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Cassava Is Not a Goitrogen in Mice[1] (42145)

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Abstract. To examine the effect of cassava on the thyroid function of mice, we fed fresh cassava root to mice and compared this diet with low iodine diet and Purina. Cassava provided a low iodine intake and increased urine thiocyanate excretion and serum thiocyanate levels. Mice on cassava lost weight. The thyroid glands of mice on cassava were not enlarged, even when normalized for body weight. The 4- and 24-hr thyroid uptakes of mice on cassava were similar to those of mice on low iodine diets. Protein-bound [^{125}I]iodine at 24 hr was high in mice on either the cassava or low iodine diets. The thyroid iodide trap (T/M) was similar in mice on cassava and low iodine diets. When thiocyanate was added *in vitro* to the incubation medium, T/M was reduced in all groups of mice; under these conditions, thiocyanate caused a dose-related inhibition of T/M. The serum thyroxine (T_4) and triiodothyronine (T_3) concentrations of mice on cassava were reduced compared with mice on Purina diet. Thyroid T_4 and T_3 contents of mice on cassava were relatively low compared with mice on Purina diet. Hepatic T_3 content and T_4 5′-monodeiodination in liver homogenates were reduced in mice on cassava compared with other groups. The data show that cassava does not cause goiter in mice. The thiocyanate formed from ingestation of cassava is insufficient to inhibit thyroid iodide transport or organification of iodide. The cassava diet leads to rapid turnover of hormonal iodine because it is a low iodine diet. It also impairs 5′-monodeiodination of T_4 which may be related to nutritional deficiency. These data in mice do not support the concept that cassava per se has goitrogenic action in man.

Cassava is a major source of food calories in many countries in the world today (1, 2). One hundred eighteen million tons of cassava were produced in 1980, and two-thirds were for human consumption. Cassava has also been reported to be a goitrogen (3). Ermans and his colleagues have carried out a series of studies of the role of cassava as a goitrogen in central Africa and concluded that it had significant goitrogenic activity in regions of low iodine intake (4, 5). Cassava contains cyanogenic glucosides which release cyanide after ingestion. The cyanide is detoxified to thiocyanate, and the thiocyanate may cause goiter by displacement of iodide from the thyroid and by blocking organic binding of iodine (6). The postulated goitrogenic effect of cassava was manifest only in the presence of severe iodine deficiency (4, 5).

Because of the widespread use of cassava as a food in less developed countries, it is important to understand its effect on the thyroid. To accomplish this, we fed cassava to mice and studied their thyroid function.

Materials and Methods. *Animals.* Swiss–Webster female and male mice were obtained from Simonsen Laboratories (Gilroy, Calif.) at 4 weeks of age and fed special diets and distilled water. These diets were Remington low iodine diet (ICN Nutritional Biochemicals, Cleveland, Ohio) reported to contain 0.07 μg iodine/g diet, Purina rodent chow (Gardena, Calif.) which has 0.7 μg iodine/g, or cassava. The cassava was frozen, peeled cassava root (Emperador from Costa Rica) obtained from a local produce market in Los Angeles (O. & P. Dist., Inc., Los Angeles, Calif.). Three different batches were used. The cyanide content of the cassava was 28 ± 6 (SD) μg/g based on assay of 11 samples. The following additional compositional data were obtained as milligram per gram wet weight (n = 5 samples): nitrogen, 1.25 ± 0.30

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(protein, 7.8 ± 1.9); sodium, 0.34 ± 0.07; potassium, 3.72 ± 0.71; calcium, 0.18 ± 0.02; magnesium, 0.22 ± 0.02; phosphorus, 0.98 ± 0.22. Iodide was undetectable (<15 ng/g wet wt).

Because the mice lost weight on the cassava diet, a series of experiments was also performed with mice fed cassava and a supplement of low iodine diet or Purina three times weekly (5 pm–8 am) to prevent weight loss.

To collect urine specimens, mice were placed in metabolic cages. These mice had been on low iodine diet for 4 weeks and were then switched to cassava or Purina for 2 days before beginning daily urine collections for 3 days.

Measurements. Serum thyroxine (T_4) and triiodothyronine (T_3) concentrations were measured by radioimmunoassays (7). Urine and serum thiocyanate (SCN) was measured by the modification of the method of Aldridge (8) described in Test Information Summary No. 966 of Bio-Science Laboratories, Van Nuys, California. Urine iodine was measured by Bio-Science Laboratories using the Autoanalyzer; the sensitivity of the method is 2 ng/ml. Cyanide was measured in fresh frozen cassava after homogenization of 2- to 5-g aliquots ((4), p. 22).

Thyroid uptake of radioiodine was measured at 4 and 24 hr after injection of 10 μCi of [^{125}I]iodide ip in 20 μl saline. The mice were gently positioned over a collimated scintillation well crystal, counted for 30 sec, and compared with a standard. At 24 hr, the mice were bled by aortic transection, the thyroids were removed and weighed. Each group of thyroid glands was pooled, suspended in 500 μl of 0.05 *M* sodium phosphate buffer, pH 7.0, and homogenized with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.) for 60 sec using the PT 7 generator. Then 100–200 μl of thyroid homogenate was digested with Pronase (3 mg/ml, Calibiochem-Behring Co., San Diego, Calif.) for 20 hr at 37°C under nitrogen (9). T_3 and T_4 content in the thyroid digest was measured by radioimmunoassay (7).

Serum [^{125}I] protein-bound iodine (PB^{125}I) was measured on 25-μl aliquots after precipitation by 1.2 ml of 10% trichloroacetic acid (TCA); after centrifuging, the precipitate was washed with 1 ml 10% TCA and then counted in a gamma well counter. Results were expressed as the percentage dose per milliliter.

After sacrifice of the mice by decapitation, thyronines were extracted from liver tissue (10) and then measured by radioimmunoassay (9). The completeness of recovery was monitored by the addition of [^{125}I]T_4 or [^{125}I]T_3 to the tissue before extraction, and the results were corrected for recovery. Recover was 53.6% for T_4 and 70.3% for T_3.

Thyroid iodide trap. The activity of the thyroid iodide trap was measured by the following method. Mice were sacrificed by decapitation. The thyroid was removed attached to the trachea, rinsed with McCoy's 5a medium with L-glutamine (Gibco Laboratories, Grand Island, N.Y.), blotted gently, and placed in 35 × 10-mm Falcon 3001 culture dishes (Oxnard, Calif.) containing 3 ml McCoy's medium, 1 m*M* methimazole to prevent organic binding, 10^{-8} *M* potassium iodide, and [^{125}I]iodide, 100,000 cpm/ml. Incubation was carried out at 37°C for 150 min in an incubator based on data showing a progressive increase of iodide accumulation up to 180 min. With each experiment, a group of control thyroids was incubated in medium containing 1 m*M* sodium thiocyanate which completely inhibited iodide transport. There were usually six mice in each group of animals on special diets. At the conclusion of the incubation, the thyroids were removed from the medium, rinsed quickly with medium without [^{125}I]iodide, dissected from the trachea, weighed, and counted. From the thyroid counts/milligram thyroid tissue and the medium counts/microliter, the thyroid/medium ratio (T/M) was calculated. In one set of experiments, thiocyanate was added to the medium *in vitro* in concentrations varying from 0.25 to 2.0 mg/dl, and the T/M was determined using thyroids from groups of mice which had been on the special diets for 18–19 days.

T_4 5′-monodeiodination was studied in liver homogenates using the technique recently reported (11).

Statistical analysis of the data was carried out by application of Student's *t* test for unpaired data and by multiple comparison tests or analysis of variance as appropriate.

Results. Mice consumed 8 to 14 g cassava

daily. On the cassava diet, the mice gradually lost weight rather than gaining weight. In comparison with the controls on low iodine, the weight of the mice on the cassava diet fell as low as 46% of the weight of the controls at 56 days of the diet (Fig. 1). When the cassava mice received a supplement of low iodine or regular diet thrice weekly, their mean weight was similar to that of the controls. The weight of mice on low iodine diet was similar to that of mice on Purina diet (data not shown).

Urine iodine excretion was very low in mice on both the low iodine and cassava diets compared with that of Purina (Fig. 2). Urine thiocyanate was markedly elevated in mice eating cassava compared with the other two groups (Fig. 2), even though the cassava was given for only 5 days. The ratio of urine SCN/I was significantly higher in mice eating the cassava diet (4765 ± 709 (SD)) than the low iodine diet (3411 ± 670), and these ratios were much greater than those of mice eating Purina (71 ± 18). Serum thiocyanate concentration was similar in mice on the Purina and low iodine diets, and it was greatly elevated in those eating cassava for 5 days (Fig. 2). In mice given these diets for 7 to 8 weeks, the serum thiocyanate level was 0.33 ± 0.02 (SE) mg/dl on Purina, 0.23 ± .02 mg/dl on low iodine diet, and 1.18 ± 0.11 mg/dl in those on cassava ($P < 0.001$ compared with other groups).

The thyroid glands of the mice on the cassava diet were consistently smaller than those of the mice on low iodine diet, even

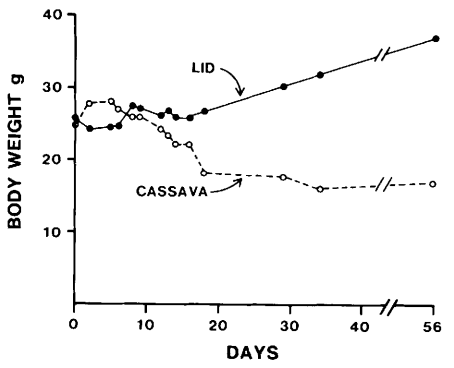

FIG. 1. Mean body weight of mice on cassava diet or low iodine diet (LID), 8 to 20 mice per group.

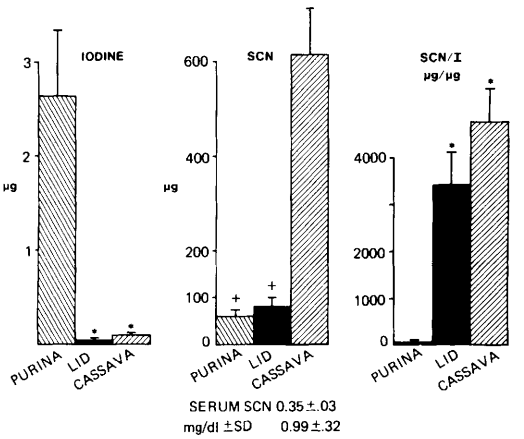

FIG. 2. Urine iodine and thiocyanate content of mice on various diets for 5 days after 4 weeks of low iodine diet (LID); daily urine collections were made during the last 3 days of the various diets; 8 mice per group; results are μg/24 hr ± SD. (*$P < 0.01$ vs Purina; †$P < 0.01$ vs cassava.)

when normalized for body weight ($P < 0.05$). Because of the weight loss sustained by the cassava mice and the likelihood that the cassava diet is deficient in protein, a group of mice on cassava received supplements of low iodine or Purina for 35 days (Fig. 3). The mice on low iodine diet had thyroid glands which were significantly larger than those on Purina; the thyroid size of the mice on cassava + low iodine was similar to those on low iodine; i.e., cassava had no additional goitrogenic effect.

The 4-hr thyroid uptakes of the mice on the diets with low iodine content (low iodine, cassava, cassava + low iodine) were higher than those of mice on Purina (Fig. 4). There was no significant difference between the group on cassava + low iodine compared with that on low iodine alone.

The serum $PB^{125}I$ was much higher in the three groups taking diets of low iodine content compared with those eating Purina (Table I). This indicates rapid turnover of hormonal iodine. There was no difference between these three groups. The mice eating cassava supplemented with Purina had a slightly but significantly greater $PB^{125}I$ than those on Purina alone.

The activity of the thyroid iodide trap was similar in the mice on low iodine diet, cassava, or cassava + low iodine diet (Fig. 5).

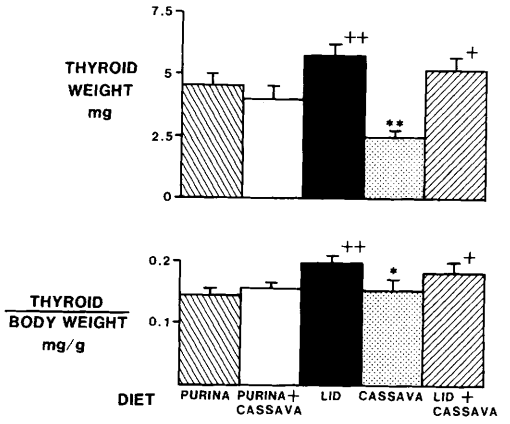

FIG. 3. Thyroid weight of mice on various diets for 34 days. Six to nine mice per group, mean ± SE; $**P < 0.01$ compared with LID (low iodine diet); $*P < 0.05$ compared with LID; $‡P < 0.01$ compared with Purina diet; $†P < 0.05$ compared with Purina.

The slight lowering of T/M (16.7 ± 7.3 (SD)) in the latter group was not significant compared with the T/M of mice on low iodine diet alone (20.8 ± 8.5). When thiocyanate was added to the incubation medium, there was a marked inhibition of the T/M at the lowest [SCN] tested, 0.25 mg/dl (Fig. 6). The inhibition of iodide trapping by thiocyanate *in vitro* was concentration dependent. The inhibition of T/M was slightly less in mice on the cassava diet than in those on low iodine diet.

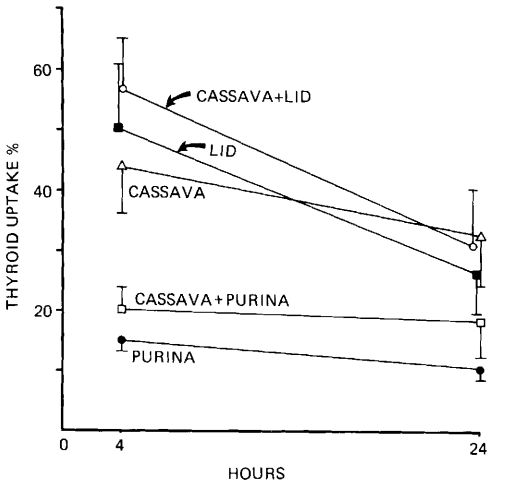

FIG. 4. Thyroid uptakes at 4 and 24 hr in mice on various diets for 25 days, six mice per group, mean ± SE. Four-hour thyroid uptakes of mice on Purina or cassava + Purina were significantly lower than the other three groups ($P < 0.01$).

TABLE I. SERUM PBI-125 OF MICE ON VARIOUS DIETS FOR 25 DAYS (SIX/GROUP, MEAN ± SD)

Diet	PBI-125 (% dose/ml)
Purina	0.20 ± 0.06
Purina + cassava	0.51 ± 0.18*
Low iodine	3.57 ± 1.10*, ns[a]
Cassava	3.15 ± 2.20*, ns
Low I + cassava	2.46 ± 0.76*, ns

[a] ns, Comparisons among these data by nonpaired *t* test were not significant.
* $P < 0.01$ vs Purina.

The serum T_4 concentration of mice on cassava was less than that of mice on Purina or cassava + Purina (Table II). Mice on low iodine diet or cassava + low iodine diet had greatly reduced serum T_4 concentrations compared with the other groups. The serum T_3 concentration of the mice on cassava was significantly lower than those of the Purina or low iodine groups.

The T_4 content of the thyroid glands of mice on low iodine diet or cassava + low iodine diet for 35 days was too low to measure (Fig. 7). It was very low in mice on cassava compared with those on Purina. The thyroid T_3 content of the mice on low iodine diet was less than that of mice on cassava. Mice on Purina had higher thyroidal T_3 and T_4 contents than the other groups.

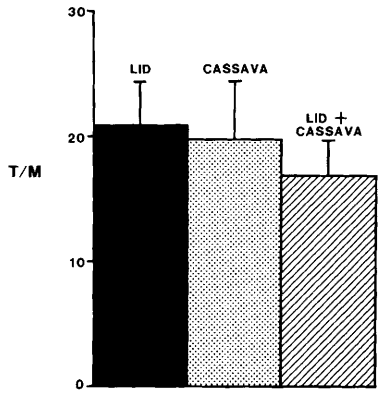

FIG. 5. Thyroid/medium (T/M) gradient of $^{125}I^-$ of mice on various diets six mice per group, mean ± SD; there were no significant differences between groups.

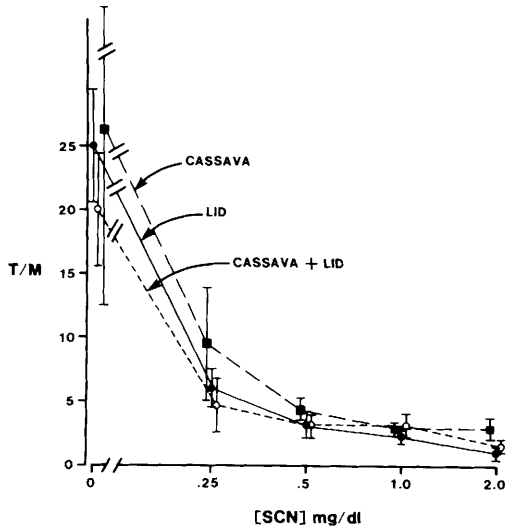

FIG. 6. Effect of thiocyanate (SCN) added *in vitro* on thyroid trap (thyroid/medium, T/M) of mice on various diets for 18 days, $n = 5$ per group, mean ± SD.

The concentration of T_4 in the liver was reduced in mice on cassava or low iodine diet compared with mice on the Purina diet (Table II). The liver T_3 concentration was significantly reduced only in mice eating cassava. The livers of mice on cassava were smaller (average 0.6 g) than those of the other mice (1.7 g); the liver weight per gram body weight was 34 mg/g for cassava mice, 51 mg/g for mice on cassava + low iodine diet, and 55 mg/g for mice on low iodine diet.

In liver homogenates of mice on the various diets, T_4 5'-monodeiodinase activity was (ng T_3/mg protein/20 min): 1.20 ± 0.15 (SD) with the low iodine diet, 0.95 ± 0.24 with the cassava supplemented with low iodine diet, and reduced to 0.39 ± 0.11 in mice eating only cassava ($P < 0.001$).

Discussion. Our studies show that cassava does not have a goitrogenic action in mice. The cassava fed to the mice had a cyanide concentration which was similar to that reported to be used in regions of endemic goiter (4). Mice on cassava had serum thiocyanate levels which were in the range of those in human subjects ingesting cassava (4). In addition, the urine thiocyanate excretion was greatly increased compared with controls. The urine iodine excretion was very low in the mice on the cassava diet and was similar to that of mice on the low iodine diet. Therefore, the cassava fed to the mice did not contain an amount of iodine which may have nullified the potential goitrogenic effect of the thiocyanate. Others have also found no increase of thyroid size of rats fed cassava roots for 3 to 4 weeks (12); in another report, the same investigators found that cassava ingestion caused goiter in rats when compared with controls on a higher iodine intake ((4), p. 98).

The high 4-hr thyroid uptake of the mice on cassava showed the relative iodine deficiency of the cassava diet compared with Purina chow. If thiocyanate generated from cassava could block the peroxidase enzyme system and the formation of thyroid hormone, then the 4- and 24-hr thyroid uptakes would have been lower than those of the controls, but this was not the case. Likewise, the increased $PB^{125}I$ of the mice on cassava showed that thyroid hormone turnover was

TABLE II. SERUM AND HEPATIC T_4 AND T_3 CONCENTRATIONS OF MICE ON VARIOUS DIETS FOR 5 TO 8 WEEKS, MEAN ± SE

Diet	No.	Serum T_4 (μg/dl)	Serum T_3 (ng/dl)	Liver[a] T_4 (ng/g)	Liver[a] T_3 (ng/g)
Purina	30	6.2 ± 0.3	67 ± 3	9.8 ± 1.1	2.8 ± 0.3
Cassava + Purina	10	5.6 ± 0.4	75 ± 5	7.5 ± 0.6	2.3 ± 0.2
Low iodine	14	2.1 ± 0.2[b]	68 ± 5	4.4 ± 0.4[b]	3.0 ± 0.2
Cassava	27	4.4 ± 0.4[b]	46 ± 4[b]	4.5 ± 0.1[b]	1.3 ± 0.1[b]
Cassava + low iodine	11	1.4 ± 0.1[b]	59 ± 8	2.8 ± 0.3[b]	2.4 ± 0.3

[a] $n = 6$ for each group.
[b] $P < 0.05$ vs Purina.

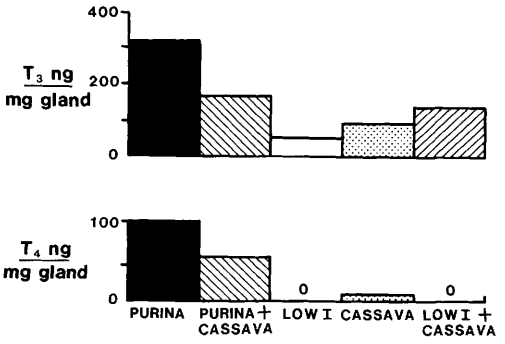

FIG. 7. T_4 and T_3 contents of mice on various diets; each bar is the mean of a pool of six thyroid glands in each group.

increased and that the amount of thyroid hormone formation was similar to that of mice on the low iodine diet.

The study of the thyroid trap showed that mice on cassava had a relatively normal T/M which was similar to that of mice on low iodine diet. If malnutrition were to cause a relative increase of T/M and thus block the inhibitory action of cassava, then the inhibitory action should have been restored in the mice on cassava supplemented with low iodine diet because they did not lose weight. However, these mice had thyroid T/M for iodide which was indistinguishable from the T/M of mice on low iodine diet alone. When the thyroids of mice on various diets were incubated in thiocyanate *in vitro,* there was marked inhibition of the thyroid trap, as has been reported with thiocyanate treatment *in vivo* and *in vitro* (6, 13, 14). Thiocyanate is not concentrated by the thyroid (14) and is rapidly cleared by the kidney so that serum thiocyanate concentrations do not increase in proportion to the administered dose of thiocyanate (16). If thyroid [SCN] were the same as the serum [SCN], then it should have been sufficient to inhibit iodide trapping. Because the thyroid uptake was similar in the mice on the cassava diet to that of mice on low iodine diet, the cassava apparently did not produce a significant block of the thyroid iodide trap *in vivo.* This suggests that thyroid thiocyanate concentrations were insufficient to block the trap or that there is some form of adaptation to the thiocyanate load. The slight reduction of the inhibitory action of thiocyanate on T/M (when added *in vitro*) by the thyroids of mice ingesting cassava supports this possibility.

Mice on cassava had reduced serum T_4 and T_3 concentrations compared with mice on Purina. The low serum T_4 concentration is attributable to the low iodine intake. The low T_3 concentration may be attributed to reduced extrathyroidal formation of T_3 from T_4, as was demonstrated in the liver homogenates of the mice eating cassava. 5'-Monodeiodination of T_4 is sensitive to carbohydrate and protein deprivation (17). Since cassava is almost entirely carbohydrate, carbohydrate deprivation per se is unlikely to explain the reduced serum T_3 concentrations in the mice. The lower hepatic T_3 content of the cassava mice may also reflect the reduction of outer ring deiodination of T_4. The thyroid T_3 content of the cassava mice was slightly greater than that of mice on the low iodine diet, so that reduced biosynthesis of T_3 by the thyroid is a less likely explanation for the effect of cassava.

The cause of the weight loss of the mice eating cassava is unclear but may be protein malnutrition or possibly a toxic effect of cassava. However, if it were a toxic effect, then the mice eating cassava supplemented with more nutritious food thrice weekly would also be expected to show some weight loss, but they did not. The protein of cassava is deficient in many essential amino acids especially methionine, cysteine, lysine, and tryptophan. The average composition has been reported to be 57 to 71% water, 30 to 35% carbohydrate, 0.2 to 1% ether extracts, 0.6 to 2.6% protein, and relatively low content of minerals and vitamins. The level of lipids in cassava is quite low, amounting to only 0.6% on the average. This is considered inadequate for animal diets consisting primarily of cassava. The recommended minimum dietary lipid requirements for animals is 1 to 2% (2).

The present results cast doubt on the hypothesis that cassava is a goitrogen because the thiocyanate generated from its ingestion displaces iodide from a thyroid with marginal iodine content due to a deficient diet. The small thyroids of mice on cassava were proportional to the reduction of body weight, probably due to protein malnutrition. Also,

cassava plus a low iodine diet did not cause more thyroid hypertrophy than the low iodine diet by itself. Our present data are consistent with the findings in a study of endemic goiter in a region of Vietnam where cassava was eaten (18). We concluded then that cassava was not a significant goitrogen in humans even in the presence of a marginally low iodine intake. We now conclude that cassava is probably by itself not a significant goitrogen at all and could make only a limited contribution to the goitrogenic effect of a severely reduced iodine intake. This conclusion must be tempered by the realization that studies in mice may not be entirely applicable to men and that other forms of cassava may possibly be goitrogenic, even in rodents. In view of the important nutritional role of cassava in the world's food supply, our data showing that cassava is not a potent goitrogen are reassuring that it is a relatively innocuous food.

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