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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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Endemic Goitre and High Cassava Diets in Eastern Nigeria

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Abstract Subsequent to the discovery of patchy distribution of endemic goitre and the low but markedly varying environmental iodine deficiency in eastern Nigeria, experimental studies of the possible goitrogenic action of dry, unfermented cassava on rats were undertaken. The results showed that cassava has an adverse action on the function of the thyroid, an action comparable to that of thionamide goitrogen.

Résumé La répartition inégale du goitre endémique et la déficience environnementale, faible mais notablement variable, de l'iode dans le Nigéria oriental, nous ont incité à entreprendre une étude expérimentale, sur des rats, de l'action du manioc séché non fermenté comme agent causatif possible du goitre. Les résultats démontrent que le manioc a une action adverse sur la fonction thyroïdienne, action comparable à celle de la thionamide.

An endemic goitre survey (Ekpechi 1964) in Nsukka Division of the east central state of Nigeria in Africa disclosed a marked variation in incidence of goitre from village to village. Also there was no consistent inverse relationship between the iodine content of the drinking water from the various villages and the incidence of goitre. Eha-Amufu village has a visible goitre incidence of 38.2% and a drinking water iodine content of $2.75 \pm 0.04 \mu\text{g/litre}$ compared to Nsukka village with a much lower visible goitre incidence of 9.3% and drinking water iodine content of $0.86 \pm 0.09 \mu\text{g/litre}$. A crude dietary survey suggested a correlation between the intake of dry, smoked, unfermented cassava and the incidence of goitre. Animal experiments designed to test the effect of dry, cooked, unfermented cassava on thyroid function were subsequently carried out at the Royal Postgraduate Medical School of London Uni-

versity and Hammersmith Hospital, London, in Professor T. Russel Fraser's Laboratory.

Preparation of Cassava

The preparation of cassava for food varies. In most areas the cassava is fermented by soaking in water for 3 days, then processed to yield a white, wet, soft, smooth, tasteless paste. The processed product is boiled in water at 160°C for 30 min, pounded, then boiled again for 30 min before pounding to a rubbery paste. It is then ready to be swallowed with gravy.

Other methods of preparation require boiling or frying after the initial fermentation. The finished product of fermented cassava is white or greyish white. Preparation of dry and unfermented cassava consumed in the areas of Nsukka Division is different. The raw cassava is peeled, smoked, then

pulverized and mixed with water to form a greyish-white paste which is then smoked for many months. To prepare a meal of unfermented cassava, the greyish white dried cake is again pulverized and added to boiling water and stirred until a bluish-black paste is formed. This is then cooled and served with gravy.

Materials and Methods

Young female albino rats (Sprague Dawley strain) weighing 170–190 g were divided into four groups of three rats each. The first group was fed 100% cassava diet (prepared in the traditional way), the second group 50% cassava diet mixed with 50% standard laboratory diet (41B cubes), the third group 100% cassava diet plus iodine supplement (10 μ g/rat per day), and the fourth group standard laboratory diet. The diets were given ad libitum for 7 days. The rats were injected intraperitoneally with 20 μ Ci 131 I 24 h before being killed, subsequently anaesthetised with ether, and exsanguinated. The thyroids were quickly removed, counted for radioactivity, and enzymatically digested. The digest was purified by passing through a 6-inch column of anionic exchange resin (Dowex 1 $\times$ 2, 200–400 mesh) and chromatographed in an ascending butanol–acetic acid–water solvent system. Estimates of the stable iodine content of the thyroids were made on aliquots of the digests using the alkaline ashing method.

Results

The rats on cassava diet alone appeared to lose appetite about the fourth day of the experiment and their general food intake was less than that of rats on either standard laboratory diet (41B cubes) or those on iodine supplement: 16 g/rat per day compared to 20 g/rat per day. The animals on iodine supplement were very active, scattering their food all over their cages and defecating and urinating more than the other animals.

Thyroid Weight

The mean thyroid weight of rats on standard laboratory diet was 7.3 ± 0.06 mg compared to 11.1 ± 1.1 mg for rats on 100% cassava, 9.1 mg for rats on 100% cassava plus iodine supplement, and 10.2 ± 1.4 mg for rats on 50% cassava.

The mean thyroid weight of rats on either 100

or 50% cassava seems to be higher than for the other groups, and even with these small groups, the thyroid weight of those on 100% cassava was probably significantly higher than the controls ($t = 2.94$, $P < .05$).

Thyroidal 131 I Uptake at 24 h

The mean thyroidal 131 I uptake of the rats on standard laboratory diet was $14.4 \pm 0.7\%$ dose, compared to 18.2% for rats on 100% cassava and $14.3 \pm 0.7\%$ dose for rats on 50% cassava.

The animals fed 100% cassava had a probably significantly higher uptake than the controls ($t = 5.5$, $P < .01$) whereas the other three groups differed very little (those on 50% cassava appeared to have a lower uptake: $t = 3.2$, $P < .05$). This higher uptake was no longer present in animals fed 100% cassava plus iodine supplement, whose uptake was normal.

Serum Protein-Bound Radioactive Iodine

The following 24-h protein-bound 131 I values are expressed as percent dose per cubic centimeter of serum: 0.028 for rats fed standard laboratory diet compared to 0.08 for those fed 100% cassava, 0.06 for those fed 100% cassava plus iodine supplement, and 0.02 for those fed 50% cassava.

The animals on 100% cassava had a PB 131 I 2.9 times that for rats on control diet. Note that the level of PB 131 I remained still higher in animals fed 100% cassava plus iodine. Only single estimations on the pooled sera of the different groups of rats could be done as the serum from one rat was not found sufficient for a valid estimation. Statistical testing of the difference in PB 131 I value between the different groups could not be done. I previously reported (1964) a mean PB 131 I of $0.017 \pm 0.006\%$ dose/ cm^3 for 15 rats on standard laboratory diet and comparing this (probably) normal value with the value of 0.08 found for rats on 100% cassava or 0.06 for rats on 100% cassava plus supplement suggests that the PB 131 I for 100% cassava-fed rats was still markedly elevated.

Thyroidal 127 I

Total concentration—The value expressed in micrograms per milligrams thyroid tissue for rats on standard laboratory diet was 0.8 compared to 0.14 for rats on 100% cassava, 0.36 for rats on

100% cassava plus iodine supplement, and 0.35 for rats on 50% cassava. As these estimates were done on the pooled homogenate of each group of three rats it was not possible to test statistical significance of the difference between the different groups, but with a coefficient of variation of 12% as found for similar estimates on 15 rats on standard laboratory diet, it is quite evident that there is a striking difference between the values of 0.14 $\mu\text{g}/\text{mg}$ for rats on 100% cassava and 0.8 $\mu\text{g}/\text{mg}$ for rats on standard laboratory diet.

Total stable—The mean total stable iodine for rats on standard laboratory diet was 10 $\mu\text{g}/\text{gland}$ or 5.9 $\mu\text{g}/100\text{ g}$ body weight compared with 2.6 $\mu\text{g}/\text{gland}$ or 1.6 $\mu\text{g}/100\text{ g}$ body weight for those on 100% cassava, 6.7 $\mu\text{g}/\text{gland}$ or 3.6 $\mu\text{g}/100\text{ g}$ body weight for rats on 50% cassava, and 5.8 $\mu\text{g}/\text{gland}$ or 3.3 $\mu\text{g}/100\text{ g}$ body weight for those on 100% cassava plus iodine supplement. This degree of depletion of iodine stores in rats fed 100% cassava is quite noteworthy.

Chromatographic Studies

As shown in Fig. 1, the animals on standard laboratory diet had an MIT:DIT ratio of 0.56 ± 0.04 compared to 1.44 ± 0.06 for rats on 100% cassava, 1.3 ± 0.02 for those on 100% cassava plus iodine supplement, and 1.4 ± 0.1 for those on 50% cassava. The MIT:DIT ratio for rats either on 100% cassava ($t = 14.6$, $P < .001$) or on 100% cassava plus iodine ($t = 12.8$, $P < .001$) is significantly higher than that for rats on standard laboratory diet.

The proportion of ^{131}I as iodothyronine (i.e. thyroxine and triiodothyronine), 35.6% of the organic for rats on standard laboratory diet, is much lower than that for the other three groups of animals. There is a high peak of activity beyond the site of thyroxine as identified with stable thyroxine (Fig. 1, top). It is likely, but not definite, that this peak corresponds to the site of triiodothyronine in this solvent system (butanol-acetic acid).

The proportion of ^{131}I as iodothyronine for rats on 100% cassava was 51% compared to 64% for rats on 100% cassava plus iodine supplement and 50% for rats on 50% cassava.

Discussion

There is recent experimental support that some antithyroid agents, such as thiourea, have a

variable effect on the radioactive iodine uptake (Slingerland et al. 1959). They reported that propylthiouracil at a concentration of 1×10^{-4} mg irregularly stimulates radioactive iodine uptake in the rat thyroid, while at higher concentration it regularly depresses uptake. Kilpatrick (1961) also reported that rats fed 100 μg of carbimazole daily for 10 days had a radioactive iodine uptake which was significantly higher than that for rats on water or 500 μg of carbimazole daily for 10 days. The figures for the 24-h uptakes were 10.1% dose for control rats, 6% dose for those on 500 μg of carbimazole, and 13.5% dose for those fed 100 μg of carbimazole.

These observations raise the possibility that small daily intakes of dietary goitrogens in endemic areas could cause an increase rather than a decrease in radioactive iodine uptake. Clement and Wishart (1956) reported that milk containing a suspected goitrogen depressed radioactive iodine uptake in adults who drank it. But the amount of milk consumed (60–80 oz) was certainly a much larger quantity than a normal adult takes daily. The larger the amount given, the greater the amount of probable goitrogen ingested, and the pattern of uptake may well change. It would be interesting to see what would happen to the uptake if Clement and Wishart had given only 10–20 oz of milk to their patients.

The probably significantly higher thyroid weight and higher thyroïdal uptake of radioactive iodine at 24 h for rats on 100% cassava, compared with rats on standard laboratory diet, suggest an effect of iodine deficiency on the thyroid. But a pure iodine-deficient diet did not significantly change the thyroid weight even after 206 days so this effect of cassava on thyroid uptake is unlikely due to its low content of iodine alone. This observation, that pure iodine deficiency does not cause an early change in thyroid weight, has been made by van Middlesworth (1952) and Money et al. (1952).

More recent work by Slingerland et al. (1959) and Kilpatrick (1961) has shown that low concentration of a goitrogen of the thionamide series can cause an increase or variable thyroïdal uptake of radioactive iodine. It is, therefore, possible that the increased uptake of ^{131}I in cassava-fed animals (100%) could have been due to the presence of a low concentration of a goitrogen in cassava.

The striking degree of thyroïdal iodine depletion caused by cassava in 7 days is unlikely to have been

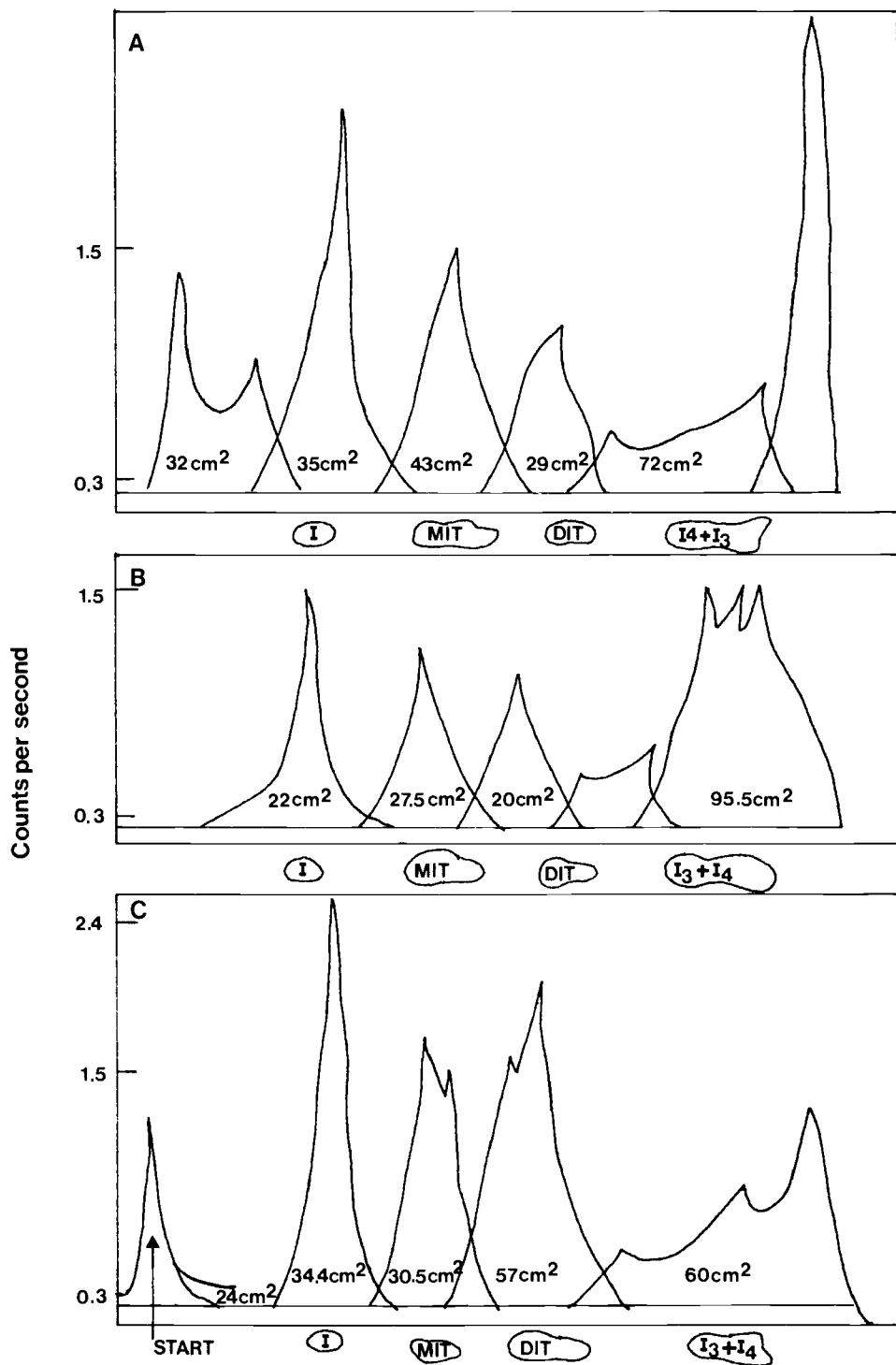


FIG. 1. Effect of cassava on thyroid hormone production in the rat (chromatograms of resin purified rat thyroid digests after 7 days on cassava).

TABLE 1. Comparison of various measures of thyroid function in rats fed on 100% cassava with similar measures on rats on 100% cassava plus iodine supplement, 50% cassava, and standard control diets over a 7-day period.

Experimental groups	Thyroid weight (mg/100 g/rat)	Thyroid digest			
		Distribution of Intrathyroidal ^{131}I at 24 h as % dose		Total $^{127}\text{I}^a$ ($\mu\text{g}/\text{mg}$ thyroid)	S PB ^{131}I % dose/cc
		Ratio MIT:DIT (mean $\pm$ SE)	Iodothyronine as % organic (mean $\pm$ SE) ^c		
Fed 100% Cassava	11.1 $\pm$ 1.1	1.44 $\pm$ 0.06 ^c	51%	0.14	0.08
50% Cassava plus 50% Control Diet (41B)	10.2 $\pm$ 1.4	1.4 $\pm$ 0.11 ^c	50%	0.35	0.02
Cassava plus iodine supplement ($\mu\text{g}/\text{rat}/\text{day}$)	9.1 $\pm$ 1	1.3 $\pm$ 0.02 ^b	65%	0.36	0.06
Control diet 41B and standard laboratory diet	7.3 $\pm$ 0.06	0.6 $\pm$ 0.02	35.6%	0.8	0.028

^aSingle estimates on pooled homogenate of three rats.

^bSingle estimates on pooled sera of three rats.

^cIodothyronines as % of organic iodoamino acids.

caused by its low iodine content. Table 1 shows that a 7-day pure iodine-deficient diet had no obvious effect on the total thyroïdal iodine. Slingerland et al. (1959) and Kilpatrick (1961) reported severe depletion of total thyroïdal iodine by thionamide goitrogen. Slingerland et al. (1959) found that the total thyroïdal iodine of the rat was reduced from 9 $\mu\text{g}/\text{gland}$ to 2.4 $\mu\text{g}/\text{gland}$ in 10 days by 0.1% propylthiouracil. Kilpatrick (1961) found that carbimazole (500 μg) reduced the total thyroïdal iodine in the rat from 15.2 to 3.6 μg in 10 days. In this respect the effect of cassava on the total iodine stores is not unlike that of either carbimazole reported by Kilpatrick or to 0.1% propylthiouracil by Slingerland et al.

The marked elevated PB ^{131}I as found in 100% cassava-fed rats could be regarded as a reflection of the low intrathyroïdal iodine stores and the rapid turnover. But this degree of elevation of PB ^{131}I was not observed with pure iodine deficiency. Such increased release of thyroïdal ^{131}I either as reflected by the PB ^{131}I or otherwise has been observed in rats fed propylthiouracil by van Middlesworth (1952) and by Escobar del Rey et al. (1961). Using ^{131}I labelled l-thyroxine, they later reported that such increased release of thyroïdal ^{131}I in rats treated with propylthiouracil

and thiouracil was due to the decreased peripheral deiodination of thyroxine. Such effects of thionamide goitrogens (propylthiouracil and thiouracil) in decreasing peripheral deiodination of thyroxine can and would cause a marked elevation of PB ^{131}I . This abnormal elevation of PB ^{131}I in 100% cassava-fed rats is, therefore, possibly due to the presence of a thionamide goitrogen in cassava, since as mentioned above, very low intrathyroïdal iodine concentration alone did not produce that degree of elevation in our other experiments.

The significant changes in the intrathyroïdal iodine metabolism are the alteration of ratio of ^{131}I MIT:DIT and the increased production of iodothyronines in all cassava-fed animals compared to the rats on standard laboratory diet. It is worthy of note that even iodine supplementation did not reverse this effect of cassava on thyroid function.

Changes in the ratio of ^{131}I MIT:DIT have been reported in a number of conditions, and it is doubtful if this change is specific to any pathological or biochemical abnormality in the thyroid. Nevertheless, this change has consistently been reported in animals fed thionamide goitrogens. Kilpatrick (1961) reported that the ratio of ^{131}I MIT:DIT for rats on 100 μg of carbimazole for 10 days was

1:34. Slingerland et al. (1959) found a ratio of 2:2 for rats on 0.1% propylthiouracil for 10 days. No one as far as we know has reported a change in this ratio in the very early stages of iodine deficiency. Leloup and Lachiver (1955) reported changes in this ratio in rats after 3 or 6 months on an iodine-deficient diet. We found a normal ratio of 0.34 in albino rats fed an iodine-deficient diet after 7 days.

Slingerland et al. (1959), discussing the change in this ratio, commented that a severe depletion of total iodine in the gland was not the cause since the MIT:DIT remained high when the total iodine was preserved by adding thyroid to propylthiouracil. A high T.S.H. as well could not account for the elevated ratio since thyroid tablets would have suppressed this. Ermans et al. (1963) found a significant correlation ($r = 0.73$, 0.02 , $P < 0.01$) between this ratio of MIT:DIT and iodine concentration per gram, but not with total iodine of human goitrous thyroid gland in "slow secretion patients" from Uele in the Congo, an endemic goitre area. On the other hand, Dimitriadou et al. (1961) did not find any change in this ratio in young Thai endemic goitres even though the iodine concentration per gram of thyroid in some patients was as low as that of Ermans et al. (1963).

After 7 months on an iodine-deficient diet the MIT:DIT ratio of rats was still below unity regardless of the fact that their iodine concentration per milligram of thyroid tissue had dropped to $0.14 \mu\text{g}$. In the light of our experimental results as stated above, the change in the ^{131}I -labelled MIT:DIT found in cassava-fed animals must have an explanation other than diminished iodine concentration or diminished total iodine. And the most likely explanation here is that cassava has an effect on this ratio not unlike that of the thionamide goitrogens.

The increased proportion of ^{131}I as iodothyronines in all cassava-fed animals compared to rats on standard laboratory diet is a reflection of the rapid iodine turnover which in turn reflects the decreased thyroïdal iodine concentration in these animals. Such an accelerated release of labelled hormone and rapid turnover in the pattern in human thyrotoxicosis and iodine deficiency has been reported by Dimitriadou et al. (1961) and by Kierderling et al. (1961). So far as we are aware such over-active synthetic function of the thyroid has not been reported with goitrogens. On the contrary, it is well established that goitro-

gens depress production of iodothyronines. Studies which recorded the depression of iodothyronine production by thionamide goitrogen have been carried out with chemically pure thionamide goitrogen and the general conclusions from such studies can not apply strictly to our studies since the cassava effect on thyroid function is a combination of its iodine deficiency as well as its probable goitrogenic content. This unusual combined effect of high MIT:DIT ratio and increased rate of production of iodothyronine in rats fed 100% cassava can probably be explained by the very low thyroïdal iodine concentration and the rapid turnover of whatever iodine that gets beyond the block between the MIT and DIT.

As can also be observed in Table 1, the rate of iodothyronine production is higher (64%) in animals fed cassava plus iodine supplement than in animals on cassava alone (51%). It seems that there are two factors responsible for this observation: the increased rate of secretion of labelled active hormone as reflected by PB ^{131}I , and the probable increased rate of production of triiodothyronine in animals on cassava alone without iodine supplement (0.08% dose against 0.06% dose).

The above observations of the effect of unfermented cassava on different parameters of thyroid function strongly suggest that unfermented cassava contains a substance with an effect not unlike that of the thionamide goitrogens. Thus, the probably increased thyroid weight, the abnormally elevated PB ^{131}I , the severe depletion of iodine stores and the higher ratio of MIT:DIT, and also the fact that iodine supplement could not reverse all the effects of cassava, are in keeping with the effect of a thionamide goitrogen.

The point has been made that food goitrogens act as permissive factors in an area of poor iodine supply. Some support to the above comes from our therapeutic trials of pot iodide in an unselected group of goitrous patients in Nsukka Division. Among 236 young goitrous adults living in this division, aged 25 and under, given 10 mg of pot iodate daily for 3 months, only 17 still had palpable glands after the trial whereas the response to iodine therapy was very poor in the older age-group from the same village. The trial was confined to 67 non-nodular goitrous males and females aged 45 years and over. Only 11 had any marked decrease in their goitre size after 3 months. A longer duration of iodine therapy may be needed

to produce significant reduction in the size of older goitres.

When sufficient research funds are available for the detailed study of iodine kinetics, and the measurement of thyroidal uptake of ^{131}I before and after cassava meals, we will be able to determine what role dry, smoked, unfermented cassava plays on the etiology of goitre in the inhabitants of this area.

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