NUTRITIONAL FACTORS INVOLVED IN
THE GOITROGENIC ACTION OF CASSAVA

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by

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THESIS

submitted in partial fulfilment of the

requirement for the degree of

MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

FACULTY OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

College of Rural Home Science

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1987

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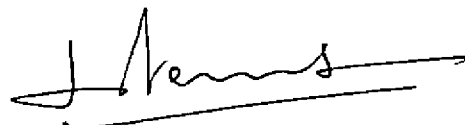any other University or Society.

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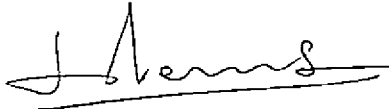College of Rural Home Science

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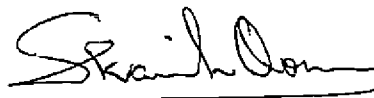APPROVED BY:

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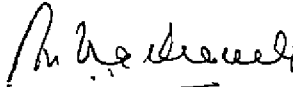
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INTRODUCTION

INTRODUCTION

Simple or endemic goitre, a morbid enlargement
of the thyroid gland was known in China as early as
3000 B.C. In India, this condition appeared to have
been known even from Vedic times and the ancient Hindu
medical experts like Charaka and Susruta were apparently
referring to goitre when describing the condition called
'galaganda' (Murthy, 1982).

Chatin (1850) convincingly proved that goitre
developed due to iodine deficiency by stimulation of
the anterior pituitary to produce and release increased
amounts of thyrotropin and thus causes a compensatory
enlargement of the thyroid gland. Baumann (1895)
analysed the thyroid gland and showed that it contained
iodine in small amounts. Through animal and human
studies it was proved that deficiency of iodine caused
goitre and this deficiency could be prevented by in-
clusion of iodine in the food.

Goitre has been an age old health problem in the southern slopes of the Himalayas caused mainly due to iodine deficient soil and food grown in this soil. Recent observations show that goitre is also distributed widely in the subcontinent with varying degrees of severity. The world's most classic and intense endemic goitre belt occurs along the slopes and foothills of the Himalayas, extending over 2400 km from Kashmir in the west to the Naga Hills in the east.

Enlarged thyroid gland (goitre) can also be caused by eating large amounts of goitrogenic foods such as turnips, cabbage, rutabaga and cassava over long periods of time. These foods, contain natural antithyroid compounds called goitrogens which inhibit the formation of thyroid hormones necessary for vital metabolic processes and which make them unsafe for human consumption.

Several health problems are said to be associated with chronic cassava ingestion namely goitre, cretinism, ataxic neuropathy and diabetes. But among millions of cassava consumers worldwide, chronic cyanide toxicity

occurs only in certain areas. This effect is enhanced
by a severely protein deficient diet, or in an area
where endemic goitre is present and where cassava is
the staple food.

Kerala, is the only state in India where cassava
is a major dietary component. It is the most popular
subsidiary food crop of the middle and low income group
of people in Kerala, being the cheapest and easily
cultivable source of food energy. According to the
latest available estimates, certain endemic goitre
pockets are identified in southern parts of Kerala
where cassava cultivation and consumption is high,
thereby making it necessary to investigate the goitro-
genic action of prolonged cassava consumption. The
present investigation was taken up as a step to study
the effect of prolonged consumption of cassava on experi-
mental animals with special reference to:

1) the antithyroid action of cassava
2) the influence of calorie, protein and iodine
   supplementation on the anti thyroid action of
   cassava.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Kerala in Southern India with approximately
twenty five million people is more populous than many
states. Cock (1985) reported that rice, cassava and
coconut predominated the Kerala diets, which were
deficient in both protein and calories. Cassava
production in India had expanded dramtically since
1950, largely because yields had risen from five
tonnes per hectare to six tonnes per hectare. According
to Cock (1985) these yields were among the highest in
the world even though Kerala had infertile soils and a
shorter growing season than many other cassava growing
areas.

Annual production of cassava in India as reported
in Agricultural Situation in India (1986) was 5.8 million
tonnes of fresh tuber and accounted for 4.5 per cent of
the total world production. It was also reported that
Kerala accounted for nearly 75 per cent of the area
under cassava cultivation and 71 per cent of the production
of cassava in India. According to the Economic Review
(1985) production of cassava in Kerala was 39.53 lakh tonnes

and the area under production was 2, 32, 753 hectares.
Production of cassava per hectare in Karnataka, Tamil
Nadu and AndhraPradesh in 1983-84 was 10.18, 31.13 and
6.03 tonnes respectively (Lakshmi and Pal, 1986). The
area under cassava production during 1984-85 were highest
in Trivandrum followed by Quilon, Kottayam, Pathanamthitta,
Idukki, Trichur and Wynad districts in Kerala (Agricultural
Situation in India, 1986).

Cock (1985) reported that Kerala is the only State
in India where cassava is used as a dietary component.
Although its use in the diet is widespread throughout the
state it is mainly consumed by poorer people, particularly
along the coastal districts where poverty is at a maximum.
According to Poulose et al. (1984) the increase in popu-
lation and decrease in production of rice has made cassava
an important food item. About one fifth of the total
calorie intake in Kerala, as had been pointed out by
Cock (1985), comes from subsidized ration rice which, per
calorie was similar in price to cassava.

People living on a cassava diet with little of
other foods are likely to suffer from protein deficiency

(Gopalan, 1979). According to Okigbo (1980) replacement of more protein rich weaning foods by cassava products should be avoided in order to safeguard young children from cassava toxicity and protein deficiency. Similarly Mata et al. (1982) reported that in Costa Rica, cassava consumption contributed little to the protein content of the diet. Maberly et al. (1976) found that there was minimal consumption of seafoods and other common food crops in certain coastal areas of Sarawak, with near dependence on cassava roots.

Cock (1985) has reported that in Kerala, cassava provided more amount of calories per adult equivalent and the total protein intake of the population was low. However, Gopalan (1979) observed that in Kerala cassava is generally eaten along with fish, which contains excellent proteins as well as iodine and thereby balancing the diet. Dorozynski (1978) reported that marine products and cassava are the two main food products in Kerala. An analysis of the dietary habits of Keralites by Poulose et al. (1984) revealed that 75 per cent of the people were regular consumers of cassava and salted fish.

Results of a survey conducted by Prema et al. (1980)
among 250 farm families in Trivandrum and Quilon districts
in Kerala indicated that cassava was used by all families
irrespective of their income.

According to Poulose et al. (1984) the first report
of endemicity of goitre in Kerala came from Idukki district
were a 33 per cent incidence of goitre was found among the
labour population in tea estates. But Kochupillai et al.
(1976) stated that endemic goitre is not prevalent in
people residing along the coastal strips of two districts
in Kerala as well as in midlands where cassava is widely
consumed.

The goitrogenic action of cassava was first sus-
pected to be due to a goitrogen present in the tuber
Ekpechi et al., 1966 and Nwokolo et al. (1966) suggested
that cassava contained a goitrogen similar to ones
present in brassica vegetables and thiourea derivatives.
Ermans (1979) gave new data on the pathogency of endemic
goitre and certain goitrogenic foods. Ermans et al.(1983)
reported that cassava ingestion was one of the key factors
in the etiology of endemic goitre and cretinism in

Central Africa. Maberly et al. (1976) observed a
goitre incidence of 74 per cent in a coastal community
of Sarawak,Malaysia . probably due to the consumption
of large quantities of cassava.

There are a number of studies by various authors
to support the fact that consumption of cassava did not
cause goitre. Studies conducted by Nestel (1973) and
Phillips (1974) indicated that goitre and cretinism were
not found in all populations whose staple food was cassava
(Kelly and Snedden, 1960; Kochupillai et al., 1980;
Medeiros - Neto and Dunn, 1980). Hennart et al. (1982)
indicated that a cassava based diet did not necessarily
result in the development of goitre. Delange et al. (1982)
reviewed the nutritional factors involved in three rural
areas in Zaire and found that chronic consumption of
cassava in large quantities did not necessarily result
in the development of endemic goitre. However they found
that poorly detoxified cassava caused an inhibition of
the penetration of iodine into the thyroid in man.

Ekpechi (1973) showed that cassava had an adverse
action on the function of the thyroid comparable to that
of thionamide goitrogen. Delange and Ermans (1971) stated

stated that the absorption of cassava grown in the
goitrous areas of Idjwi island inhibited the penetration
of iodine into the human thyroid. Ermans (1979) reported
that in severe goitrous endemic areas in Zaire prolonged
consumption of cassava affected the adaptation process
of the thyroid gland.

The first reference to the noxious principle
namely 'hydrocyanic acid' present in cassava was made by
Clusius in 1605 and later by Henry and Charland in 1836.
This compound was identified by Peckolt (1886).
Dunstan et al. (1906) isolated another glucoside and
an enzyme linamarase or linase which was capable of
hydrolysing the glucoside from cassava. Linamarin from
Cassava peel, in a pure form was isolated by Wood (1966).
Clapp et al. (1966) observed that among the cyanogenic
plants, cassava in which the principal glucoside was
linamarin was the most important in terms of human intake.
Van der velden et al. (1973) observed that the human body
detoxified the cyanide which was liberated from the
cyanogenic glucoside, linamarin, contained in cassava.
The cyanide in cassava roots and tissues was mainly
found in a bound form as a cyanogenic glucoside (linamarin),

which accounted for approximately 90 per cent of the total cyanide content, the remainder being present as free cyanide (Nartey, 1978). Lancaster et al. (1982) studied the ill effects of cyanogenic glucosides of cassava which released cyanide on hydrolysis. Cock (1985) reported that raw cassava contained the glycosides linamarin and lotausteralin, which when in contact with linamarase, was converted into a poison.

Delange et al. (1982) had also found that the antithyroid action of cassava was due to the release of thiocyanate from the cyanide (hydrocyanic acid) produced by the hyodrolysis of linamarin. They also stated that the efficiency of the detoxification process would be effected by processing, the varieties used and the portions commonly consumed. Cassava was classified into three categories based on its hydro-cyanic acid content as innocuous tubers with less than 50 mg hydrocyanic acid/kg fresh peeled roots, moderately poisonous ones with 50-100 mg hydrocyanic acid/kg and dangerously poisonous ones over 100 mg hydrocyanic acid/kg (Bolhius (1954), De Bruijn (1971) and Coursey (1973)).

Oyenuga and Amazigo (1957) and (Oke, 1973) found
that hydrocyanic acid in cassava varied according to
season. Carmody (1900) found that sweet cassava
varieties might contain as much cyanide as the bitter
ones. He stated that in the former, the cyanide was
concentrated in the peels and outer cortical layers,
while in bitter ones it was distributed uniformly in
the root, hence peeling the sweet varieties reduced
the cyanide considerably.

De Bruijn (1971) had shown that the glucoside
content varied between the proximal (near the peduncle)
and the distal parts of the roots, although the varia-
tions appeared to be purely random. He also confirmed
that the hydrocyanic acid content of fresh peel, pulp
and whole cassava root was consistently higher in bitter
varieties than in sweet strains. Evaluation of 24
varieties of cassava for their hydrocyanic acid content
by Dharmalingam et al. (1973) revealed a wide variation
ranging from 10 to 55 ug/g of hydrocyanic acid in the
fresh and 105 to 375 ug/g in the rind. Low toxic levels
of hydrocyanic acid in the edible portion of the tubers
contributed to their suitability for raw consumption.
It was also observed that hydrocyanic acid in the flesh
had no direct relationship with that of the rind in the
varieties studied.

According to Muthuswamy et al. (1973) varietal differences ranged from 5 to 125 ppm with an average of 41.21 ppm on a fresh weight basis. While Anon (1962) reported that bitter types contained 770 ppm while sweet types contained less than 160 ppm on a fresh weight basis. Aw-yong as quoted by Mahendranathan (1971) stated that the flesh of the sweet type contained about 70 ppm, while the bitter type about 200 to 300 ppm.

Grace (1977) stated that the glucoside content in the cassava plant was markedly increased by drought and potassium deficiency.

Bourdoux et al. (1980) stated that there was no significant correlation between the weight and the length of the roots and their hydrocyanic acid content. The study also showed that the majority of glucoside was eliminated at different stages of preparation. The usefulness of cassava as food and feed increased by adding Mg^{2+} during processing. Hollo et al. (1981) found that Mg^{2+} activated linamarinase which in turn degraded the (CN^-) containing linamarin, liberating hydrocyanic acid, thus degrading toxicity of the product.

Cock (1985) repeated that although occasional deaths
from consuming raw cassava roots had been reported the
traditional processing and cooking methods reduced the
cyanide levels.

It was reported that the variation of cyanide
content in cassava was influenced more by practices
than by variety (Phillips, 1974.; Yeoh et al., 1974).
Gomez (1983) discovered that processing of cassava
roots led to the rapid conversion of bound cyanide to
free cyanide, which was then released. The cyanide
content of the processed products, therefore was con-
siderably lower than that of the fresh roots. In his
research Normanha (1965) had found that intoxication
due to excess consumption of bitter cassava was due to
insufficient cooking time, resulting in partial hydrolysis
of the cyanogenic glucoside or in total liberation of
hydrocyanic acid. Stanbury (1985) pointed out that
poorly prepared cassava yielded an abundance of cyanide
which was converted to thiocyanate after ingestion,
which competed with iodide for entry into the thyroid.

In an experiment to extract cassava starch
Arguedas et al. (1982) found that the dried end
product contained less per cent of cyanide than had
been present in the raw material. The International
Institute of Tropical Agriculture (1983) found that
in cassava products that had undergone fermentation
before being processed (Chickwangue, foofoo, ntuka)
both total and free hydrocyanic acid were almost
totally eliminated. Studies conducted on different
processing methods at CTCRI (1983) depicted that baking
and steaming reduced cyanoglucoside content by 20 per
cent, drying at 65°C by 27 per cent and boiling in water
by 50 per cent. They also discovered that cyanoglucoside
retention decreased with the decrease in size of the
processed cassava pieces. The importance of the modes
of preparation and processing of cassava had been studied
by Bourdoux et al. (1982). They found that there were
higher concentrations of thiocyanate in serum and urine
of the population of Bas Zaire, Kivu and Ubangi when
compared to the control populations in Kinshasa and
Brussels. They suggested that this was nearly due to
different detoxification methods used in these areas.

A survey conducted by Prema et al. (1980) among 250 farm families in Trivandrum and Quilon districts revealed that steaming and deep frying methods of cooking were commonly used for preparing cassava. Fermented preparations and salted preparations with cassava were not commonly used by these farm families.

The effect of dry heat methods like baking, boiling, roasting and frying were found to be significantly superior to both steaming and boiling in detoxification of cassava as reported by Vimalakumari et al. (1980). They also found that tarmaind pulp and lime juice were highly effective in reducing hydrocyanic acid, and the addition of papaya also decreased it significantly. Besides this, sugar and honey when added also produced similar results.

A long term feeding trial of seven months by Tewe (1975) demonstrated that fresh and dried cassava diets with upto 402.3 ppm hydrocyanic acid did not cause pathological changes in the thyroid of female rats and their offspring.

An epidemic of spastic paraparesis associated with chronic cyanide intoxication was revealed by Casadei et al. (1984). They suggested that detoxification of the bitter variety by sun drying was inadequate because of general food shortage and metabolic detoxification which was probably reduced owing to the absence of sulphur.

El Tinay et al. (1984) studied the extent of loss of hydrocyanic acid during fermentation of cassava tubers from both sweet and bitter varieties in the traditional method (whole unpeeled tubers) compared with the fermentation of peeled tubers and crushed pulps with or without the addition of water. The loss of cyanide from the whole sweet tuber was not significant after 5 days. There was a marked decrease in free cyanide in the first day of fermentation of the peeled tubers compared to whole tubers.

Gomez and Valdivieso (1985) studied the effect of drying temperature and loading rates on cyanide elimination from cassava whole root chips, one with high and the other with intermediate cyanide. They found that

the main factor determining cyanide elimination was
the loading rate rather than the drying temperature.
Artificial drying at the temperature and loadings
assayed allowed total cyanide loss of 87 per cent and
69 per cent for chips of intermediate and high cyanide
varieties respectively. According to Nambisan et al.
(1985) baked, fried and steamed tubers retained maximum
(> 80 per cent) cyanoglucoside. They also found that
retention varied from 30 - 60 per cent depending on
chip thickness in sun dried chips. In the case of
cassava boiled in water smaller chip size and sufficient
water was found to be ideal for maximum cyanoglucoside
removal. They further stated that more than 95 per cent
cyanoglucoside was removed by crushing the fresh tuber
and subsequent sun drying.

Cock (1985) stated that the principal method of
detoxification of cyanide from cassava to thiocyanate
in the blood was by the action of an enzyme called
rhodanese. Reinwein (1961), Auriga et al. (1975) had
found that rhodanese was widely distributed in the body
with the highest concentration in liver and kidney.

Himwich and Saunders (1948) had explained that, the
susceptibility to hydrocyanic acid intoxication varied
from species to species probably due to the variation
in the amount of the enzyme rhodanese in the liver.
According to studies done at CTCRI (1982) rhodanese
activity of cerebrum and cerebellum was maximum in
rabbits fed with raw cassava meal and the enzyme activity
in liver was more in animals fed with cooked cassava
meal. They also found that the rhodanese activity was
high in animals fed with raw cassava meal when compared
to the control. Izokun-Ethiobhio and Ugochukwu (1984)
through animal experiments found that the reaction
catalysed might not be the main pathway of detoxification
of cyanide. Rutkowiski et al. (1985) suggested that
hepatic rhodanese was not principally involved in the
detoxification of cyanide even when exogenous thio-
sulphate was provided. Further studies by Rutkowiski
et al. (1986) had shown that a healthy liver was not
essential to give protection from cyanide. The mito-
chondrial membrane might be a barrier since lysedmito-
chondria had increased rhodanese activity. They also
stated that the major detoxification pathway for cyanide
was a biotransformation to the less toxic thiocyanate.

Tewe and Maner (1978) found that rhodanese activity was consistently higher in growing rats fed fresh and dried cassava based diets. Bourdoux et al. (1980) found that in the presence of its specific enzyme, cyanide was detoxified and this provoked an increase in serum and urinary thiocyanate levels. According to Mahadevan et al. (1980) prolonged feeding of rats with a variety of cassava containing high hydrocyanic acid led to increased levels of plasma thiocyanate. Increased serum thiocyanate levels associated with cassava based diets in rats and humans were reported by Ermans et al. (1980). Significant elevation in plasma thiocyanate level in animals fed a cassava diet was observed in CTCRI (1982). Osuntokun (1973) reported significant rise in plasma thiocyanate and urinary thiocyanate when cassava consumption increased.

Investigations by Tewe (1984) on African giant rats had shown that serum, organ and urinary thiocyanate were higher ($p < 0.08$) on cassava based diets. Serum urea concentrations increased proportionally with dietary

hydrocyanic acid level but no pathological lesions were observed in spleen, kidney, liver and thyroid glands of the animals. Ermans (1973) discovered that there was a moderate increase of plasma thiocyanate in rats fed cassava for a long term. Mahoney et al. (1983) confirmed in their study that the urine thiocyanate excretion of the non goitrous population on a low cassava diet was significantly lower than that found in an endemic goitrous population. Guzman (1975) found that the urinary excretion of thiocyanate was closely related to the protein quality, food intake and weight gain in a bitter cassava based diet. Courtois et al. (1983) reported that urinary thiocyanate excretion increased in proportion to the increased consumption of unsoaked roots. Smith (1961) and Cock (1985) reported that this thiocyanate excreted in the urine played its toxic role by using up body sulphur in detoxification, thereby increasing the body's demand for sulphur containing amino acids or by interfering with the iodine uptake of the thyroid resulting in goitre. According to Cock (1985) in both cases high cassava consumption aggravated problems associated with low levels of sulphur amino acids

and iodine in the diet. According to Lang (1933);
Wheeler et al. (1975) and Barett et al. (1978) the
requirements for iodine and sulphur aminoacids were
slightly increased when cassava consumption was high.
Studies by Lancaster et al. (1982) and Gomez and Valdivieso
(1983) revealed that cassava had a low protein content
and a low content of sulphur containing amino acids and
the cyanogenic glucosides contained in a cassava based
diet produced an above normal nutritional need for
certain sulphur bearing amino acids. Spath (1971)
pointed out that methionine and cystine in particular
appeared to be the sources of sulphur for thiosulphates
used by the body in the detoxification of cyanides. He
also reported that populations depending primarily on
cassava ought to have reliable sources of sulphur
bearing protein such as fish. Maner (1972) found that
sixty per cent of cassava in the diet affected body
weight gain and efficiency and that supplementation
with methionine improved the condition probably by
providing the sulphur needed for detoxification of
hydrocyanic acid. Hamid and Jalaludin (1972) found
that methionine was the limiting factor on high

cassava rations. Adegbola (1977) found that added methonine helped to improve the protein quality of cassava based diets. Hennart et al. (1982) found that even extreme protein calorie malnutrition in humans apparently did not seem to critically impair the endogenous conversion to thioyanate of hydro-cyanic acid released from cassava. According to Tewe (1984) the interaction of cassava peel and protein deficiency decreased white blood cell count. Tewe et al. (1984) found that cyanide intake in pigs had significant effects on thyroid and protein metabolism.

Meister (1953) revealed the other ways of detoxification which involved sulphur containing amino acids like cysteine and cystine. Fielder and Wood (1956) stated that 3 mercaptopyruvate formed from deamination or transamination of cysteine combined with cyanide by the action of a sulphur transferase to form thiocyanate and pyruvic acid. According to Meister et al. (1954) this enzyme also has been found principally in liver, kidneys, spleen and pancreas. Wood and Cooley (1956) reported that cystine was capable of

reacting directly with cyanide, leading to a cleavage
of the disulfide bond and formation of cysteine and beta
thiocyanoalanine which decomposed to thiocyanate and
could be excreted as such in the urine.

Delange et al. (1980) found that thiocyanate
inhibited the iodine accumulation in the thyroid so
that the long term effects were similar to those of
iodine deficiency, when the iodine-thiocyanate ratio
fell below a critical level goitre developed.
Ermans (1979) carried out studies in severe goitrous
endemic areas in Zaire and in iodine deficient rats
and the results showed that in iodine deficiency as
well as in prolonged consumption of cassava along with
iodine deficiency there was endogenous production of
abnormal amounts of thiocyanate and a marked decrease
of thiocyanate blood levels. Hennart et al. (1982)
their study conducted in Bas Zaire, Kivu and Ubangi
showed that the dietary supply of iodine also played a
crucial role in the development of goitre in cassava
based diets. They indicated that goitre did not
necessarily develop provided that the iodine intake
was high enough or the hydrocyanic acid content of

cassava was low enough. Ermans et al. (1973) revealed
that ingestion of cassava entailed marked depletion of
iodine stores. They also found that depletion was
fairly moderate in iodine supplemented rats and
severe in iodine deficient rats. The study also
depicted that chronic ingestion of thiocyanate did
not necessarily cause blocking of the thyroidad pump
but increased the iodine uptake by the gland. Ermans
et al. (1983) reported that the main difficulty en-
countered was to distinguish between the goitrogenic
effects of thiocyanate and those of iodine deficiency.
Bourdoux et al. (1980) found that linamarin in the
presence of its specific enzyme decreased radioactive
iodine uptake by the thyroid, whereas Ermans (1979)
stated that the thyroid uptake of ^{125}I remained
unchanged during prolonged consumption of cassava in
rats. Delange et al. (1982) commented that the iodine -
thiocyanate ratio was determined by the level of iodine
intake and hydrocyanic acid content of cassava.

Underwood (1962) stated that serum protein
bound iodine levels varied significantly with species,
age, pregnancy, level of thyroid activity and diet but
not with sex. Agarwal et al. (1982) reported that serum
PBI was found to decrease as the severity of goitre in-
creased in goitrous children. In rats fed cassava for

a long term Ermans (1973) discovered that there was
depletion of thyroidal iodine stores, major abnorma-
lities of intrathyroidal metabolism, reduction of
plasma PBI^{127} while thyroidal ^{131}I uptake was not
inhibited at all. Experiments conducted by Ekpechi
et al. (1966) indicated marked decreases in precursor
and hormone iodine stores and increases in serum
protein bound iodine and thyroid weight after feeding
cassava with iodine supplements.

In the presence of iodine deficiency the
risk of irreversible alterations to the central nervous
system of young children associated with cassava in-
gestion by pregnant women had been studied by Colinet
et al. (1982). They studied the role of hydrocyanic acid
and found that in the cerebellum there was decrease of
protein, RNA and cholesterol which slowed down cellular
growth when hydrocyanic acid was given with an iodine
deficient diet. These anomalies were not observed when
the same hydrocyanic acid overload was administered in
the presence of a normal supply of iodine. Serum
cholesterol levels were found to increase with severity
of goitre in children of both sexes by Agarwal et al. (1982)
in Gorakhpur. In rats fed cassava an increase in serum
high density lipoprotein cholesterol and a decrease in

low density lipoprotein and very low density lipo-
protein cholesterol were seen as compared with those
fed rice. Premakumari and Kurup (1982) also stated
that the activity of some hepatic lipogenic enzymes
increased while activity of lipoprotein lipase and
triglyceride lipase in the extra hepatic tissues
decreased. Faecal excretion of neutral sterols and
bile acids was significantly more in rats fed cassava.
Another important study done at CTCRI (1982) revealed
that cholesterol and phospholipid values were reduced
significantly when rabbits were fed cooked cassava
meal. According to Adamson et al. (1983) cassava based
diets resulted in the highest cholesterol levels when
compared to yam and alfalfa diets.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study on 'Nutritional Factors involved in the Goitrogenic Action of Cassava' was based on the assessment of:

I. Antithyroid action of cassava.

II. Influence of calorie, protein and iodine supplementation on the antithyroid action of cassava.

A. The antithyroid action of cassava is influenced by the hydrocyanic acid present in the tubers. Therefore to assess the antithyroid action of cassava the following experiments were planned:

1. Estimation of hydrocyanic acid (HCN) content in different varieties of cassava.

2. Effect of cooking on hydrocyanic acid content of different varieties of cassava.

3. Effect of addition of different ingredients on the hydrocyanic acid content of cassava.

B. Antithyroid action of cassava was further assessed by:

I. Conducting feeding experiments with two varieties of raw, cooked and dry cassava.

II. Conducting feeding experiments with protein and iodine free cassava based diets.

III. Conducting feeding experiments with cassava based diets supplemented with iodine and protein.

The different varieties of cassava selected for the feeding trials were:

1. Manihot esculenta Crantz variety M-4, it has a low hydrocyanic acid content and is the popular staple because of its quality.

2. Manihot esculenta Crantz variety H-165 it has a high content of hydrocyanic acid.

1. Hydrocyanic acid content in different varieties of cassava.Estimation of Hydrocyanic acid(HCN).

Fresh cassava tubers of six varieties were analysed for the hydrocyanic acid (HCN) content by the method of Indira and Sinha (1969).

One gram of fresh tuber sample (without rind) was homogenised with distilled water in a mortar with pestle and transferred into a 500 ml conical flask immediately (volume of homogenate being 25 ml). A Whatman No.1 filter paper strip (10 x 2cm), soaked in alkaline sodium picrate (25g of sodium carbonate and 5g of picric acid powder in 1 litre of distilled water), dried and suspended under a suitable rubber cork (with a bent pin) was placed on the mouth of the flask and closed tightly. The strips were kept overnight (18 hours) and were eluted in 60 ml distilled water. Absorbency was recorded in a colorimeter using a green filter (515-550 mu). The amount of hydrocyanic acid was calculated from a standard curve, drawn using standard KCN solution.

Reagent Blank:

A Whatman No.1 filter paper strip (10 x 2cm), soaked in alkaline sodium picrate, was dried and suspended under a suitable rubber cork (with a bent pin) was placed on the mouth of the 500 ml conical flask (containing 25 ml distilled water) and closed tightly.

It was kept overnight and the strip was eluted in 60ml distilled water. Absorbency was recorded in a colorimeter using a green filter (515-550 mu).

The different varieties of cassava used for analysis are given below:

1. Manihot esculenta Crantz - variety M-4. This is the most popular variety consumed and has a low hydrocyanic acid content.

2. Manihot esculenta Crantz variety H-165. This has a high hydrocyanic acid content.

The remaining four varieties were chosen because they are the common locally available varieties.

3. Manihot esculenta Crantz variety Kalikalan.
4. Manihot esculenta Crantz variety Panniyur.
5. Manihot esculenta Crantz variety Nyarukku.
6. Manihot esculenta Crantz variety Pravuvella.

2. Effect of cooking or processing on hydrocyanic acid (HCN) content of different varieties of cassava.

Fresh cassava tubers of two varieties namely Manihot esculenta Crantz variety M-4 and Manihot esculenta

Crantz variety H-165 were cooked and samples analysed
for the Hydrocyanic acid content.

Calculation of loss or gain in hydrocyanic acid content.

The difference in hydrocyanic acid content
between the fresh raw sample and the cooked or processed
sample was calculated and the percentage was worked out
from that.

The different methods of cooking selected for
the experiment were baking, grilling, boiling, sundrying,
steaming, soaking, soaking and sundrying, frying.

2.1. BAKING:

A set of triplicate samples of peeled cassava
of 5g each were placed in an oven at a temperature of
60°C and kept for 20 minutes. The experiment was
repeated at different baking temperatures of 105°C and
165°C for the same duration. The three sets of baked
samples were removed after 20 minutes and cooled.

2.2. GRILLING:

Triplicate samples of 10g each of peeled cassava of _Manihot_ _esculenta_ Crantz variety M-4 and _Manihot_ _esculenta_ Crantz variety H-165 were grilled on hot coals for ten minutes.

2.3. BOILING:

Triplicate samples of 5g of peeled cassava tuber of _Manihot_ _esculenta_ Crantz variety M-4 and _Manihot_ _esculenta_ Crantz variety H-165 were boiled with 50 ml of water for 15 minutes. The experiment was repeated with 30 ml of water. The cassava samples were cooked until the water was completely absorbed. The cooked samples of the two experiments were cooled.

2.4. SUNDRYING:

Triplicate samples of 5g of peeled cassava pieces of _Manihot_ _esculenta_ Crantz variety M-4 and _Manihot_ _esculenta_ Crantz variety H-165 were added to 50 ml of just boiling water and boiled for 3 minutes. All the samples were removed and sundried for five hours.

Triplicate samples of 5g of peeled cassava pieces of Manihot esculenta Crantz variety M-4 and Manihot esculenta Crantz variety H-165 were sundried for 5 hours without any prior treatment.

Triplicate samples of 5g of peeled cassava pieces of Manihot esculenta Crantz variety M-4 and Manihot esculenta Crantz variety H-165 were steamed for three minutes and sundried for 5 hours.

2.5. STEAMING:

Triplicate samples of 10g of peeled cassava pieces were steamed for 20 minutes and cooled.

2.6. SOAKING:

500g of peeled cassava of the two varieties Manihot esculenta Crantz variety M-4 and Mahihot esculenta Crantz variety H-165 were soaked in water for 4 days. Each day triplicate samples of 5 g of the soaked cassava was taken for analysis.

2.7. SOAKING AND SUNDRYING:

500g of peeled cassava of two varieties <u>Manihot</u>
<u>esculenta</u> Crantz variety M-4 and <u>Manihot</u> <u>esculenta</u> Crantz
variety H-165 was soaked for 4 days. Each day triplicate
samples of 5g of the soaked cassava was taken and sun-
dried for 5 hours.

2.8. FRYING:

. Shallow fat frying - Triplicate samples of 10g
of peeled fresh cassava of two varieties <u>Manihot</u> <u>esculenta</u>
Crantz variety M-4 and <u>Manihot</u> <u>esculenta</u> Crantz variety
H-165 were cut into thin slices and shallow fried in
10ml of oil, then cooled. Deep fat frying - Triplicate
samples of 10g of peeled fresh cassava of two varieties
<u>Manihot</u> <u>esculenta</u> Crantz variety M-4 and <u>Manihot</u> <u>esculenta</u>
Crantz variety H-165 were cut into thin slices and deep
fried in 40 ml of oil and cooled.

From all the samples of cassava cooked by different
methods, 1 g cassava was weighed and the hydrocyanic acid
content was determined by the method of Indira and Sinha
(1969). Triplicate samples of every treatment were used
for this estimation.

REAGENT BLANK:

A Whatman No.1 filter paper soaked in alkaline
sodium picrate was dried and suspended from a rubber
cork (with a bent pin). The cork was placed on the
mouth of a 500 ml conical flask (containing 25 ml water)
and closed tightly. The strip was kept overnight and
eluted in 60 ml distilled water. Absorbency was recorded
in a colorimeter using a green filter (515-550 mu).

3. Effect of addition of different ingredients on the
   hydrocyanic acid content of cassava.

The effect of addition of ingredients such as
common salt, sugar, baking soda (Sodium bicarbonate),
tomato,lime juice, tamarind pulp, vinegar, honey,
drumstick leaves and raw papaya while cooking on hydro-
cyanic acid content of fresh cassava tubers was assessed.

Cassava tubers of two varieties namely Manihot
esculenta Crantz variety M-4 and Manihot esculenta Crantz
variety H-165 were treated identically. In the experi-
ments 5g of peeled cassava was boiled in 50ml of water
for 25 minutes with added materials such as:

1. 3g of common salt
2. 5g of sugar
3. 500mg of sodium bicarbonate (baking soda)
4. 5g tomato
5. 5ml lime juice
6. 5ml tamarind pulp
7. 5ml vinegar
8. 5ml honey
9. 5g drumstick leaves
10. 5g raw papaya
11. 5g of peeled cassava was boiled in 50 ml
    water for 25 minutes as control.

Triplicate sets of 1g each of the samples from the above 11 treatments were taken and analysed for the hydrocyanic acid content by the method of Indira and Sinha (1969).

Antithyroid action of cassava was further assessed by conducting feeding trials to study the effect of linamarin on iodine metabolism in rats fed cassava diets. Cassava contains a cyanogenic glucoside linamarin which on hydrolysis yields hydrocyanic acid (HCN), a cyanide containing compound. In the body hydrocyanic acid is detoxified to a less toxic compound - thiocyanate. This detoxification takes places by the action of hepatic rhodanese.

To assess the antithyroid action of cassava feeding trials were conducted with two varieties of raw, cooked and sundried cassava.

Male albino rats (Sprague - Dawley strain weight 50-70 g) were divided into seven groups of six rats each and fed cassava in three different forms namely raw, cooked in water and sundried (Fig. I). The hydrocyanic acid content of the three samples were analysed. Two varieties of cassava namely Manihot esculenta Crantz variety M-4 and Manihot esculenta Crantz variety H-165 were chosen for the experiment.

The rats were fed as follows:-

Group I   - Cooked cassava (M-4)   (TEST DIET)
Group II - Raw cassava   (M-4)   (TEST DIET)
Group III - Dried cassava (M-4)   (TEST DIET)
Group IV - Cooked cassava (H-165)(TEST DIET)
Group V   - Raw cassava   (H-165)(TEST DIET)
Group VI - Dried cassava (H-165)(TEST DIET)
Group VII - Control Diet   (No Cassava)

Three rats were housed in each cage with wire mesh floors (Fig.II).

Fig. I. Different forms of cassava namely cooked, raw and sundried.

Fig. II. Rats housed in cage with wire mesh floor.

The diets used for the experiment had the following composition:

Ingredients	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII
Cassava (g)	50	50	50	50	50	50	-
Variety	M-4	M-4	M-4	H-165	H-165	H-165	-
Form	Cooked	Raw	Dried	Cooked	Raw	Dried	-
Skim milk(g)	33	33	33	33	33	33	52.6
Groundnut Oil (g)	9	9	9	9	9	9	9.0
Vitamin Mixture(g)	4	4	4	4	4	4	4.0
*Dextrin (g)	-	-	-	-	-	-	30.4
**Mineral Mixture(g)	4	4	4	4	4	4	4.0
	100	100	100	100	100	100	100

* In the control diet group VII Dextrin was used instead of cassava.

** The test diets did not contain iodine in the mineral mix, while the control diet contained iodine.

The composition of the Wesson's salt mixture used in the above diets is as follows:

	Wesson's salt mixture(g/kg)
Sodium Chloride	105.00
Potassium Chloride	120.00
Potassium dihydrogen Phosphate	310.00
Calcium Phosphate	149.00
Calcium Carbonate	210.00
Manganese Sulphate (anhydrous)	0.20
Potassium Aluminium Sulphate	0.09
Magnesium Sulphate (anhydrous)	90.00
Ferrous Phosphate	14.70
Copper Sulphate	0.39
Sodium Fluoride	0.57
Potassium Iodide (only in the control diet)	0.05

In addition the following trace elements were also added per kg diet.

Zinc Chloride	15.00 mg
Cobalt Chloride	0.15 mg

The vitamin mixture had the following composition (mg/100 g diet).

Thiamine	0.8
Riboflavin	0.8
Pyridoxine hydrochloride	0.6
Niacin	5.0
Calcium Pantothenate	4.0
Inositol	20.0
Folic Acid	0.4
Vitamin B_{12}	2.0 μg
Biotin	20.0 ug
Retinyl acetate	1000 IU
Ergocalciferol	150 IU
Alpha tocopherol	12.0
Menadione	0.3
Choline Chloride	200.0

Water was provided ad libitum. In group VII (control diet) the recommended dietary allowance of protein 20 per cent for rats was provided and iodine was added in the mineral mix. The protein intake in the test diets was fixed at 12.5 per cent.

The rats were maintained on the respective diets for thirty days. At the end of this period the weights were recorded, (Fig.III) and they were deprived of food overnight, then stunned by a blow at the back of the neck and killed by decapitation. The blood was collected and kept for the serum to separate out.

The tissues were removed to ice cold containes for the following estimations.

1) Weight of different tissues

2) Rhodanese enzyme activity of liver, kidney and spleen.

3) Total liver protein

4) Serum thiocyanate

5) Liver and serum cholesterol

1.   Weight of different tissues were recorded. The accuracy was observed to 1 g.

2.   Rhodanese activity of tissues liver, kidney and spleen were estimated by the method of Sörbo (1953).

Fig. III. Weighing of rat in Triple Beam Balance.

Reagents:

0.125 M $Na_2 S_2 O_3$.

0.5% bovine serum albumin (stock solution).

0.20 M $KH_2 PO_4$.

0.25 M KCN.

38 per cent formaldehyde

Ferric nitrate reagent. 100g of $Fe(No_3)_3$. $9H_2O+$

200 ml of 65 per cent HNO_3 per 1000 ml.

Enzyme: Dilute the enzyme in the presence of 0.0125 M thiosulfate and 0.025 per cent albumin (which protect the enzyme against inactivation by dilution) to obtain 0.3 to 1.6 R.U./ml.

Definition of unit and specific activity: One rhodanese unit (R.U) is defined as that amount of enzyme which forms 10 microequivalents of thiocyanate under the above conditions. Specific activity is expressed as rhodanese units per milligram dry weight, with purified enzyme preparations.

Procedure: One ml of $Na_2 S_2 O_3$, 0.5 ml of $KH_2 PO_4$ and 0.5 ml of KCN are mixed in a 50 ml Erlenmeyer flask.

One-half ml of the enzyme was added, and the reaction was stopped after 5 minutes at 20° by the addition of 0.5 ml 38 per cent formaldehyde. Then 2.5 ml of Ferric nitrate reagent and 25 ml of distilled water were added, and the optical density at 460 mu was determined. One micro equivalent of thiocyanate in the test gives the optical density, 0.104. The colour is stable for atleast 1 hour. A blank determination is always carried out by adding the formaldehyde to the test before the enzyme. With crude extracts or tissue homegenates it is necessary to remove the interferring turbidity by centrifugation or filteration before the optical density is determined.

Enzyme proteins were estimated by the Biuret Method.

Reagents:

1. Biuret reagent: Dissolved 4.25 g of potassium sodium tartarate (KNa $C_4 H_4 O_6$ $4H_2 O$), 1.5g of cupric sulphate (Cu $SO_4 . 5H_2O$) and 2.5 g Potassium Iodide in about 500 ml of distilled water. Dissolved 4 g of NaOH in the solution and made up the volume to 1 litre.

2. Standard: The standard protein solution may be either a pooled normal human serum (standardised by Kjeldahl

method) or a solution of pure albumin in saline.

Procedure: To 0.1 ml aliquots of standard, test plasma and blank (saline or distilled water) added 5 ml of biuret reagent. Mixed well and kept for 30 minutes. Read absorbance of test and standard against blank at 540 nm.

3. Total liver protein was estimated by Nesselerisation.

Total liver protein was estimated in the dry defatted tissue by microkjeldahl digestion followed by Nesselerisation.

4. Serum thiocyanate was estimated by the method of Aldridge (1944).

Reagents:

1. Ferric nitrate reagent - 100 g Ferric nitrate $9H_2O$ + 200 ml of 65 per cent HNO_3 are dissolved in 1000 ml water.

2. Thiocyanate standard - 20 mg Ammonium Thiocyanate was dissolved in distilled water (100 ml) - 20mg/ 100 ml or 200 ug/ml.

<u>Assay</u>: Separated the plasma from 5 ml of blood by centrifuging the blood collected in a fluoride or oxalate test tube for 15 minutes. Removed the supernatant plasma with a capillary pipette.

To 0.5 ml of plasma add 5.0 ml of ferric nitrate reagent. Mixed well read at 460 mu. The colour was stable for 1 hour.

5. <u>Extraction of tissues for lipid estimation</u>:

a) <u>Extraction of serum</u>:

The serum was extracted according to the procedure of Folch <u>et</u> <u>al</u>. (1957).
'n' ml of the serum sample was added dropwise to 5 'n' volume of methanol in a stoppered tube.. To this 5 'n' volume of choloroform was added so that the proportion of chloroform to methanol was 2:1 (V/V). It was filtered and the residue was washed with chloroform: methanol at least three times.

To the filterate, 0.02 per cent calcium chloride (20 per cent of the total volume of the extract) was added

in a stoppered tube, mixed vigorously and allowed to
stand for some time. The aqueous upper phase was removed
with a pasteur pipette and the lower layer was washed
three times each time with 5 ml of chloroform: methanol:
calcium chloride solution (3:48:48 v/v). The washed
lower layer of chloroform was evaporated to dryness and
the residue was dissolved in a known volume of chloroform
(1 ml of serum coresponds to 25 ml of chloroform solution).
From this, aliquots were used for lipid analysis.

b) Extraction of liver for lipid estimation:

The tissue was homogenised with washed, powdered
glass and extracted with chloroform: methanol (2:1) and
processed as described for serum. 0.25g of the tissue
corresponded to 25 ml of the extract in the case of liver.

Estimation of Cholesterol:

Total cholesterol was estimated in the tissues
by the method of Abell et al. (1952).

Reagents:

     1) 33 per cent Potassium Hydroxide (KOH)

     2) Absolute ethanol.

3) Ethanolic KOH - 6 ml of 33 per cent KOH in
water was added to 94 ml of absolute ethanol.

4) Petroleum ether (60 - 80°C) (AR).

5) Colour reagent - 20 ml of acetic anhydride was
cooled in ice.

1 ml of concentrated sulphuric acid was added
to this with shaking.

It was again cooled for 10 minutes and 10 ml of
glacial acetic acid was added and allowed to attain
room temperature.

An aliquot from the lipid extract was pipetted
out into a glass stoppered centifuge tube and evaporated
to dryness. 5 ml of ethanolic KOH was added, stoppered
and was shaken well. It was then warmed in a water bath
at 37-40°C for 55 minutes. After cooling to room tem-
perature, 10 ml of petroleum ether (60-80°C) was added
and mixed. 5 ml of water was added to this and shaken
vigorously for one minute. It was then centrifuged at
a low speed for 5 minutes. 4 ml of the petroleum ether
layer was pipetted out into a test tube and evaporated
to dryness at 60°C. A standard (2mg cholesterol/ml) was

also treated in the same manner. 6 ml of colour reagent
was added to each tube and kept at 25°C after thorough
shaking. 6 ml of colour reagent was taken as the blank.
After 30-35 minutes, the optical density was read at 620 nm.

Antithyroid action of cassava was further
assessed by conducting feeding trials with protein and
iodine free cassava diets as well as feeding trials with
diets supplemented with iodine and protein. After the
preliminary study Manihot esculenta Crantz variety M-4
of cassava was selected and a feeding experiment was
conducted for 90 days on rats.

Male albino rats (Sprague – Dawley strain, weight
40-50 g) were divided into 5 groups of 6 rats each and
fed M-4 variety of cassava in the cooked form. The rats
were divided into the following groups:

Group I   - Cassava alone
Group II   - Cassava + Iodine
Group III - Cassava + Protein
Group IV   - Cassava + Iodine + Protein
Group V   - Control diet.

Three rats were housed in each cage with wire
mesh floors.

The diets used had the following composition:

Ingredients	Group I	Group II	Group III	Group IV	Group V
Cassava (g)	69.85	69.85	30.4	30.4	-
Variety	M-4	M-4	M-4	M-4	-
Form	Cooked	Cooked	Cooked	Cooked	-
*Dextrin(g)	-	-	-	-	30.4
Skim milk(g)	13.15	13.15	**52.6	**52.6	**52.6
Groundnut Oil (g)	9.00	9.00	9.0	9.0	9.0
Vitamin mixture(g)	4.0	4.0	4.0	4.0	4.0
Mineral mixture(g)	***4.0	***4.0	***4.0	4.0	4.0
	100	100	100	100	100

*   Group I (control diet) contains Dextrin
     instead of Cassava.

**. In Group III, IV and V 20 per cent of
     protein was provided which is the recommended
     allowance of protein for rats while in
     Group I and II (low protein diets) protein
     was provided only for existence. Each rat
     was fed with 15 g of diet (Fig.IV)

*** In Group I and Group III the mineral
     mixture does not contain potassium iodide.

Water was provided ad libitum.

Fig. IV. Weighing of the diet.

The rats were maintained on the respective diets
for 90 days. At the end of this period weights were
recorded and they were deprived of food overnight,urine
samples were collected (Fig.Ⅴ). The rats stunned by a
blow at the back of the neck and killed by decapitation.
The blood was collected and kept for the serum to separate
out. The tissues were removed to ice cold containers for
the following estimations.

1. Weight of different tissues were recorded.

2. The rhodanese enzyme activity in liver, kidney
   and spleen were estimated by the method of
   Sörbo (1953) as described earlier.

3. Total liver protein was estimated by nesse-
   lerisation as described earlier.

4. Serum and urinary thiocyanate were estimated
   by the method of Aldridge (1944) as described
   earlier.

5. Liver and Serum cholesterol were estimated by
   the method of Abell (1952) as described earlier.

6. Serum protein bound iodine was estimated by the
   method of Foss et al. (1960) as described below.

Reagents:

1. 10 per cent Zinc sulphate (fresh) solution
   Dissolve 100g of $ZnSO_4$, $7H_2O$ in water and make
   upto 1 litre.

Fig. V.   Rat housed in metabolic cage.

2. Sodium Hydroxide, 0.5 N

3. Potassium Hydroxide, 2 N

4. Sodium Arsenite 0.1 N, 6.50 g per litre or dissolve 4.95 g of Arsenious oxide in 25 ml of sodium hydroxide and make to a litre with water.

5. Sulphuric acid - Hydrochloric acid mixture. Pour 98 ml concentrated H_2SO_4 into about 350 ml distilled water, cool, add 27 ml concentrated HCl and make to 500 ml with water.

6. Ceric Ammonium Sulphate - this should have an extinction as the standard blank of about 0.8. Foss et al. (1960) used 12.65 g of the dihydrate $Ce_2 (SO_4)_3$, $2(NH_4)_2 SO_4$, $2H_2O$ per litre in 1.6 N sulphuric acid but the exact concentration may be a little different from this and should be determined.

7. Iodide standards - Dissolved 130.8 mg of potassium iodide in water and made upto 1 litre. These solutions contain 100 micrograms of iodine per ml. Prepared a more dilute stock solution by diluting 2 ml of this to one litre.

The standards should be prepared for use at least weekly by diluting 10, 20 and 30 ml of the dilute stock solution to 100 ml with water. These now contain 0.02, 0.04 and 0.06 ug of iodine per ml. All these solutions should be kept in the refrigerator.

Procedure: Measured 1 ml of serum into a pyrex centrifuge tube (125 x 15 mm), diluted with 7 ml of distilled water and added 1 ml of 10 per cent zinc sulphate. Mixed with a narrow glass rod (about 2 mm in diameter), added 1 ml of 0.5 N sodium hydroxide and mixed well. Removed any material adhering to the rod by rotating it against the inner wall of the tube allowed to stand about 15 minutes then centrifuged for 10 minutes at 2,000 rpm. Decanted the supernatant fluid. Added 10 ml of distilled water and resuspended the protein precipitate with the stirring rod already used. It was not stirred vigorously but only sufficiently to obtain a uniform suspension. Centrifuged again and discarded the supernatant fluid. Carried out two further washings in the same way. After finally pouring off the supernatant added 1 ml of 2N potassium hydroxide and stirred with the same stirring rod. Washed down the rod with 0.5 to 1.0 ml of water added drop by drop down the rod. Placed the tubes overnight in an oven at 100-105°C to drive off water. After thorough drying, ashed in a muffle furnace. Placed in the furnace cold with the oven door closed and no draught during the heating. Vapour containing iodine forms during the latter part of the heating up and conditions aimed to keep this in the tubes. Heat at 660°C. Brought to this temperature

in 30-45 minutes. Heated for one hour. Opened the door
at 5, 24, 45 minutes after reaching 600°C. Then removed
and allowed to cool to room temperature. Added 10 ml of
water and stirred well with the glass rod, removing any
material from the walls of the tube, centrifuged for 10
minutes. Pipetted duplicate 4 ml portions (equal to 0.4 ml
serum) of the clear supernatant fluid into pyrex test tubes.
To each added 0.5 ml of arsenite and then slowly 1 ml of
the sulphuric acid, hydrochloric acid. Mixed and placed
the tubes in a constant temperature bath at $37 \pm 0.1°C$ for
10 minutes. Warmed the ammonium sulphate in the same way,
then at 1 minute intervals added 1 ml of this to each of
the tubes, mixed quickly by flicking with the fingers. At
a fixed time which varies with the reagents but is in the
period 15-20 minutes and which gives a fall in extinction
from about 0.8 to 0.2 with the strongest of standards read
the extinction at 420 mm in an SP 600 spectrophotometer or
similar instrument. Ran a reagent blank in duplicate with
each set of tests using 1 ml of water instead of 1 ml of
serum put through the entire procedure. To the standards
add 0.4 ml 2N potassium hydroxide and proceed with the
addition of arsenite etc. as for the test.

Statistical analysis was done using student's 't'
test (Bennet and Franklin (1967)).

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

A. The antithyroid action of cassava is influenced
   by the hydrocyanic acid present in the tubers.
   Therefore to assess the antithyroid action of
   cassava the following experiments were planned.

1. Estimation of hydrocyanic acid (HCN) content
   in different varieties of cassava.

Table 1 presents the hydrocyanic acid content
of fresh cassava tubers that are commonly used in Kerala.

Table 1. Hydrocyanic acid content of six varieties of
         cassava.

No.	Variety	Hydrocyanic acid content + SEM mg/ 100 g (fresh weight basis)	Per cent
1	M-4	15.81 ± 0.49	15.81
2	H-165	28.1 ± 0.70	28.1
3	Kalikalan	17.34 ± 0.50	17.34
4	Panniyur	18.29 ± 0.54	18.29
5	Nyarukku	17.63 ± 0.51	17.63
6	Pravuvella	18.58 ± 0.57	18.58

Mean of three determinations.

As revealed in the table there was considerable difference in the hydrocyanic acid content of the freshly peeled varieties among which H-165 rated most bitter due to its high hydrocyanic acid content (28.1 mg per cent). M-4 had the least hydrocyanic acid content (15.81 mg per cent) which made it more acceptable for consumption. Hydrocyanic acid content of the other popular varieties like Kalikalan, Panniyur, Nyarukku and Pravuvella were in between the former and latter varieties (17.34, 18.29, 17.63 and 18.58 mg per cent respectively) (Fig.VI). It is generally agreed that the amount of hydrocyanic acid in cassava tubers varied greatly due to a number of factors including the variety of the cassava and environmental conditions under which it was grown (Bourdoux et al. (1982) and ARC (1967 and 1969)). In the utilisation of cassava as food, the high hydrocyanic acid content is very detrimental as it imparts residual bitter taste even after cooking. However tubers with high hydrocyanic acid content can be used for the manufacture of industrial starch and starch derived products like adhesives.

FIG. VI GRAPHICAL REPRESENTATION OF HYDROCYANIC ACID CONTENT OF SIX VARIETIES OF CASSAVA
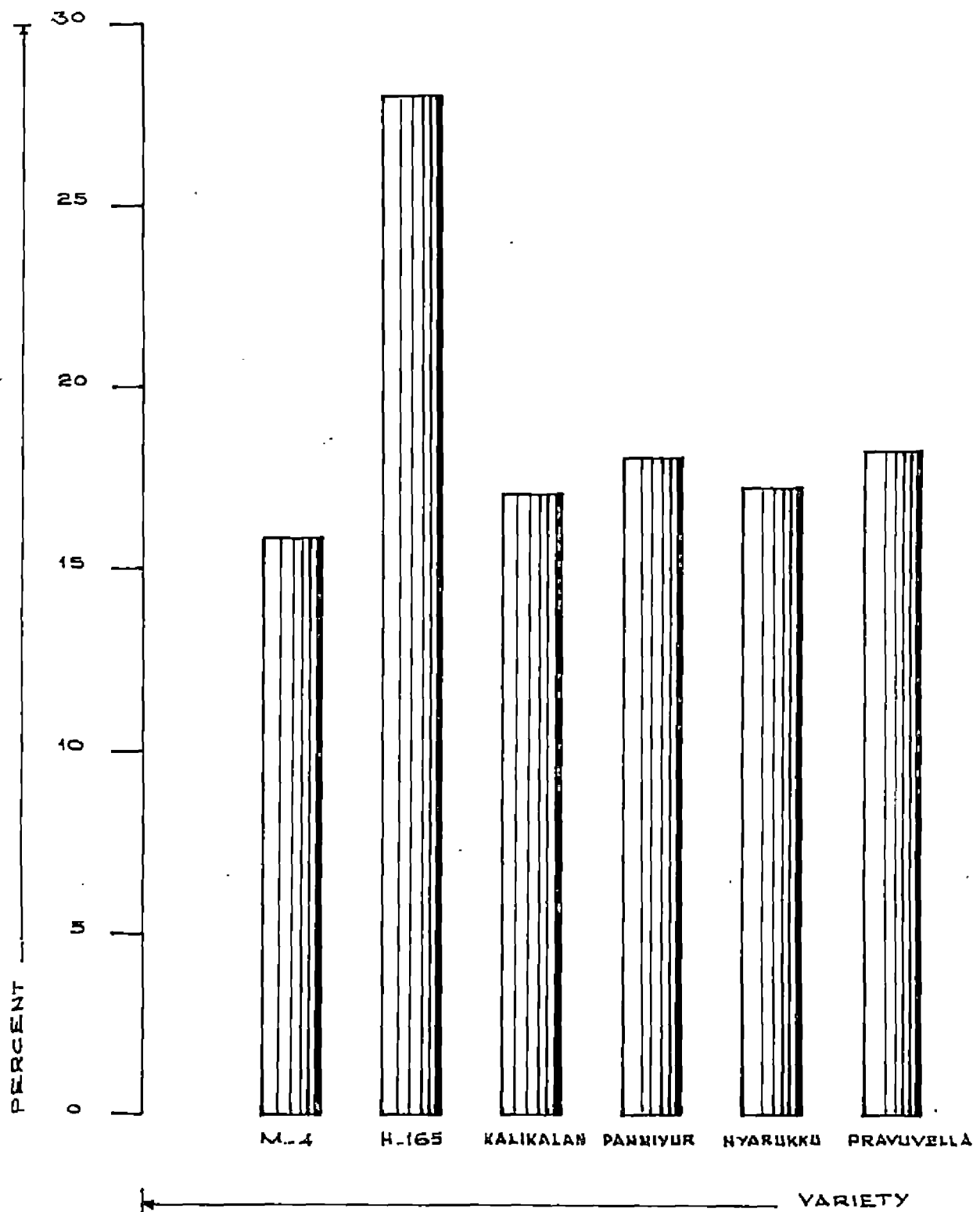
2. Effect of cooking on hydrocyanic acid content of different varieties of cassava.

Table 2.1 presents the effect of baking on hydrocyanic acid content of M-4 and H-165 varieties.

Table 2.1. Effect of baking on hydrocyanic acid content of cassava.

No.	Variety	Tempe-rature	Hydrocyanic acid content ± SEM mg/100 g	Per cent
1	M-4	Fresh	15.81 ± 0.49	
2		60°	17.1 ± 0.58	8.16
		105°	10.14 ± 0.25	35.86
		165°	2.21 ± 0.04	86.0
2	H-165	Fresh	28.1 ± 0.70	
		60°	30.1 ± 0.90	7.1
		105°	18.5 ± 0.59	34.2
		165°	8.23 ± 0.18	70.7

Mean of 3 determinations

In both M-4 and H-165 varieties, slight heating (60°C) produced an increase in the hydrocyanic acid

content due to the loss of water (8.16 and 7.1 per cent increase respectively). At 105°C in both varieties however about 35 and 34 per cent of the initial hydrocyanic acid content was lost, and at 165°C about 86 per cent of the initial hydrocyanic acid content in M-4 variety and about 70 per cent in H-165 variety was released. This finding is in line with the results obtained by previous studies conducted by Vimalakumari et al. (1980) where there was 82 per cent loss in hydrocyanic acid content while baking at high temperatures. These latter temperatures were chosen because they exceeded the decomposition temperature reported for linamarase at 72°C (Joachim and Pandittesekere (1944) and linamarin at 150°C (Cerighelli, 1955). Such temperatures however are never achieved by the traditional cooking methods of cassava.

Table 2.2 presents the effect of grilling on hydrocyanic acid content of M-4 and H-165 varieties.

Table 2.2. Effect of grilling on hydrocyanic acid
content of cassava.

Variety	Treatment	Hydrocyanic acid content ± SEM (mg/100 g)	Per cent
M-4	1 Fresh	15.81 ± 0.49	
	2 Grilled	8.26 ± 0.21[a]	47.75
H-165	1 Fresh	28.1 ± 0.7	
	2 Grilled	11.32 ± 0.33[a]	59.72

Mean of 3 determinations
a=p<0.01

Group 1 is compared to Group 2 in each variety.

't' value for table 2.2

t between groups	t value
M-4 1 and 2	14.16
H-165 1 and 2	21.68

As depicted in table 2.2 during grilling of M-4
variety, 47.75 per cent of initial hydrocyanic acid
content was lost while for H-165 the reduction is

59.72 per cent. Reduction in hydrocyanic acid

content while grilling was found to be significant

in both varieties.

Table 2.3 presents the effect of boiling on

hydrocyanic acid content of M-4 and H-165 varieties.

Table 2.3. Effect of boiling on hydrocyanic acid

content of cassava.

Variety	Treatment	Hydrocyanic acid content $\pm$ SEM (mg/100 g)	Per cent
M-4	1 uncooked	15.81 ± 0.49	
	2 discarding cooking water	6.93 ± 0.22^{a}	56.2
	3 cooked with enough water	14.84 ± 0.43	6.1
H-165	1 uncooked	28.1 ± 0.7	
	2 discarding cooking water	16.03 ± 0.45^{a}	43.0
	3 cooked with enough water	26.95 ± 0.65	7.3

Mean of 3 determinations.

Group 2 and 3 were compared with Group 1
in each variety.

$a = p < 0.01$.

't' value for table 2.3

t between groups	t value
M-4	
1 and 2	16.53
1 and 3	1.49
H-165	
1 and 2	14.50
1 and 3	1.20

The free hydrocyanic acid content of fresh tubers were rapidly removed in boiling water. In M-4 variety the hydrocyanic acid content was reduced to 6.93 mg per cent and in H-165 variety 16.03 mg per cent, when cooking water was removed and this reduction in hydrocyanic acid content was found to be statistically significant. Whereas there was no significant change in the hydrocyanic acid content of cassava cooked with just sufficient water without discarding cooking water in both M-4 and H-165 varieties. Earlier reports of hydrocyanic acid losses through boiling were quite variable losses of 9 to 100 per cent by Raymond et al. (1941) and Paula and Rangel (1939), 50-80 per cent by Joachim and Pandittesekere (1944) and Pieris et al. (1974) and losses of 10 per cent

by De Bruijn (1971). The percentage loss of hydrocyanic
acid by the boiling and discarding method was found
to be 80 per cent. This is the traditional cooking
method adopted for cooking cassava. In Kerala, it
can be assumed that on an average 50 per cent of the
hydrocyanic acid content from the cassava varieties
were removed before consumption.

Table 2.4 presents the effect of sundrying on
hydrocyanic acid content of M-4 and H-165 varieties.

Table 2.4.   Effect of sundrying on hydrocyanic acid
              content of cassava.

Variety	Treatment	Hydrocyanic acid content ± SEM (mg/100 g)	Per cent Gain	Per cent Loss
M-4	1 Raw	15.81 ± 0.49		
	2 Raw sundrying	17.8 ± 0.52	12.58	-
	3 Boiling and sundrying	4.1 ± 0.12^{a}	-	74.1
	4 Steaming and sundrying	5.5 ± 0.17^{a}	-	65.2
H-165	1 Raw	28.1 ± 0.7		
	2 Raw sundrying	30.04 ± 0.84	7.0	-
	3 Boiling and sundrying	10.7 ± 0.37^{a}	-	62
	4 Steaming and sundrying	11.3 ± 0.33^{a}	-	60

Mean of 3 determinations.
Groups 2, 3 and 4 were compared with Group 1
in each variety.
a=p< 0.01.

't' value for table 2.4

t between groups	t value
M-4	
1 and 2	2.78
1 and 3	23.21
1 and 4	19.87
H-165	
1 and 2	1.77
1 and 3	21.97
1 and 4	21.70

It was observed that there was an increase in the hydrocyanic acid content in both varieties when sundried. In M-4 variety the increase was 12.58 per cent where as in H-165 variety the increase was 7 per cent. The main effect of drying was the removal of water from the roots. Consequently a large part of the hydrocyanic acid remained in the roots and the apparent increase in hydrocyanic acid content resulted only from disappearance of water. Bourdoux et al. (1982) also observed an increase in the hydrocyanic acid content of the roots dried for 1-2 days.

There was a significant decrease in hydrocyanic
acid content when the roots were boiled for 3 minutes
and then sundried. In M-4 variety about 74 per cent
of the initial hydrocyanic acid was lost and in H-165
the reduction was about 62 per cent. Paula and Rangel
(1939) had found that steeping in boiling water for
short periods before sundrying could reduce the hydro-
cyanic acid content to a considerable degree espe-
cially if the root was grated as well. It is one of
the most popular methods of processing cassava for
storage purposes which is usually known as "Vattukappa"
in Kerala.

Again by steaming and sundrying, there was con-
siderable reduction in the hydrocyanic acid content
in both varieties and the results were comparable to
boiling and sundrying. Razafimaherry (1953) reported
that the Madagascar food product 'bournoka' which was
prepared from steamed sundried cassava product was
free of hydrocyanic acid.

Table 2.5 presents the effect of steaming on
the hydrocyanic acid content of M-4 and H-165 varieties.

Table 2.5. Effect of steaming on the hydrocyanic

acid content of cassava.

No.	Variety	Hydrocyanic acid content ± SEM (mg/100 g)	Per cent
1	M-4		
	1 raw	15.81 ± 0.49	
	2 steamed	4.9 ± 0.09[a]	69
2	H-165		
	1 raw	28.1 ± 0.70	
	2 steamed	8.1 ± 0.16[a]	71

Mean of 3 determinations.

Group 2 was compared to Group 1 in each variety.

a=p< 0.01

't' value for table 2.5

t between groups	t value
M-4	
1 and 2	21.83
H-165	
1 and 2	27.83

By steaming,the hydrocyanic acid content in M-4
variety was reduced significantly to 4.9 mg per cent
and in H-165 variety 8.1 mg per cent. In Kerala and

Sri Lanka, traditional breakfast preparations such as pittu, hoppers and string hoppers (Idiappam) are also prepared from cassava by steaming which will detoxify most of the hydrocyanic acid present in them.

Table 2.6 presents the effect of soaking on the hydrocyanic acid content of cassava.

Table 2.6. Effect of soaking on the hydrocyanic acid content of cassava.

Soaking period (days)	Hydrocyanic acid content mg/100 g ± SEM			
	M-4 per cent		H-165 per cent	
0	15.81 ± 0.49		28.1 ± 0.70	
1	7.35 ± 0.21	53.5	17.1 ± 0.44	39.14
2	5.48 ± 0.15	65.3	13.17± 0.33	53.13
3	3.11 ± 0.07	80.3	8.76± 0.18	68.8
4	1.04 ± 0.02	93.42	4.13± 0.08	85.3

Mean of 3 determinations

To explore the effects of soaking on detoxification the remaining hydrocyanic acid content of roots soaked for 1-4 days was measured. Soaking for only

one day released 53 per cent of the initial hydrocyanic
acid content of M-4 variety and 39.1 per cent in H-165
variety. The release was gradual and soaking for
4 days decreased the hydrocyanic acid content by about
93.4 per cent in M-4 and 85.3 per cent in H-165.
Soaking for more than 5 days was tested but the roots
decomposed entirely. Similar results were obtained
by Bourdoux et al. (1982) and he observed that the
low hydrocyanic acid content after 4 days of soaking
was actually due to the release of the linamarin
originally present and not a result of the deactivation
or release of the enzyme.

Table 2.7 presents the effect of soaking and
sundrying on hydrocyanic acid content of M-4 and H-165
varieties.

Table 2.7. Effect of soaking and sundrying on the
hydrocyanic acid content of cassava.

Soaking and sundrying period(days)	Remaining hydrocyanic acid content (mg/100g)+SEM			
	M-4 per cent		H-165 per cent	
0	15.81 ± 0.49		28.1 ± 0.70	
1	5.14 ± 0.13	67.48	13.85 ± 0.30	50.71
2	3.27 ± 0.07	79.32	9.41 ± 0.19	66.51
3	1.98 ± 0.04	87.34	4.14 ± 0.08	85.26
4	0.905± 0.02	94.27	2.76 ± 0.07	90.17

Mean of 3 determinations.

The effect of soaking and sundrying as a detoxification procedure was studied. It was found that the hydrocyanic acid content of M-4 roots soaked for only one day and then sundried was decreased to about 67.48 per cent while that of H-165 decreased to 50.71 per cent. The hydrocyanic acid content decreased as the soaking period increased and by the end of 4 days soaking, the dried products of M-4 variety lost about 93.42 per cent of the initial hydrocyanic acid and of H-165 variety 85.3 per cent of hydrocyanic acid was lost. In this context soaking and sundrying may be regarded as the most efficient detoxification process for cassava. However, this method of detoxification is not popular in Kerala.

Table 2.8 presents the effect of frying on the hydrocyanic acid content of M-4 and H-165 varieties.

Table 2.8. Effect of frying on the hydrocyanic acid content of cassava.

Variety	Treatments	Hydrocyanic acid content $\pm$ SEM (mg/100 g)	Per cent
M-4	1 Raw	15.81 $\pm$ 0.49	
	2 Shallow frying	9.43 $\pm$ 0.24[a]	40.4
	3 Deep frying	5.89 $\pm$ 0.12[a]	62.7
H-165	1 Raw	28.1 $\pm$ 0.70	
	2 Shallow frying	19.8 $\pm$ 0.47[b]	29.5
	3 Deep frying	10.81 $\pm$ 0.24[a]	61.5

Mean of 3 determinations.

Group 2 and 3 were compared to Group 1 in each variety.

a=p< 0.01.

b=p between 0.01 and 0.05.

't' value for table 2.8

t between groups	t value
M-4	
1 and 2	11.73
1 and 3	19.68
H-165	
1 and 2	9.81
1 and 3	23.16

In observing the effect of frying on the
hydrocyanic acid content of cassava it was seen
that there was a significant reduction in hydrocyanic
acid content in both shallow fat frying and deep fat
frying. It is to be noted that in deep frying method,
the percentage loss of hydrocyanic acid from the two
varieties was found to be 62.7 per cent and 61.5
per cent respectively, even though there was wide
variation in the hydrocyanic acid content of fresh
tubers of these two varieties. From this it can be
assumed that cassava varieties with high hydrocyanic
acid content can be safely used for the preparation
of "chips" which is a very popular snack, in the state.
The effect of the type of fat used for frying was not
studied in the present investigation. But data is
available from Nigeria that the effect of hydrocyanic
acid toxicity was counteracted by using palm oil in
the preparation of cassava foods (Formunyan (1982)).

3. Effect of addition of different ingredients on
   the hydrocyanic acid content of cassava.

Table 3 presents data on the influence of some
of the added materials while cooking on the hydrocyanic
acid content of cooked cassava of M-4 and H-165 varieties.

Table 3. Effect of added materials on the hydrocyanic
acid content of cooked cassava.

No.	Added in-gredients	M-4	Per cent	H-165	Per cent
		Hydrocyanic acid content(mg/100 g) ± SEM			
1	Boiled cassava alone	6.93 ± 0.22		16.03 ± 0.45	
2	Common salt	4.01 ± 0.17	42.13	7.52 ± 1.26	53.08
3	Cooking soda	3.47 ± 0.13	49.92	5.98 ± 1.01	62.69
4	Tomato	3.04 ± 0.11	56.13	5.91 ± 1.86	63.13
5	Lime juice	1.84 ± 0.03	73.44	3.48 ± 1.41	78.29
6	Tamarind pulp	1.91 ± 0.04	72.44	3.53 ± 0.08	77.98
7	Vinegar	3.04 ± 0.11	56.13	5.74 ± 1.75	64.19
8	Honey	2.11 ± 0.07	69.55	4.06 ± 1.73	74.67
9	Drumstick leaves	3.51 ± 0.14	49.35	6.11 ± 2.04	61.88
10	Papaya	2.48 ± 0.09	64.21	4.21 ± 1.08	73.74
11	Sugar	1.94 ± 0.05	72.00	3.78 ± 1.01	76.42

Mean of 3 determinations.

The data revealed that all the materials added, that were commonly used for cooking,were effective in reducing the hydrocyanic acid content. The most common ingredient added to foods while cooking is common salt. In the present experiment though it reduces the hydro-cyanic acid content, the loss was only 42.13 per cent in M-4 and in the case of H-165 it was 53.08 per cent. Acid containing foods such as lime juice and tamarind pulp were found to be more effective in detoxifying hydrocyanic acid. While using lime juice with M-4 variety the loss was 73.44 per cent and in H-165 the loss was 78 per cent. This was the most effective cooking material which reduced the hydrocyanic acid content to a significant extent. Lime juice was followed by the tamarind pulp which is usually added in curry preparations. In M-4 variety tamarind pulp reduced the hydrocyanic acid content upto 72.44 per cent and in H-165 77.98 per cent. Sugar and honey were also able to decrease the hydrocyanic acid content, the loss being 72 per cent and 76.42 per cent for sugar and 69.55 per cent and 74.67 per cent in case of honey for M-4 and H-165 respectively. While adding papaya,

73.74 per cent of the hydrocyanic was lost for H-165
variety but the loss was only 64.21 per cent in the
case of M-4 variety. These results are in agreement
with the observations of Adricens (1946) and Vimala-
kumari et al. (1980). The added materials selected
for the experiment were ingredients generally used
in the conventional preparations of cassava and hence
it can be assumed that the preparations made with
these ingredients may have less hydrocyanic acid content.

B. Assessment of the antithyroid action of cassava
was done by conducting feeding experiment with
two varieties (M-4 and H-165) of raw, cooked and
sun dried cassava. The results of the feeding
experiment are discussed under the following
lines.

1. Gain in weight of rats fed on different forms
of cassava diets and variation in weight of
different tissues.

Table 4.1. Gain in weight of rats fed different forms of cassava diets.

Variety	Groups	Particulars	Gain in weight(g)±SEM
M-4	I	Cooked	82.46 ± 1.65^a
	II	Raw	67.9 ± 1.83^a
	III	Sundried	63.5 ± 1.90^a
H-165	IV	Cooked	70.41 ± 2.11^a
	V	Raw	68.23 ± 1.90^a
	VI	Sundried	67.88 ± 1.83^a
Control	VII	No cassava	94.8 ± 2.37

Average of the values from 6 rats $\pm$ SEM.
Groups I, II, III, IV, V and VI were compared to Group VII.
$a = p < 0.01$

't' value for table 4.1.

t between groups	t value
VII and I	4.27
VII and II	8.98
VII and III	10.29
VII and IV	7.68
VII and V	8.74
VII and VI	8.99

As revealed in the table there was significant
reduction in the body weight of animals fed different
forms of cassava diets when compared to the control.
The gain in weight of the two experimental groups were
statistically compared to the control group and the
most significant result was found between groups fed dried
cassava (M-4) and control, followed by Group V and
II. All these three groups contained M-4 variety or H-165
cassava which was the bitter variety. Among the three
forms of cassava diets, weight gained by the animals
fed cooked cassava was maximum followed by raw cassava
diet and lastly by sundried cassava. Similar trends
were shown when two varieties of cassava in three forms
were fed compared to the control group. The
weight gained by the animals in the control group was
highest. The difference in weight gain could be due to
protein deficiency. In the experimental groups, 12.5
per cent protein was supplied whereas the recommended
allowance for rats was 20 per cent (NIN, 1983). The
recommended allowance of 20 per cent protein was included
in the control diet. In addition to a protein deficient
diet, the presence of cyanogenic glycosides in cassava

would cause further protein deficiency because sulphur containing amino acids such as methionine and cysteine are already deficient in cassava and they are required for cyanide detoxification (Geevarghese, 1983). This may be the reason for the low weight gain in rats fed on different cassava based diets.

Table 4.2. Tissue weights(g) of rats fed different forms of cassava diets.

Variety	Group	Parti-culars	Tissue weight(g) $\pm$ SEM		
			Liver	Kidney	Spleen
M-4	I	Cooked	5.24 ± 0.17^a	1.84 ± 0.43	0.69 ± 0.003
	II	Raw	4.99 ± 0.11^a	1.65 ± 0.23	0.65 ± 0.001^a
	III	Sund-ried	5.11 ± 0.14^a	1.78 ± 0.21	0.67 ± 0.010
H-165	IV	Cooked	5.15 ± 0.15^a	1.73 ± 0.84	0.675 ± 0.028
	V	Raw	4.77 ± 0.09^a	1.54 ± 0.03^a	0.641 ± 0.002^a
	VI	Sun-dried	5.08 ± 0.13^a	1.62 ± 0.01	0.66 ± 0.007^a
Control	VII	No ca-ssava	7.18 ± 0.25	1.99 ± 0.04	0.70 ± 0.007

Average of the values of 6 rats $\pm$ SEM.
Groups I, II, III, IV, V and VI were
     compared to Group VII.
a=p< 0.01.

't' value for table 4.2.

t between groups	t value		
	Liver	Kidney	Spleen
VII and I	6.417	0.347	1.313
VII and II	8.018	1.456	7.071
VII and III	7.224	0.045	2.458
VII and IV	6.963	0.309	1.039
VII and V	9.070	9.000	8.104
VII and VI	7.452	0.228	4.041

As revealed in Table 4.2 weights of the liver in
the test diets were significantly lower than that of
the control group. The group which was fed cooked M-4
variety was ranked next to control. This group was
followed by the group fed cooked H-165. variety. In
both the varieties sundried cassava groups gave better
results when compared to the groups fed raw cassava.
However the weight of the liver tissues of all the
experimental groups were significantly lower (at 0.01
level), compared to the control group.

The difference in the weight of kidneys of the
experimental groups and control group was not significant
except for Group V which was fed raw H-165 cassava.

Among the three test groups, in both the varieties, gain in weight was highest for groups fed cooked cassava followed by groups fed sundried cassava and raw cassava. Similar trend was observed in the gain in weight of liver tissue also.

The weights of the spleen of animals belonging to groups fed raw M-4 and H-165 varieties as well as sundried H-165, were significantly lower than the control group. The trend in weight gain of spleen was similar to the results observed in the case of liver and kidney.

The difference in weight gain especially in the case of the liver is probably due to protein deficiency since only 12.5 per cent of protein was provided in the experimental groups whereas the control group was given the recommended quantity of protein. Another notable point in this context is that the cassava itself is deficient in sulphur containing amino groups which are required for the detoxification of hydrocyanic acid to thiocyanate in the body.

2. Rhodanese enzyme activity in liver, kidney
and spleen.

The principal detoxification pathway of hy-
drocyanic acid in the body was controlled by rhoda-
nese or thiosulphate cyanide - sulphur transferase,
which catalyses the reaction by favouring the
transfer of sulphur atom, coming from a donor
(thiosulphate, persulphide) to a nucleophilic
acceptor (cyanide ion). Thiosulphate ($S_2O_3^{--}$) was
formed from sulphur containing amino acids
(Lang, 1933).

$$S_2O_3^{--} + CN^- \xrightarrow{\text{rhodanese}} SCN^- + SO_3^{--}$$
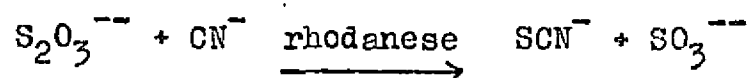
The activity of the enzyme rhodanese in
different tissues of experimental animals fed
different cassava diets was assessed and the
details are presented in Table 5 and Fig.VII.

Table 5. Activity of enzyme Rhodanese in liver, kidney and spleen of rats fed different forms of cassava diets.

Variety	Group	Parti-culars	Rhodanase activity (R.U) $\pm$ SEM		
			Liver	Kidney	Spleen
M-4	I	Cooked	0.417 ± 0.004	0.391 ± 0.00	0.45 ± 0.018
	II	Raw	0.501 ± 0.005^b	0.41 ± 0.02	0.48 ± 0.007
	III	Sun-dried	0.51 ± 0.005^a	0.42 ± 0.08^b	0.49 ± 0.008
H-165	IV	Cooked	0.571 ± 0.001^a	0.41 ± 0.10^b	0.47 ± 0.005
	V	Raw	0.58 ± 0.010^a	0.48 ± 0.05	0.51 ± 0.04
	VI	Sun-dried	0.58 ± 0.011^a	0.48 ± 0.04^a	0.49 ± 0.056
Control	VII	No ca-ssava	0.48 ± 0.004	0.38 ± 0.01	0.45 ± 0.08

Average of the values of 6 rats $\pm$ SEM

Groups I, II, III, IV, V and VI were compared to Group VII.

a=p< 0.01.

b=p between 0.01 and 0.05.

't' value for Table 5.

t between groups	t value		
	Liver	Kidney	Spleen
VII and I	1.46	1.007	0.0
VII and II	3.27	1.342	0.37
VII and III	4.37	3.265	0.49
VII and IV	6.15	3.134	0.25
VII and V	8.15	1.821	0.67
VII and VI	8.15	4.123	0.41

FIG. VII GRAPHICAL REPRESENTATION OF RHODANESE
ACTIVITY IN LIVER, KIDNEY AND SPLEEN
OF RATS FED DIFFERENT FORMS OF
CASSAVA DIETS.
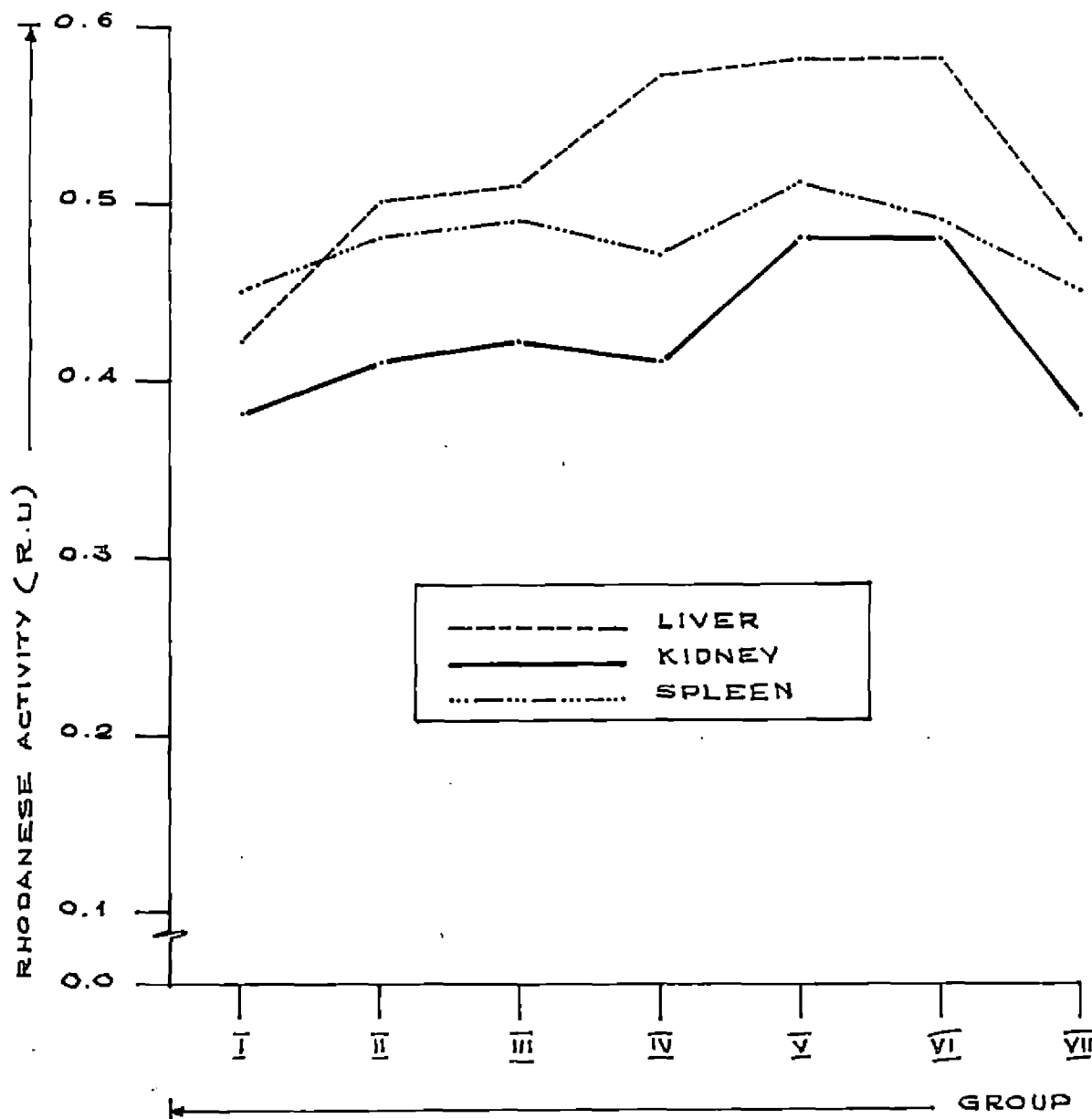
As revealed in Table 5, compared to the control group there was slight increase in the activity of the enzyme in groups fed cassava diets. The activity of the enzyme was found to be more in groups fed sundried cassava which had the highest hydrocyanic acid content in both the varieties. Among the experimental groups the enzyme activity was lowest in groups fed cooked cassava diets of both varieties, probably because the detoxification of hydrocyanic acid occurred during cooking.

Experiments conducted by Wheeler et al. (1975) revealed that sulphur containing amino acids are essential for the activity of enzyme rhodanese.

Among the three tissues in which rhodanese activity was estimated, liver was found to have higher concentration of the enzyme. The results were well supported by the earlier studies of Auriga and Koj (1975) which revealed that concentration of the enzyme was more in the liver.

The statistical analysis of the results indicated that the enzyme activity was significantly high

only in liver and kidney. Comparison between the two varieties of cassava also revealed that the activity of rhodanese was significantly higher in H-165 which contained higher concentrations of hydrocyanic acid.

3. Total liver protein.

Table 6 presents the values of liver proteins estimated in rats fed cassava diets.

Table 6. Liver proteins in rats fed different forms of cassava diets.

Variety	Groups	Particulars	Liver proteins mg/g+SEM
M-4	I	Cooked	273.45 ± 4.81
	II	Raw	268.89 ± 3.94
	III	Sundried	261.84 ± 4.54[b]
H-165	IV	Cooked	270.85 ± 4.05
	V	Raw	269.95 ± 5.04
	VI	Sundried	268.74 ± 3.02
Control	VII	No cassava	283.30 ± 4.85

Average of the values of 6 rats ± SEM.
Group I, II, III, IV, V and VI were compared with Group VII.
b=p between 0.01 and 0.05

't' value for table 6.

t between groups	t value
VII and I	1.442
VII and II	2.306
VII and III	3.230
VII and IV	1.970
VII and V	1.909
VII and VI	2.548

From Table 6 it was found that the group fed raw cassava, M-4 variety had the highest liver protein value (273.45 mg/g). The group which was fed dried cassava (M-4) had the lowest liver protein content (261.84 mg/g). It was significantly lower than the control group which had a liver protein content of 283.3 mg/g. Similar results were observed in H-165 based diets also. Group IV which was fed the cooked H-165 diet had the highest liver protein content (270.85 mg/g), followed by Groups V and VI. However the results were not statistically significant except in Group III where the diet was sundried M-4 variety of cassava. The slight differences present among the groups may be due to protein deficiency of the diet.

A nutritional factor that may have been involved in the goitrogenic action of cassava was the protein intake since the endogenous conversion of cyanide (hydrocyanic acid) into thiocyanate required sulphur amino acids (Ermans et al. 1972; Oke, 1973; Bourdoux et al., 1980). This may have been the reason for low liver protein concentration in groups fed cassava diet with high hydrocyanic acid content. Suitable processing of cassava can help to minimise this effect by effective detoxification of hydrocyanic acid present.

4. Serum thiocyanate.

Table 7 and Fig.VIII revealed the serum thiocyanate levels in rats fed cassava diets.

Table 7. Concentration of thiocyanate in serum of rats fed different forms of cassava diets.

Variety	Group	Particulars	Thiocyanate mg/dl
M-4	I	Cooked	0.741 ± 0.015
	II	Raw	0.762 ± 0.019^{b}
	III	Sundried	0.754 ± 0.017
H-165	IV	Cooked	0.82 ± 0.025^{a}
	V	Raw	0.861 ± 0.028^{a}
	VI	Sundried	0.845 ± 0.026^{a}
Control	VII	No cassava	0.7 ± 0.014

Average of the values from 6 rats $\pm$ SEM
Groups I, II, III, IV, V and VI were compared to Group VII
a=p< 0.01
b=p between 0.01 and 0.05

't' value for table 7.

t between groups	t value
VII and I	2.011
VII and II	2.622
VII and III	2.423
VII and IV	4.239
VII and V	4.977
VII and VI	4.307

As revealed in Table 7, serum thiocyanate levels were found to be slightly increased in rats fed cassava diets. In both M-4 and H-165 varieties, thiocyanate level was high in rats fed raw form of cassava that is 0.762 mg/dl and 0.861 mg/dl respectively. But this level decreased to 0.754 mg/dl and 0.845 mg/dl when the feeding was conducted with dried cassava. However the results were not significant in the case of groups fed cooked M-4 cassava and sundried M-4 cassava. In groups fed raw M-4, the concentration of serum thiocyanate level, when compared to control, was increased significantly only at 0.05 per cent level. The results of feeding cooked cassava were comparable to that of the control, indicating detoxification of cyanide during cooking. While in the case of H-165 variety of cassava the difference in concentration of thiocyanate was statistically significant for all the three groups at 0.01 level.

FIG. VIII GRAPHICAL REPRESENTATION OF CONCENTRATION OF THIOCYANATE IN SERUM OF RATS FED DIFFERENT FORMS OF CASSAVA DIETS
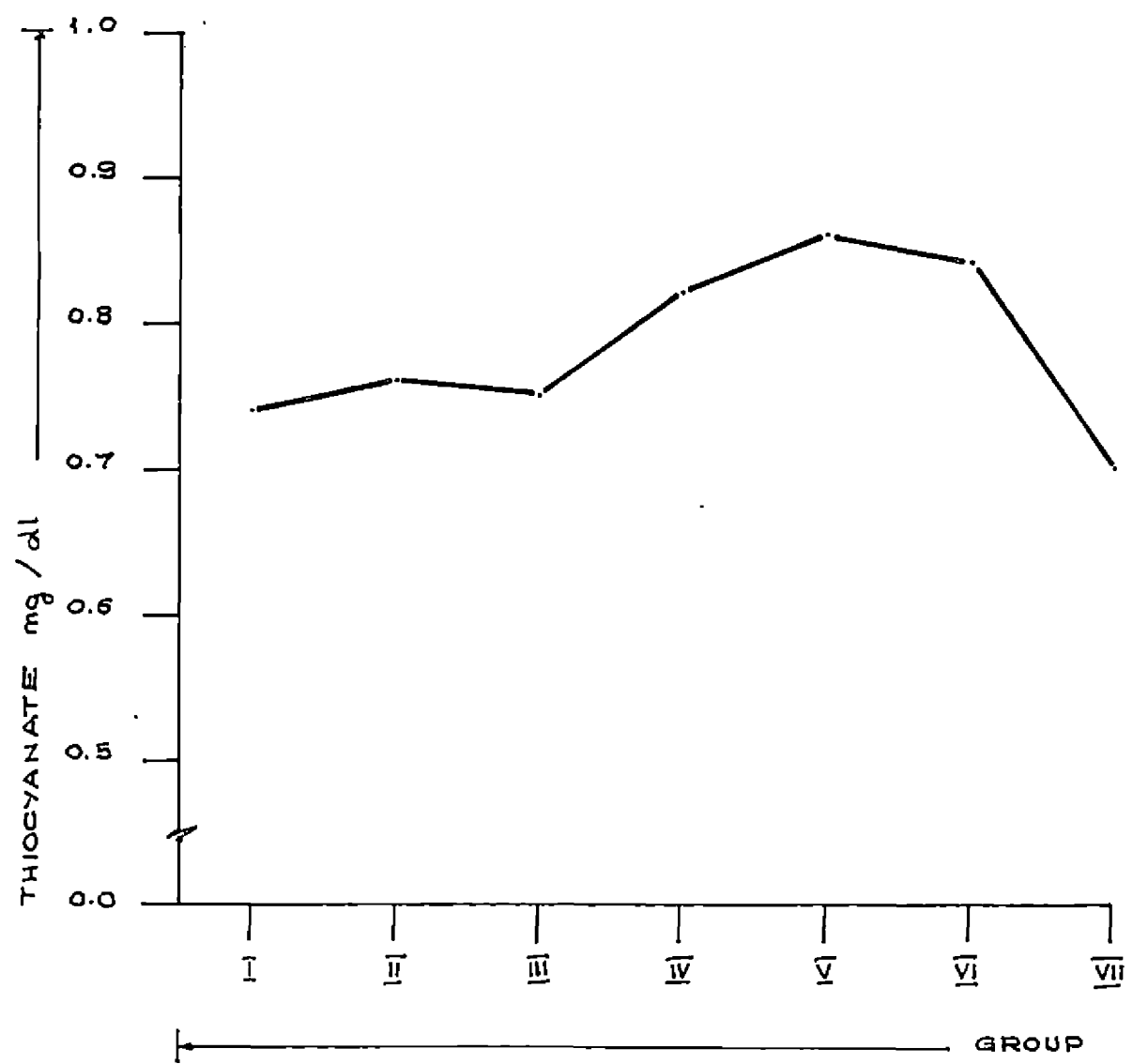
The inhibitory action of thiocyanate on thyroid uptake had been clearly shown experimentally by several scientists (Delange et al., 1980). The antithyroid effect of cassava is directly linked to endogenous thiocyanate production following cassava ingestion. The toxicity of cassava resulted from the degradation of linamarin and the concomitant production of cyanide. Sublethal doses of cyanide activates the body's own mechanisms of detoxification that ensure the transformation of cyanide into less toxic substances principally thiocyanate. Halmi (1961), Wollman (1962) and Scranton et al. (1969) had shown that thiocyanate even in low concentrations, would inhibit the iodide transport.

5. Liver and serum cholesterol.

Table 8 presents the liver and serum cholesterol levels of rats fed on different cassava diets.

Table 8. Concentration of cholesterol in serum and liver in rats fed different forms of cassava diets.

Variety	Groups	Particulars	Cholesterol ± SEM	
			Serum mg/100 ml	Liver mg/100 g
M-4	I	Cooked	63.01 ± 1.88	360.81 ± 5.21
	II	Raw	70.11 ± 2.23^b	371.55 ± 3.56
	III	Sundried	67.21 ± 3.11	368.44 ± 4.21
H-165	IV	Cooked	65.33 ± 1.33	364.11 ± 3.14
	V	Raw	70.96 ± 2.18^b	372.98 ± 2.22
	VI	Sundried	68.35 ± 2.13	369.37 ± 3.47
Control	VII	No cassava	62.92 ± 1.47	359.91 ± 7.56

Average of the values from 6 rats ± SEM
Groups I, II, III, IV, V and VI were compared
     to Group VII
b=p between 0.01 and 0.05.

't' value for table 8

t between groups	t value	
	Serum	Liver
VII and I	0.038	0.098
VII and II	2.691	1.393
VII and III	1.247	0.986
VII and IV	1.216	0.513
VII and V	3.058	1.659
VII and VI	2.098	1.137

The results indicated that there was increase in
serum cholesterol levels in rats fed cassava diets,
when compared to the control group. However the increase
was statistically significant only in the case of groups
fed raw cassava in both varieties. Similar trends were
shown in the cholesterol content of liver also. But
there was no significant change in the cholesterol level
of liver of all the groups. This may be due to the low
hydrocyanic acid content in M-4 variety and more over
by cooking 50 per cent of the initial hydrocyanic acid
was lost. This result is in line with the study done
on children in Gorakhpur where they observed significant
increase in serum cholesterol values, when severity of
goitre increased (Agarwal et al., 1982).

II. Influence of calorie protein deficiency and effect
    of Iodine supplementation on the antithyroid action
    of Cassava.

Antithyroid action of cassava was further
assessed by feeding cooked M-4 diets for 90 days with-
out protein and iodine and supplemented with protein
and iodine. The results of this study are discussed
on the following lines.

1. Gain in weight of rats and variation in weight of
   different tissues.

Table 9.1 presents the gain in weight of rats
fed protein and iodine free cassava diets and cassava
diets supplemented with iodine and protein.

Table 9.1. Gain in weight of rats fed protein and
           iodine free diets and diets supplemented
           with protein and iodine.

Groups	Particulars	Gain in weight(g)±SEM
I	Cassava alone	163.42 ± 3.89^a
II	Cassava + Iodine	168.91 ± 3.52^a
III	Cassava ± Protein	190.54 ± 2.58
IV	Cassava ± Protein + Iodine	191.93 ± 3.76
V	No Cassava	192.81 ± 3.08

Average of the values from 6 rats ± SEM
Group I, II, III and IV were compared to
Group V.
a=p< 0.01.

't' value for table 9.1

t between groups	t value
V and I	5.9
V and II	5.1
V and III	0.6
V and IV	0.2

As revealed in Table 9.1 there was no significant difference in weight gain among Groups III, IV when compared to Group V probably because the recommended allowance of protein was met in the diets provided to these groups. The gain in weight was lowest in the group fed cassava alone followed by Group II where cassava was supplemented with iodine. Gain in weight of the control group was significantly greater when compared to these two groups. This may be due to the low protein and high cassava levels of the diets of the Groups I and II. It was also to be noted that the presence of cyanogenic glycosides in cassava would cause further protein deficiency.

Table 9.2 presents the tissue weights of rats fed protein and iodine free cassava diets and diets supplemented with protein and iodine.

Table 9.2. Tissue weights of rats fed protein and iodine free cassava diets and cassava diets supplemented with protein and iodine.

Groups	Particulars	Tissue weight(g)		
		Liver	Kidney	Spleen
I	Cassava alone	$4.01 + 1.39^a$	1.43 ± 0.20	1.08 ± 0.43
II	Cassava + Iodine	4.25 ± 1.87^a	1.54 ± 0.04	1.17 ± 0.05
III	Cassava + Protein	5.48 ± 2.07	2.01 ± 0.08	1.35 ± 0.01
IV	Cassava + Protein + Iodine	7.47 ± 3.21	2.21 ± 0.06	1.48 ± 0.08
V	No cassava	7.58 ± 0.16	2.31 ± 0.43	1.50 ± 0.08

Average of the values of 6 rats $\pm$ SEM.
Group I, II, III and IV were compared to Group V
$a = p < 0.01$.

't' value for table 9.2

t between groups	t value		
	Liver	Kidney	Spleen
V and I	4.95	1.86	0.18
V and II	6.25	1.78	0.03
V and III	1.01	0.69	1.78
V and IV	0.03	0.23	2.25

As revealed in Table 9.2 the weight of liver in the Groups I and II were significantly lower than that of the control group while the weights of liver of the remaining two, Groups III and IV were comparable to the control. Protein level of the diet may have been responsible for the variation.

In the case of other tissues like kidney and spleen, there was slight reduction in the weight of tissues but the difference was not statistically significant when compared to the control. However, the trend in weight gain of kidney and spleen were similar to the results observed in the case of liver.

2. Rhodanese enzyme activity in liver, kidney and spleen.

The major pathway for the detoxification of hydro-
cyanic acid injected through cassava was by the action of
the enzyme rhodanese with the production of thiocyanate.
It utilizes sulphur containing amino acids for its activity.

Table 10 and Fig.IX reveal the activity of the
enzyme rhodanese in different tissues of rats fed protein
and iodine free cassava diets and diets supplemented with
protein and iodine.

Table 10. Activity of the enzyme rhodanese in liver, kidney
and spleen in rats fed protein and iodine free
cassava diets and cassava diets supplemented with
protein and iodine.

Groups	Particulars	Rhodanese activity (R.U)+SEM		
		Liver	Kidney	Spleen
I	Cassava alone	0.48 ± 0.008	0.348 ± 0.014	0.458 ± 0.081
II	Cassava+Iodine	0.499 ± 0.012	0.355 ± 0.01	0.462 ± 0.101
III	Cassava+Protein	0.534 ± 0.012^b	0.428 ± 0.091^a	0.545 ± 0.05^a
IV	Cassava+Iodine+Protein	0.51 ± 0.010^b	0.401 ± 0.007^a	0.512 ± 0.02^a
V	No Cassava	0.49 ± 0.009	0.341 ± 0.008	0.456 ± 0.071

Average of the values from 6 rats $\pm$ SEM

Group I, II, III and IV were compared to Group V

a=p< 0.01

b=p between 0.01 and 0.05

't' value for table 10.

t between groups	t value		
	Liver	Kidney	Spleen
V and I	0.83	0.43	0.02
V and II	1.39	1.09	0.05
V and III	2.89	7.95	7.73
V and IV	2.59	5.64	4.13

As revealed in Table 10, the activity of the enzyme rhodanese was found to be higher in all the test groups except in Group I for liver which was fed cassava alone. The rhodanese activity was significantly higher in groups fed protein supplemented diets. The rhodanese activity was significantly higher at 0.01 level in Kidney and spleen, while in the case of liver the significance was at 0.05 per cent level. The increased activity of the enzyme may be due to the presence of protein in the diet. Since protein thus available would be utilised for the detoxification of hydrocyanic acid to thiocyanate. In protein free cassava diets (Groups I and II) the activity of the enzyme was low due to the absence of protein. Experimentally it has been proved that

FIG. IX GRAPHICAL REPRESENTATION OF RHODANESE
ACTIVITY IN LIVER, KIDNEY AND SPLEEN OF RATS
FED PROTEIN AND IODINE FREE CASSAVA DIETS
AND DIETS SUPPLEMENTED WITH PROTEIN AND IODINE
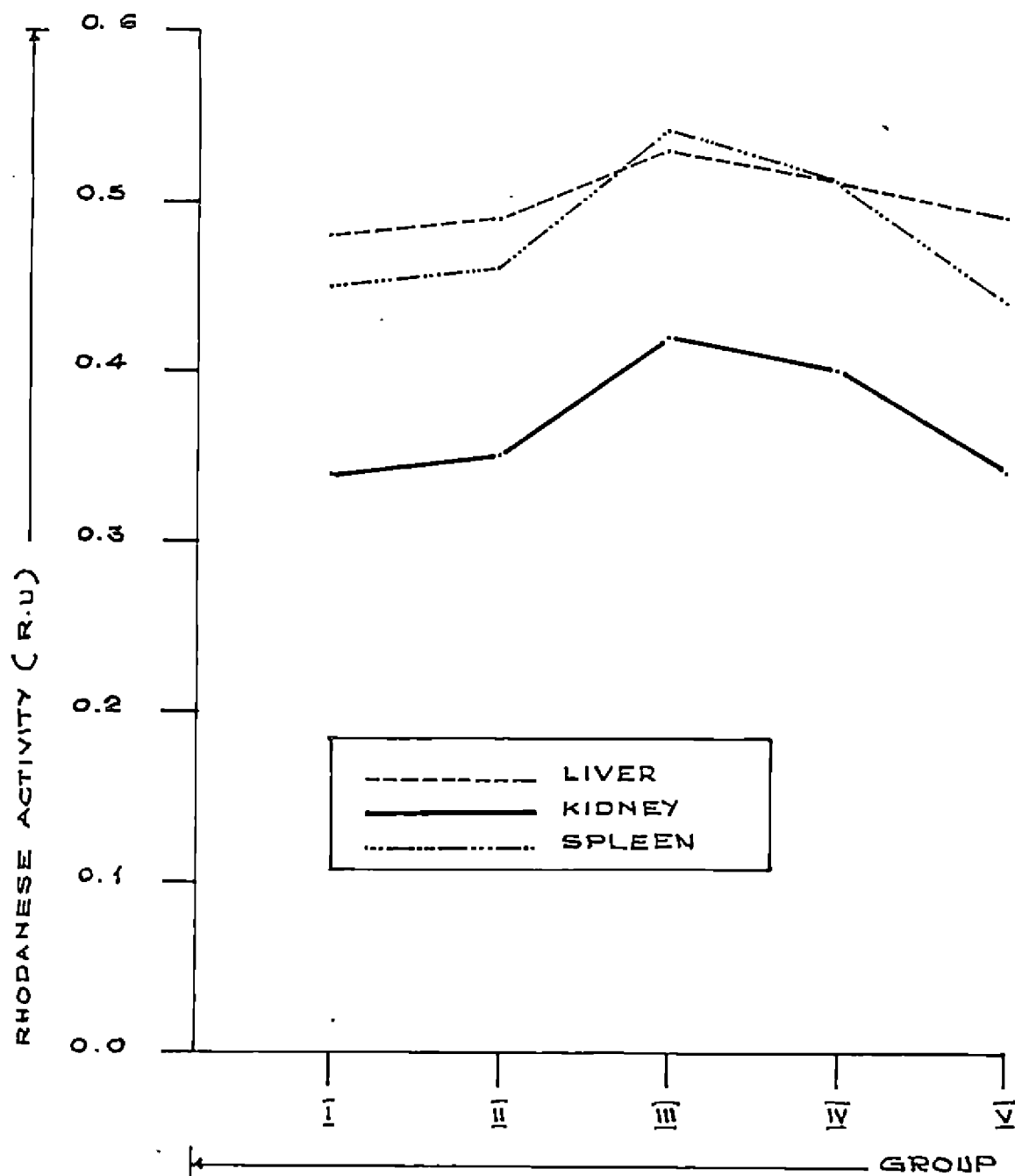
protein deficiency protects against the antithyroid
action of cassava by reducing the quantity of thio-
cyanate arising from hydrocyanic acid (Tewe, 1976).
It has also been shown experimentally that the
presence of protein deficiency impairs the development
of goitre due to a goitrogenic diet (Aschkenazy et al.,
1962, Cowan and Margossian, 1966 and Shrader et al.,
1977).

3. Total liver protein in rats fed protein and iodine
   free cassava diets and cassava diets supplemented
   with protein and iodine.

Table 11 gives the liver protein concentration
in rats fed protein and iodine free cassava diets and
cassava diets supplemented with protein and iodine.

Table 11. Liver protein concentration in rats fed
protein and iodine free cassava diets and
cassava diets supplemented with protein
and iodine.

Groups	Particulars	Liver protein mg/g+SEM
I	Cassava alone	198.99 ± 7.56^a
II	Cassava+Iodine	200.82 ± 8.03^a
III	Cassava+Protein	291.3 ± 9.90
IV	Cassava+Protein+Iodine	294.5 ± 8.25
V	No cassava	294.8 ± 10.42

Average of the values of 6 rats $\pm$ SEM

Group I, II, III and IV were compared to Group V

a=p< 0.01

't' value for table 11

t between groups	t value
V and I	7.68
V and II	7.37
V and III	0.45
V and IV	0.25

As depicted in Table 11, liver protein was found to be significantly lower in groups fed cassava diets without protein in Group I and II while liver protein content of Groups III and IV were comparable to the control group. The low liver protein concentration in Group I and II may be due to a protein deficient diet.

4. Serum and urinary thiocyanate levels in rats fed protein and iodine free cassava diets and cassava diets supplemented with protein and iodine.

Table 12 and Fig.X) present the serum and urinary thiocyanate levels in rats fed protein and iodine free cassava diets and diets supplemented with protein and iodine.

Table 12. Serum and urinary thiocyanate levels in rats fed protein and iodine free cassava diets and cassava diets supplemented with protein and iodine.

| Groups	Particulars	Thiocyanate mg/dl	
		Serum	Urine
I	Cassava alone	0.752 ± 0.017	1.064 ± 0.029
II	Cassava+Iodine	0.721 ± 0.025	1.034 ± 0.032
III	Cassava+Protein	0.755 ± 0.031	1.08 ± 0.029
IV	Cassava+Protein+ Iodine	0.732 ± 0.019	1.034 ± 0.041
V	No Cassava	0.720 ± 0.017	1.01 ± 0.037

Average of the values of 6 rats ± SEM

Group I, II, III and IV were compared to Group V.

't' value for table 12

t between groups	t value	
	Serum	Urine
V and I	1.331	1.149
V and II	0.033	0.49
V and III	0.98	1.489
V and IV	0.47	0.54

As revealed in Table 12, serum and urinary thiocyanate level was found to be higher in all the test groups, compared to control group. However the variation was not statistically significant. In Group III where cassava diet supplemented with protein alone was fed, the serum and urinary thiocyanate levels were the highest among the test groups. This may be due to the increased activity of the enzyme rhodanese as reported in Table 12 in the present study. Studies conducted by Wheeler et al.(1975) and Barrett et al. (1978) had revealed that in the presence of sulphur containing amino acids, this enzyme is responsible for the conversion of hydrocyanic acid to thiocyanate. Earlier studies conducted by Smith (1961)

FIG. X GRAPHICAL REPRESENTATION OF CONCENTRATION OF SERUM AND URINARY THIOCYANATE OF RATS FED PROTEIN AND IODINE FREE CASSAVA DIETS AND DIETS SUPPLEMENTED WITH PROTEIN AND IODINE
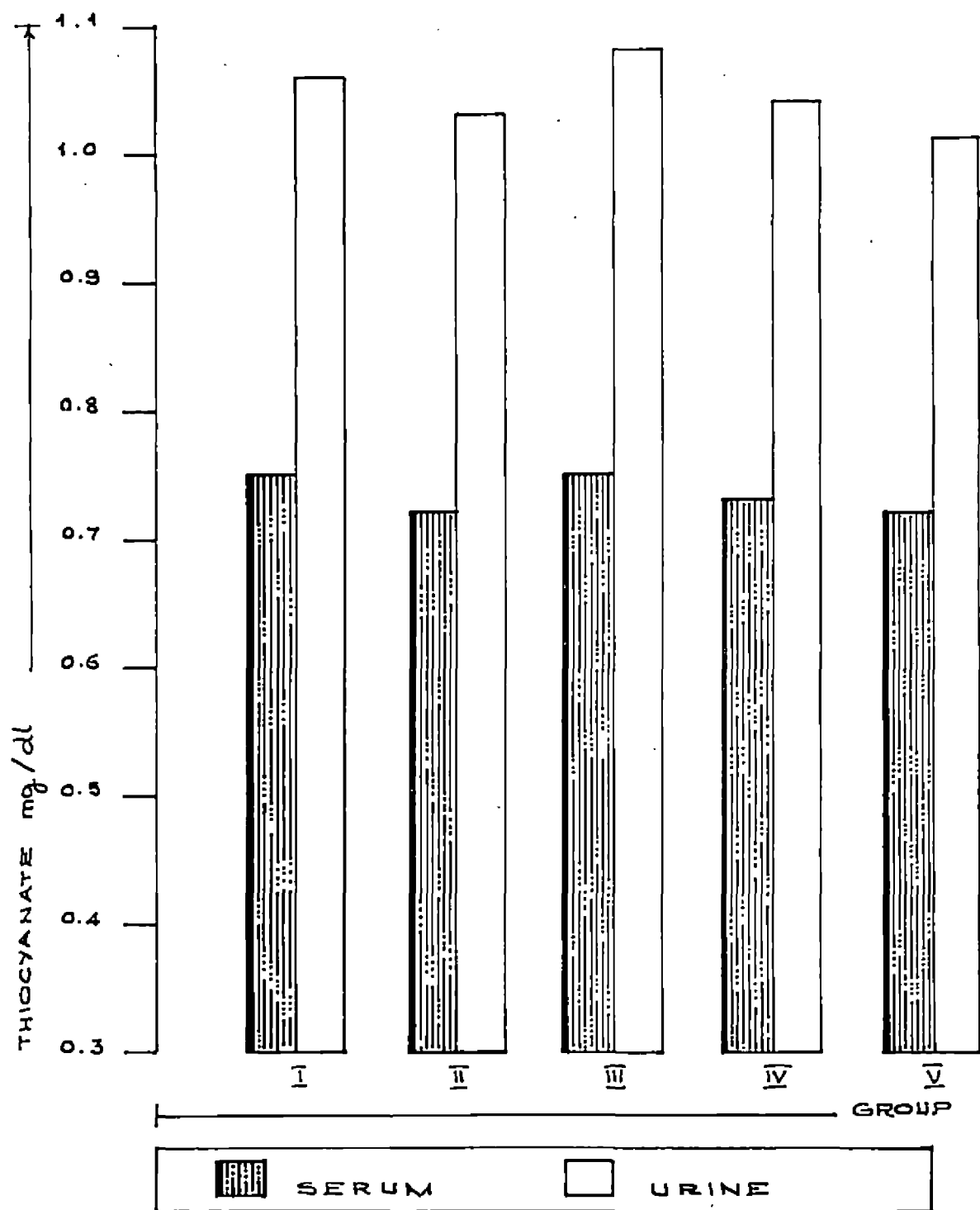
had already proved that thiocyanate, the product of
this pathway of detoxification was eliminated from
the body mainly through excretion in urine. Probably
the higher level of urinary thiocyanate in Group III
may be because of this detoxification pathway.

In Group II where cassava diet was supplemented
with iodine alone, the serum and urinary thiocyanate
levels were nearer to the control values suggesting
that a cassava based diet did not necessarily result
in the development of goitre, provided that the iodine
intake was high enough.

In Group I where there was protein and iodine
deficiency again, there was increase in the serum and
urinary thiocyanate level and the values were nearer
to the ones attained in Group III. However in this
case, the increased level of serum and urinary thio-
cyanate may be due to protein deficiency since in
protein calorie malnutrition, plasma iodine is in-
creased through a reduction in thyroid uptake and
kidney clearance (Benmiloud et al. (1982).

5. Liver and Serum cholesterol of rats fed protein
   and iodine free cassava diets and cassava diets
   supplemented with iodine and protein.

Table 13 presents the liver and serum chole-
sterol levels in rats fed protein and iodine free
cassava diets and cassava diets supplemented with
iodine and protein.

Table 13. Liver and serum cholesterol levels in rats
          fed protein and iodine free cassava diets
          and cassava diets supplemented with protein
          and iodine.

Groups	Cholesterol ± SEM	
	Serum mg/100 ml	Liver mg/100 g
I	66.99 ± 1.60	359.22 ± 11.14
II	66.98 ± 1.67	358.63 ± 14.70
III	64.23 ± 2.31	353.87 ± 13.45
IV	64.85 ± 1.62	354.12 ± 13.10
V	62.31 ± 1.56	350.11 ± 13.65

Average of the values from 6 rats ± SEM

Group I, II, III and IV were compared to Group V

't' value for table 13

t between groups	t value	
	Serum	Liver
V and I	2.13	0.52
V and II	2.04	0.42
V and III	0.69	0.19
V and IV	1.5	0.21

The results presented in Table 13 indicated an increase in the liver and serum cholesterol levels in the test groups. The increase was highest in Group I where cassava diet without protein and iodine was fed, but this increase was not significant when compared to the control group.

In Group II, where cassava diet was supplemented with iodine alone the values were comparable to Group I. But in groups III and IV where the cassava diet was supplemented with protein, the values for serum and liver cholesterol levels decreased but the results were higher than the values obtained for the control group. These results were in line with the study

conducted by Agarwal et al. (1982) among children in
Gorakhpur where significant increase in serum chole-
sterol was observed as the severity of goitre increased.

6. Serum protein bound iodine in rats fed protein
   and iodine free cassava diets and cassava diets
   supplemented with protein and iodine.

Table 14 and Fig.XI present the serum protein
bound iodine in rats fed protein and iodine free cassava
diets and cassava diets supplemented with protein and
iodine.

Table 14. Serum protein bound iodine in rats fed
          protein and iodine free cassava diets and
          cassava diets supplemented with protein
          and iodine.

Group	Particulars	Protein bound iodine ug/100ml
I	Cassava alone	0.065 ± 0.002^a
II	Cassava+Iodine	0.078 ± 0.004
III	Cassava+Protein	0.071 ± 0.003^b
IV	Cassava+Iodine+Protein	0.081 ± 0.004
V	No cassava	0.084 ± 0.002

Average of the values of 6 rats $\pm$ SEM

Group I, II, III and IV were compared to Group V

a=p< 0.01

b=p between 0.01 and 0.05

' 't' value for table 14

t between groups	t value
V and I	6.718
V and II	1.341
V and III	3.606
V and IV	0.671

As revealed in Table 14 the least value for protein bound iodine was obtained by Group I, which was fed cassava alone and this was followed by Group III which was fed cassava and protein. No iodine was supplemented in these groups. The values obtained for the iodine supplemented groups were comparable to that of the control which had the highest value. The difference between Group I and control was at 0.01 per cent level and the difference between Group III and control was at 0.05 per cent level. These results are in line with the studies conducted by Ermans et al. (1973) where they revealed that PBI decreased with increased iodine deficiency.

FIG. XI   GRAPHICAL REPRESENTATION OF SERUM PROTEIN BOUND IODINE IN RATS FED PROTEIN AND IODINE FREE CASSAVA DIETS AND DIETS SUPPLEMENTED WITH PROTEIN AND IODINE
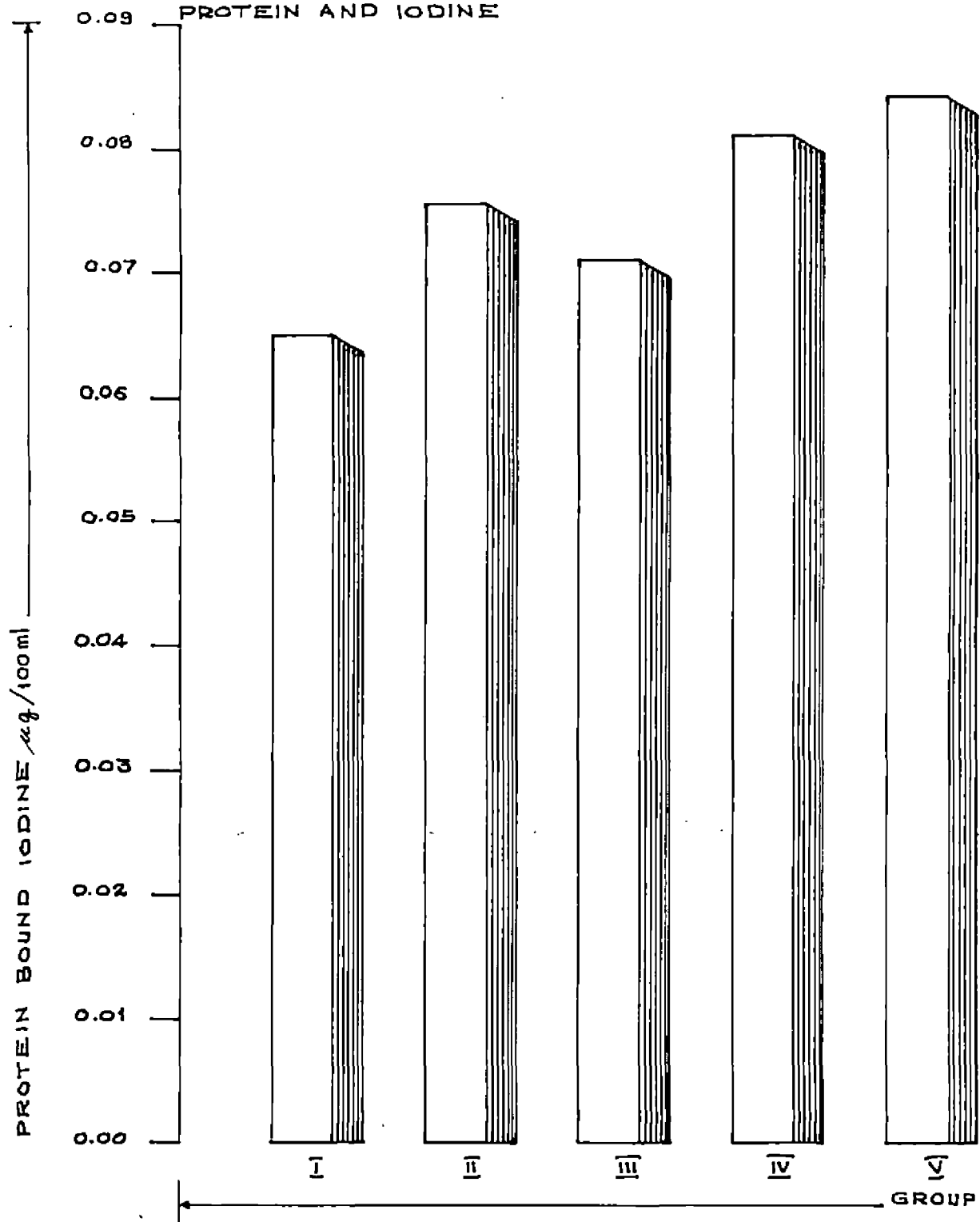
SUMMARY

SUMMARY

Cassava contains a cyanogenic glucoside, linamarin, the degradation of which releases hydrocyanic acid. The detoxification of hydrocyanic acid in the living body results in the formation of thiocyanate which induces antithyroid action, resulting in endemic goitre. Kerala is the only state in India where cassava is used as a subsidiary staple food by the poorer sections among whom protein calorie malnutrition is prevalent. Endemic goitre is reported in certain districts in the state where cassava consumption is high.

The present investigation was therefore undertaken to assess the goitrogenic effect of cassava by studying:

    i) the antithyroid action of cassava;
    ii) the influence of calorie, protein and iodine supplementation on the antithyroid action of cassava.

Hydrocyanic acid present in cassava is mainly responsible for its antithyroid action and therefore the hydrocyanic acid content in six varieties of cassava commonly used in Kerala was assessed. From this, M-4

which is very popular in Kerala and H-165 which contains
the highest concentration of hydrocyanic acid were sele-
cted for further studies. The influence of different
processing and cooking methods on hydrocyanic acid
content of these two varieties were assessed. The
results revealed that soaking and sundrying for 4 days
was the most effective detoxification method. The effect
of addition of different ingredients used for cooking,
or food tasters, on the hydrocyanic acid content of
cassava was assessed. The results of the experiment
indicated that lime juice was the most effective ingre-
dient in reducing hydrocyanic acid content.

Antithyroid action of cassava was further
assessed by conducting suitable animal experiments.
A preliminary feeding experiment of thirty days duration
was conducted using two varieties of cassava namely M-4
and H-165. The cassava varieties were fed as raw,
cooked and in sundried forms. The experimental diet
was deficient in iodine and protein. The control group
was provided dextrin instead of cassava with recommended
allowance of protein and iodine. The results of the study
revealed that the gain in weight as well as the weight of
liver, in the test groups were significantly lower than
the control group.

The kidney weights of the group fed raw
H-165 cassava and the spleen weights of group fed raw
M-4 and H-165 as well as sundried H-165 were signifi-
cantly lower than those of the control group.

Rhodanese is the enzyme responsible for the
detoxification of hydrocyanic acid to thiocyanate and
the activity of this enzyme in liver, kidney and spleen
was found to be influenced by the hydrocyanic acid
content of the test diets. Similarly liver protein
values were decreased as the hydrocyanic acid content
of the test diets increased. Serum thiocyanate levels
and serum and liver cholesterol levels were found to
increase as the hydrocyanic acid content of the test
diets were increased.

The effect of protein and iodine on cassava
based diets were studied by conducting an experiment
of ninety days duration. The M-4 variety which is very
popular in Kerala was selected for the study. Among
the four groups included in the study, in the first
two Groups (Group I and II) the diets were predominated
by cassava (69.85 per cent) while in the remaining

two groups 39.45 per cent of cassava was replaced by protein food. In Group II and Group IV the diets were supplemented with iodine also. In the control group (Group V) cassava was replaced by dextrin. The proportion of protein food present in the control was similar to that of Groups III and IV.

The enzyme rhodanese activity was lower in the animals fed cassava alone while the enzyme activity was higher in the protein supplemented Groups III and IV probably because rhodanese requires sulphur containing amino acids for its activity and this was provided in the protein supplemented diets. The serum and urinary thiocyanate levels were found to be highest in Group III in which the diet was supplemented with protein alone. This may be due to the increased activity of the enzyme rhodanese in the presence of sulphur containing amino acids which converts hydrocyanic acid to thiocyanate.

The serum and liver cholesterol levels were highest in Group I which was deficient in both protein and iodine.

Serum protein bound iodine was lowest in Group I which was fed cassava alone without protein and iodine supplementation followed by Group III fed cassava supplemented with protein but without iodine.

The salient findings of the present study indicate
that, among different processing methods, soaking for
3 or 4 days helps to remove hydrocyanic acid from the
tuber. This method is at present not popular in Kerala
due to a certain degree of fermentation of the tuber.
Experiments with various food tasters indicate that
incorporation of different food additives for giving
final taste to the prepared food often helps to reduce
hydrocyanic acid content.

The results of the animal experiments throw light
on the fact that prolonged consumption of cassava with
high hydrocyanic acid content and improperly processed
cassava chips may cause goitre. This health hazard can
be obviated if the cassava based diet is well supplemented
with protein and iodine.

The present day population explosion demands
popularisation of high yielding varieties of food crops
like H-165 which contain higher amounts of hydrocyanic
acid. The ill effects of hydrocyanic acid in such
varieties are mitigated to some extent by proper methods
of processing and by including protein and iodine with
food items in the meals.

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NUTRITIONAL FACTORS INVOLVED IN THE GOITROGENIC ACTION OF CASSAVA

by

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ABSTRACT

The present investigation was undertaken to assess the goitrogenic action of cassava. The study this antithyroid action of cassava hydrocyanic acid content of six varieties of cassava commonly used in Kerala were assessed. M-4 a popular variety and H-165 which contains the highest concentration of hydrocyanic acid were selected for further experiments. Under various processing methods, soaking and sundrying for four days was found to be the most effective detoxification method. Among the various food tasters tested, lime juice was found to be the most effective ingredient in reducing hydrocyanic acid content. A preliminary feeding trial was conducted for thirty days. In this experiment the two varieties of cassava M-4 and H-165 were fed in three different forms cooked, raw and sundried. The diets were deficient in protein and iodine. The results revealed that gain in weight as well as the weight of tissues like liver, were significantly lower in the test diets when compared to the control.

The kidney weights of the group fed raw H-165 cassava and the spleen weights of groups fed raw M-4 and H-165 as well as sundried H-165 were significantly lower than those of the control group.

The enzyme rhodanese activity was influenced by the hydrocyanic acid content of the test diets. Liver protein values decreased as hydrocyanic acid content increased. Serum thiocyanate levels and serum and liver cholesterol levels increased as hydrocyanic acid content of the test diets increased.

An experiment of ninety days duration was conducted to study the effect of protein and iodine on cassava based diets M-4 variety of cassava was used for the experiment. The salient findings of the study indicated that in the diets in which cassava predominated, gain in weight of rats, weight of liver, and concentration of liver protein were significantly lower when compared to the control. Enzyme rhodanese activity was lower in animals fed only cassava and higher in protein supplemented diets. Serum and urinary thiocyanate were highest in the cassava diet supplemented with protein. Serum and liver cholesterol levels were highest in the group deficient in both protein and iodine. Serum protein bound iodine was lowest for the group fed cassava alone without protein and iodine supplementation.