers. The utilisation of these tissues as a source of staple food therefore represents a serious health hazard which can be overcome only by an intensive search for mutant cultivars or varieties in which cyanogenesis is genetically suppressed. An example of varieties of a plant species exhibiting this phenomenon is found in the family Mimosaceae. The South African *Acacia sieberia* var. *woodii* and the Australian *Acacia glaucescens* are both cyanophoric (Rimington 1935; Finnemore and Cox 1928); analysis of organic solvent extracts from leaves and phyllodes indicate that these two plants synthesise and store high levels of the cyanogenic glucosides acacipetalin, sambunigrin, and prunasin. However, the West African *Acacia sieberiana* var. *villosa* does not appear to contain cyanogenic glucosides in its tissues (Nartey 1972 unpublished data).

The production of non-cyanophoric cassava varieties capable of synthesising and storing higher levels of protein in tubers—via mutation studies, intensive screening, and breeding—will undoubtedly minimise the occurrence of diseases engendered by chronic cyanide intoxication and protein deficiency in the developing countries.

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