INFLUENCE OF DIETARY SUPPLEMENTATION OF PROTEIN AND IODINE ON TAPIOCA TOXICITY

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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DECLARATION

I hereby declare that the thesis entitled **"INFLUENCE OF DIETARY SUPPLEMENTATION OF PROTEIN AND IODINE ON TAPIOCA TOXICITY"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship or other similar title, of any other University or Society.

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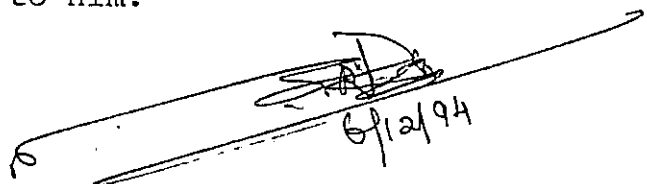
CERTIFICATE

Certified that this thesis entitled **"INFLUENCE OF DIETARY SUPPLEMENTATION OF PROTEIN AND IODINE ON TAPIOCA TOXICITY"** is a record of research work done independently by **A. Lateef**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



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A. LATEEF

To my beloved Mother

and in loving memory of

my Father.

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CHAPTER 1

INTRODUCTION

Tapioca or cassava (*Manihot esculenta* Crantz) is one of the most extensively cultivated tuber crops in about 80 developing countries (FAO, 1980) and forms world's fifth major staple food crop for over 800 million people (Phillips, 1982). This popular tuber crop has also gained eminence as a major source of energy for livestock (Tewe, 1984). However, an important factor which limits the utilization of tapioca as a major component in food is its toxicity due to the presence of cyanogenic glycosides (CNG) linamarin and lotaustralin. These cyanogenic glycosides can easily be hydrolysed either endogenously by the enzyme linamarase (Nambisan and Sundaresan, 1985) or by intestinal microbial activity (Padmaja and Panikkar, 1989b) or even by rumen microflora (Allison, 1978) to liberate toxic hydrocyanic acid (HCN). Varieties of tapioca differ considerably in their CNG content. Several high yielding varieties and hybrids also have relatively high cyanide concentrations in their tubers. The literature available on the variation in CNG content of the varieties grown in India is scanty. The existence of remarkably high levels of CNG in different varieties of tapioca that are used for human consumption and as animal feed became a subject of

considerable clinical and nutritional interest, and an elucidation of their role in livestock performance assumed much economic relevance. Various pathological conditions have been found to be associated with prolonged consumption of tapioca (Dorozynski, 1978; Geevarghese, 1982; Gilbert, 1984 and Shenoy et al., 1993) in human beings.

In order to ascertain the effects of cyanide contained in tapioca on the performance of animals several feeding trials employing tapioca meals prepared from either low or high cyanide containing varieties have been carried out on growing rats (Tewe and Maner, 1978), pigs (Gomez, 1982 and Tewe, 1982), African giant rats (Tewe, 1984) and rabbit (Ratnakumar, 1989). Increased concentration of serum thiocyanate has been reported to be associated with feeding tapioca based diets implicating the significance of this goitrogenic substance in influencing iodine metabolism.

However, these studies did not elucidate clearly the nutritional basis of tapioca to induce goitre and the ill effects on the performance of livestock maintained on tapioca-based diets. Further, the role of iodine alone or in combination with protein in a tapioca-based diet has not yet been elucidated. Moreover, work on these lines in ruminants especially in goats is scanty eventhough the breakdown of CNG

is more effective in ruminants than in non-ruminants (Nestel, 1973 and Kingsbury, 1975).

Hence it was considered better to investigate the ways and means by which tapioca is made less toxic and more wholesome as a dietary ingredient by combining this with various levels of protein and iodine in the diet.

Present investigation

In the present investigation following three series of experiments were carried out to elucidate the physiological and histological changes brought about by cyanide present in tapioca and to ascertain how best the same could be modified by the addition of different levels of dietary proteins and iodine.

1. To assess the variation in the cyanogenic glycoside content (in terms of cyanide) among different varieties of tapioca, generally available locally, and to assess the extent of retention of CNG in tapioca, subjected to processes like boiling and sun drying.

2. To study the goitrogenic and diabetogenic effects of tapioca in rats due to its prolonged ingestion. Its nutritional, endocrinological, biochemical and histopathological effects were studied in rats. The

nutritional basis of tapioca to induce goitrogenic and diabetogenic effects was the main objective of the investigation. The role of iodine and protein in a tapioca-based diet to ameliorate the cyanide toxicity in rats was investigated.

3. To study the effect of different levels of dietary protein along with iodine on a tapioca-based diet in kids. The study included growth rate, endocrinological and biochemical parameters in blood.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 Tapioca (Cassava)

Cassava (*Manihot esculenta* Crantz) belonging to the order Euphorbiaceae, was a native of Brazil and introduced to West Africa by Portugese. It is now cultivated extensively in almost every part of the tropical countries, where it has become almost a staple food (Oke, 1982) for over 800 million people (Phillips, 1982). Cassava was introduced to Kerala by Portugese navigators and was named 'Kappal Kizhangu' after the Portugese ship 'Kappal' that was believed to have sailed to Kerala coast in 17th century A.D. (Geevarghese, 1986).

2.1.1 Cassava production

The world production of cassava was estimated to be 124.987 million metric tons in 1985. FAO (1980) projected the production to be 135.513 million metric tons by the turn of the decade 1990. India stood fifth in the world production of cassava with an estimated yield of 4.058 million metric tons in 1980 (Geevarghese, 1986), eighty per cent of which was produced in Kerala.

2.1.2 Cassava consumption

The world demand of cassava for human consumption was 61.671 million metric tons in 1975 which was projected to attain the level of 88.715 million metric tons by the year 1990 with an average annual increment of 2.4 per cent (FAO, 1980). Annual per capita consumption of cassava was greatest in Africa, on an average 102 kg. Out of the total world production of cassava, 65 per cent was utilized for human consumption, 19 per cent as animal feed, 10 per cent for industrial purposes (manufacture of commercial starch, glucose and ethanol) and the rest 6 per cent was waste (Geevarghese, 1986).

2.1.3 Cyanogenic glycosides in cassava

The toxic nature of tubers and edible leaves of cassava was known from the days of cultivation of the plant. Cassava synthesizes and stores cyanogenic materials in all its vegetative tissues especially in their leaves and tubers.

Turnock (1937) identified linamarin [2-(β-D gluco-pyranosyloxy) iso-butyro-nitrile] as the principal cyanogen compound (96%) of cassava. Methyl linamarin or Lotaustralin which is closely related to linamarin was also found to be present (4%) in lower proportion (Nartey, 1968). The total concentration of cyanogenic glycosides (CNG) was reported to

be fluctuating during growth of the plants, mainly due to changes in ecological conditions (DeBruijn, 1973).

The presence of cyanogenic glycosides was the main factor standing in the way of large scale commercial exploitation of cassava as a dietary supplement for carbohydrate in man and animals. Further more, the cyanogenic glycosides appeared to be accompanied always by the glycosidase enzyme linamarase which was instrumental in liberating hydrocyanic acid from cyanogenic glycosides upon hydrolysis (Coursey, 1973; Swan and Lewis, 1976; Nambisan and Sundaresan, 1985).

2.1.4 Influence of variety of cassava on cyanogenic glycoside (CNG) content

The CNG content in cassava tuber varies with different conditions like variety of cassava, its mode and time of cultivation and harvesting the usufructs including the leaves as animal feeds.

The amount of cyanide might be as high as 2000 ppm in extremely bitter varieties (Rogers, 1963). Several high yielding varieties and hybrids (Kawano, 1978) also had relatively high cyanide concentrations in their roots (Gomez and Valdivieso, 1983a). They are bitter cultivars, which are unsuitable for human consumption, but can be acceptable as

animal feed provided they are processed adequately (Gomez et al., 1983) to reduce the CNG content. Thus, the most important determining factor for the cyanide concentration in roots appeared to be varietal (Gomez et al., 1984).

Based on organoleptic evaluation, Nambisan and Sundaresan (1991) grouped tubers of cassava varieties into three classes (1) non-bitter (2) bitter and (3) very bitter. The cassava strains listed under very bitter group were 'R 9 S_1(2)', 'R 17 OP(40)', 'R 17 OP(23)', 'R 20 OP(9)', 'R 20 S_1(11)', 'R 35 S_1(16)', (18)', (33)', (38)', (10)', 'R 35 OP(33)', (38)', 'R 63 OP(2)', 'R 40 OP(20)', and '172'. The CNG content in these strains ranged from 320 to 1100 µg cyanide/g of fresh tuber. The strains under bitter group were '118', '14/75', '158', and 'H-165' which contained 100 to 180 µg cyanide/g while the non-bitter strains viz. 'Kalikalan', 'H-1687', 'H-2304', 'M-4' contained 27.5 to 77.5 µg cyanide/g of fresh tuber.

Determination of cyanogenic glycoside content in terms of cyanide in fresh tubers of H-165, H-2304 (Sree Sahya) and H-1687 (Sree Visakh) showed that it was 140 to 160, 82.5 to 90.0 and 50 to 58.2 µg of cyanide/g fresh tuber respectively (Nambisan and Sundaresan, 1985, 1991), while the estimated content in M4 was only 32.5 to 50 µg cyanide/g fresh tuber (Nambisan and Sundaresan, 1991).

2.1.5 Effect of different processing methods on the CNG retention in cassava

The extent of utilization of cassava tubers for both human and animal consumption was limited by the presence of their cyanogenic glycosides (bound cyanide about 90%), HCN (free cyanide about 10%) and acetone cyanhydrin (Nartey, 1978) in tubers, leaves and tender parts of the plant. As a result, the tubers have to be processed by methods that reduce their toxicity and improve their palatability (Coursey, 1973; Maduagwu and Adewale, 1980). Since the skin of the tuber was found to contain higher levels of CNG, the risk of toxicity could markedly be reduced by removing the skin prior to processing. Similarly processing of cassava by boiling, sun drying, oven drying etc., decreased their cyanide contents markedly by the linamarin present in them getting hydrolysed by linamarase. However, processing at higher temperatures inactivated the enzyme leading to incomplete hydrolysis of CNG (Swan and Lewis, 1976).

A number of studies have indicated that a variety of traditional processing methods like drying, soaking, boiling, roasting, fermenting etc., were ineffective to remove all the bound form of cyanide, resulting in residual quantities still remaining, which could be more than sufficient to cause toxicity (Bourdox et al., 1982ab). At the same time Oke

(1982) found that it was difficult to remove the last traces of CNG from cassava without making it unsuitable for human as well as animal consumption.

2.1.5.1 Effect of boiling on cyanogenic glycosides (CNG)

Joachim and Panditeskere (1944) reported that cyanide content of tubers of a variety of cassava reduced from 103-332 ppm to 27-87 ppm on boiling.

But Oke (1982) noted that cooking at a temperature of about 72°C, destroyed the enzyme linamarase leaving about 90 per cent of the CNG still intact in the tubers.

Nambisan and Sundaresan (1985, 1991) showed that in the process of boiling cassava in water, smaller chip size with sufficient water was the ideal condition for maximum removal of CNG. Even then there was 25-75 per cent retention of CNG, depending on the chip size, and 24-70 per cent of retention depending on the volume of water used for boiling. Furthermore, it was pointed out that most of the CNG lost in the process of boiling could be recovered from the water in which it was boiled, indicating and supporting the view that glycosides were heat stable and therefore, very little degradation could be effected by boiling. The loss of CNG in the process of boiling was due to their solubility in water. The enzyme being inactivated at boiling temperature,

apparently did not contribute much in the degradation of CNG during this short period of processing. On the other hand, when extremely bitter tubers were processed by boiling, water was changed two or three times until the bitterness was reduced to an acceptable level. In their study boiling was found to reduce 55.5, 52.7 and 52.7 percentages of CNG respectively from its original concentration in H-165, H-2304 and H-1687 varieties.

2.1.5.2 Effect of sun drying on cyanogenic glycosides (CNG)

Cooke and Maduagwu (1978) found that drying at high temperature led to retention of more CNG because of rapid depletion of moisture essential for enzyme action on CNG. However, air drying at lower temperatures such as sun drying removed 29 per cent CNG.

Gomez (1982) indicated that sun drying on a concrete floor; oven drying at 60°C; and to lesser extent drying on inclined trays led to reduction in the total cyanide content of the dried chips of cassava. The reduction in total cyanide content was to the order of 10-30 per cent of the initial cyanide content in the fresh chips.

Oke (1982) suggested that simple drying of sliced or rasped roots would be capable of removing about 90 per cent of

the CNG at 60°C but would be less effective if heating was carried out at 100°C, due to denaturing of the enzyme.

Gomez (1984) found that sun drying of cassava led to greatest losses of cyanide in both low and high cyanide containing varieties. This was because at longer drying times with enough moisture and comparatively mild temperature condition the endogenous linamarase remained active for longer time and hydrolytic cleavage of CNG was facilitated.

Nambisan and Sundaresan (1985 and 1991) observed that the CNG retention in sun dried chips of cultivars H-165, H-2304 and H-1687 varied from 30-60 per cent depending upon the chip thickness and the temperature at which chips were dried. They further pointed out that faster drying at high temperatures resulted in depletion of moisture essential for enzyme action on CNG. As a result the CNG retention was more at 70°C than at 50°C.

2.2 Hydrolysis of cyanogenic glycosides (CNG)

A perusal of literature on the nutritional aspects of cassava reveals that it contains cyanogenic glycosides which upon hydrolysis yields hydrocyanic acid (HCN).

2.2.1 Cyanide detoxification

The most extensively studied mechanism for cyanide detoxification at non-fatal levels is through a series of reactions in which cyanide accepts sulfur from inorganic and organic sulfur donors with the resultant formation of a relatively less toxic thiocyanate. These reactions are catalysed by the enzyme rhodanase (Thiosulfate sulfur transferase) and 3-mercaptopyruvate sulfur transferase (Nartey, 1973; Conn, 1978).

McMillan and Geevarghese (1979) determined the effect of dietary protein level on thiocyanate production in rats which were given potassium cyanide in drinking water. It was noted that the excretion of thiocyanate, expressed as percentage of the cyanide ingested was clearly lower in animals kept on low protein (10%) diet compared to those maintained on high protein (20%) diet. The reduced urinary thiocyanate excretion in rats given low protein diet was due to deficiency of sulfur-containing amino acids which altered cyanide detoxification.

Geevarghese (1982) pointed out that the presence of cyanogenic glycosides in cassava would cause protein deficiency further because sulfur containing amino acids such

as methionine and cysteine, already deficient in cassava, are required for cyanide detoxification.

2.2.2 Antithyroid action of thiocyanate

Wyngaarden et al. (1953) reported that thiocyanate competed with iodide in its transport to the thyroid gland, leading to depressed iodide uptake by the thyroid for the formation of thyroxine.

Means et al. (1963) observed that thiocyanate and its precursors interfered with the accumulation of iodide by the thyroid which could be overcome by an excess of iodide.

Ekpechi et al. (1966) showed that the antithyroid action in rats fed chronically with cassava was related to the endogenous production of thiocyanate from cyanide in cassava.

Montgomery (1969) noticed an appreciable increase in contents of cyanide and thiocyanate pool in the body consequent to the ingestion of cyanogenic glycosides.

Radeleff (1970) observed accumulation of toxic levels of cyanide in the body in spite of adequate levels of the enzyme rhodanase in the system which converts cyanide into much less toxic thiocyanate. However, it may be noted that thiocyanate is also a toxicant because it acts in a different pattern by disturbing the thyroid function.

Green (1971) described the mechanism of action of thiocyanate in the thyroid tissue. Thiocyanate given to intact animals was proved to be goitrogenic. When iodine intake was low, it could limit iodine accumulation so markedly that it led to decreased levels of circulating hormone thyroxine and therefore to activation of TSH secretion leading ultimately to thyroid enlargement or goitre. A high iodine intake, however, reversed the goitrogenic effect by allowing sufficient iodide to enter by diffusion for normal rates of hormone synthesis to be maintained, despite the presence of transport inhibitors. Furthermore, thiocyante was found to be an inhibitor of organic iodinations at concentrations slightly higher than those which inhibited iodide transport. In addition, thiocyanate appeared to be a competitive substrate for the thyroidal iodide peroxidase (involved in thyroid hormone synthesis), explaining both its biotransformation and its ability to inhibit iodinations.

Dorozynski (1978) also attributed the antithyroid effect of cassava to thiocyanate which inhibited iodide uptake by the thyroid gland.

Tewe and Maner (1978) from their studies on albino rats using fresh and dried cassava-based diets showed that serum thiocyanate concentration and rhodanase activity were consistently higher in growing rats.

Ermans et al. (1980) compared the long-term effects of cassava consumption (10 g/day) with the effects of administration of graded doses of thiocyanate in rats. The results indicated that plasma thiocyanate above a critical threshold level (1 mg/dl) led to rapid urinary elimination of thiocyanate as a result of an efficient renal adaptive mechanism developed during chronic thiocyanate overload. This indicated that circulating thiocyanate concentration was not a quantitative measure of thiocyanate load. However, rapid elimination of thiocyanate was accompanied by severe depletion of iodine stores which also occurred in iodine supplemented rats. The iodine depletion could partly be caused by the enhancement of the renal clearance of iodide. This could be due to competitive action of thiocyanate on renal tubular resorption of iodide with the result that along with thiocyanate iodide ion is also excreted in urine leading to its depletion in the gland stores. This caused abnormalities of iodoaminoacids i.e., increased MIT/DIT ratio, and decreased concentration of T_4. The anomalies induced simultaneously on the metabolism of iodine and thiocyanate after chronic ingestion of cassava were much similar to those produced by chronic loading of the body with well defined amounts of thiocyanate. This confirmed the hypothesis that the antithyroid action of cassava was due to the endogenous production and action of thiocyanate. They concluded that

prolonged consumption of cassava produced modifications of the thiocyanate kinetics in rats. In experimental condition, the essential action of cassava, as that of thiocyanate, was to induce an iodine depletion which could be partially corrected by extra iodine supplementation.

Udupa et al. (1983) observed that goitre was developed in experimental animals given thiocyanate along with food and water. Here thiocyanate did not get concentrated in the thyroid but acted like a competitive inhibitor to the accumulation of iodide. This caused lack of iodide and so there was a decrease in the formation of thyroid hormone which ultimately led to hyperplasia of the gland under the influence of increased secretion of thyroid stimulating hormone (TSH).

Basu et al. (1984) based on their experiment in albino rats concluded that thiocyanate inhibited the iodine transport and enhanced excretion of the accumulated iodide. With the thiocyanate level maintained at a high level (6-8 mg/dl), iodide was excreted mainly through the kidneys leading apparently even to a negative iodine balance.

2.2.3 Iodine deficiency and cassava-based diet

Dorozynski (1978) speculated that continued intake of cassava in man could cause endemic goitre, cretinism and mental retardation in the absence of adequate iodine intake.

Ermans (1980) concluded that iodine deficiency along with ingestion of goitrogenic substances produced not only goitre and abnormal thyroid metabolism but also severe abnormalities in pregnant women particularly during fetal as well as post-natal periods - congenital hypothyroidism in their offspring.

Delange et al. (1982) studied the role of iodine in a cassava-based diet. They demonstrated that in the presence of a cassava-based diet, the development of goitre was critically related to the balance between the dietary supplies of iodine and endogenous production of thiocyanate from cassava. Iodine deficiency in a more frequent diet with extreme ingestion of poorly detoxified cassava containing a very high concentration of cyanide resulted in the development not only of endemic goitre but also of endemic cretinism. It may be noted that following iodine supplement higher than 60 µg/day, goitre was not abnormally prevalent even in the presence of a high thiocyanate supply through cassava consumption. Such a situation probably accounted for the absence of endemic goitre in many populations in the world where cassava constituted a staple food.

Tewe (1982) from his studies on rats and pigs indicated that nutritionally unbalanced cassava diets with specific iodine deficiency, aggravated cassava induced thyroid

toxicity. Such dietary regimen over long periods, reduced serum iodide levels and produced lesions in thyroid with deleterious effects on the overall productivity of animals. He further postulated that protein deficiency could also play an important role in iodine metabolism because it appeared to be involved in the production of tyrosyl derivatives of the thyroid hormones. The role of some essential amino acids, notably tyrosine, in the production of goitrous symptoms in human and animal populations on cassava diets could, therefore, be vital.

Gilbert (1984) noted that cassava consumed extensively in Zaire could be the high risk factor involved in the production of goitre, particularly in those areas where iodine deficiency prevailed. He also opined that whatever be the attributes of cassava as a food crop, they are far out-weighed by the incidence of hypothyroidism resulting from the chronic cyanide toxicity induced by it.

Paulose et al. (1984) from a survey study on the incidence of goitre in Kottayam and Idukki districts of Kerala, suggested that there might be some relationship between cassava consumption and incidence of goitre in these areas, since about 75 per cent people examined were consuming cassava regularly.

2.2.4 Thyroid

Seo et al. (1977) studied the relationship between the body and organ weights in normal female rats. Thyroid weight was found to be 7.0 ± 2.2 mg/100 g body weight. Thus, at an average body weight of 184 ± 4 g, the average thyroid weight was 12.9 ± 4.1 mg.

Annamma Mathew (1979) noted a significant ($P<0.01$) increase in the weight of thyroid of male albino rats fed on a diet containing 85 per cent tapioca and 1 per cent protein. The significant increase in the thyroid weight was attributed to the effect of thiocyante resulting from cyanide detoxification. However, no alteration in the weight of thyroid was observed in the group of rats reared on 59 per cent tapioca and 27 per cent protein containing diet.

2.2.4.1 Hypothyroidism

Ingbar (1971) suggested that altered T_4 concentrations in blood might be resulted from variations in the rate of entry of T_4 into the blood. With few exceptions, this was associated with primary hypothyroidism. Decrease in T_4 concentrations reflected decreased secretion of T_4 by the thyroid. However, in patients with hypothyroidism produced as secondary development to diseases of pituitary gland the severity of the thyroid insufficiency and the extent of

decrease in the levels of serum T_4 were less than those in the usual patients with primary hypothyroidism.

Kochupillai et al. (1973) observed an inverse relationship of the level of circulating thyroxine and a direct relationship of pituitary reserve of TSH with the size of goitre.

According to Ingbar and Woeber (1981) severe thyroid failure was characteristically associated with decreases in both T_3 and T_4 concentrations. But in less severe hypothyroidism the reduction in serum T_3 concentrations was less dramatic than that of T_4. Thus, in mild or moderate hypothyroidism, low levels of serum T_4 might be accompanied by an increased serum TSH concentration. T_3 concentration during this condition might be near normal, normal or even elevated. So it was believed that measurement of serum T_4 concentration may turn out to be an aid in the diagnosis of hypothyroidism.

Soto et al. (1981) suggested that clinical hypothyroidism might be manifested in some patients even when their serum (total and free) T_3 was clearly in the normal range and serum T_4 was subnormal. Serum TSH in inhabitants of endemic goitre regions was negatively correlated with serum T_4 and not at all with T_3. Therefore, it was believed that serum T_4 and TSH determinations were much more important than the

measurement of T_3 in diagnosis of clinical hypothyroidism (Chopra, 1981; Kourdies, 1981 and Larsen, 1981).

2.2.4.1.1 Effect of hypothyroidism on growth hormone secretion

Growth hormone concentration was observed to be reduced in subjects with hypothyroidism (Schooley, 1966).

Porterfield and Hendrich (1976) suggested that the impairment of fetal metabolism occurring in maternal hypothyroidism might be due, in part, to insufficient maternal growth hormone secretion. However, maternal treatment with growth hormone alone in the absence of sufficient thyroid hormone did not totally correct all of the observed fetal abnormalities.

Coiro et al. (1979) studied growth hormone (GH) levels in the serum of thyroidectomized rats using RIA and biological criteria simultaneously. The data suggested that after thyroidectomy plasma concentration of GH could be maintained at normal or near normal level until pituitary reserves were depleted. Growth virtually ceased even when serum levels of GH were still in the normal range, probably from lack of the synergistic effects of T_4 whose levels declined rapidly and reached barely detectable levels by the 4th day of thyroidectomy. Deficiency of T_4 (thyroxine) reduced the

pituitary concentrations of GH slowly but became barely detectable by the 14th post operative day. Serum GH concentrations were also markedly reduced but remained well within the detectable limits. This confirmed that thyroidectomy decreased the amount of GH in the pituitary and serum of rats and that the synergistic effects of T_4 was of utmost importance in sustaining growth.

Burstein et al. (1979) recorded considerable loss in body weight in hypothyroidism induced in rats by propylthiouracil. A significant drop in the growth hormone level was described as the cause for the loss of body weight.

2.2.4.1.2 Metabolic alterations in hypothyroidism

Metzger and Freinkel (1971) observed that in most instances, the turnover rates of metabolites were slowed, and final "steady-state" levels were dependent upon the relative preponderance of the reduced anabolism over diminished catabolism during the development of hypothyroidism and the establishment of a new equilibrium. Thus, the net metabolism favoured accumulation of fat in hypothyroidism while the accumulation of protein was compromised. Appetite was usually reduced in proportion to metabolism.

Udupa et al. (1983) noticed that the decrease in the concentration of circulating thyroid hormones produced

profound metabolic changes in the body. It caused decrease of both anabolism and catabolism of protein, fat and carbohydrates, the decrease in rate of catabolism was more, leading to greater accumulation of fats in the body.

2.2.4.1.2.1 Alterations in protein metabolism

Crispel and Wilson (1964) observed reduction in both anabolism and catabolism of protein in hypothyroidism in man and found that the reduction in catabolism was more pronounced.

Metzger and Freinkel (1971) concluded that the retardation in growth in hypothyroid children was due more to the profound impairment of synthesis of new protein rather than their breakdown. This was also linked to the deficiency of growth hormone secretion which was considered as the pre-requisite principle for the formation of new protein and therefore growth.

Udupa et al. (1983) observed that in hypothyroidism, there was usually a positive nitrogen balance. The main reason for this was the marked decrease in the protein catabolism which indirectly influenced the anabolic processes. The ultimate result was decrease in the turnover of proteins.

2.2.4.1.2.2 Alterations in carbohydrate metabolism

Ensinck and Williams (1981) concluded that the cause of hypoglycemia in thyroid hormone deficient subjects was probably multifactorial in origin, involving a reduction in delivery of gluconeogenic precursors like lactate with a defect in their conversion to glucose in the liver at the instance of modified activity of pyruvate carboxylase.

Udupa et al. (1983) stated that the utilization of carbohydrate might be decreased in the absence of thyroid hormone which normally augments the action of insulin and epinephrine.

2.2.4.1.2.2.1 Effect of cyanide on blood glucose

Annamma Mathew (1979) found no alterations in blood glucose levels of male albino rats maintained on diets containing either 27 per cent protein with 59 per cent tapioca or 1 per cent protein with 85 per cent tapioca. The cyanide content of tapioca was 120 µg cyanide/g tuber. The study suggested that most of the cyanide present in the diets was efficiently detoxified and excreted by the body. The quantity of the cyanide escaped detoxification was stated to be insufficient to produce hyperglycemia.

McMillan and Geevarghese (1979) examined the effect of cyanide on blood glucose concentration in rats exposed chronically to 12 mM potassium cyanide in drinking water. Mild hyperglycemia was observed after initial exposure to cyanide-containing drinking water. Blood glucose concentration above 200 mg/dl occurred occasionally, but hyperglycemia failed to persist in any of the animals during later part of the study. In another trial, rapid onset of marked hyperglycemia was seen to persist for more than an hour after intraperitoneal injection of 15 μM potassium cyanide per kg. The failure of persistent hyperglycemia was due to the reason that rats were capable of surviving after regular ingestion of substantial amounts of cyanide by continuous neutralization of atleast 20 times a lethal amount day after day. Despite evidence of recurrent hyperglycemia during chronic ingestion of cyanide, rats did not develop diabetes. Age and species differences in the susceptibility of the pancreatic islets to hyperglycemic damage was said to account for this failure.

2.2.4.1.2.3 Alterations in lipid metabolism

Ingbar and Woeber (1981) described the effects of hypothyroidism on lipid metabolism. According to them thyroid homones appeared to affect virtually all aspects of lipid metabolism, including synthesis, mobilization and degradation.

But degradation was affected more than synthesis, the net effect of hormone deficiency being an increase in the stores of most lipids with their high concentrations in plasma. This was true for triglycerides, phospholipids and cholesterol.

2.2.4.1.2.3.1 Alterations in cholesterol

Hellman et al. (1959) had pointed out that, in hypothyroidism, adrenal cortex secreted proportionately more etiocholanolone and less androsterone than normal. This realignment of steroidogenesis might make some contribution to the hyper-cholesterolemia of myxedema since plasma cholesterol levels could be lowered by the administration of large doses of androsterone.

Meittinen (1968) suggested that hypercholesterolemia of myxedema might be ascribed to a period in which the reduction of cholesterol breakdown was greater than the diminution in cholesterol synthesis.

Sobel and Braunwald (1971) indicated that hypothyroidism was usually associated with hypercholesterolemia and other aberrations in lipid metabolism. Since the synthesis of cholesterol was reduced, the net increase in serum cholesterol appeared to reflect the markedly diminished rate of catabolism and excretion of bile acid.

Mason and Wilkinson (1973) pointed out that determination of serum cholesterol was a valuable index in the diagnosis of variations in thyroid function since synthesis of cholesterol was inversely proportional to the thyroid activity.

According to Ingbar and Woeber (1981) one of the classic effects of thyroid hormones was to lower the concentration of cholesterol in plasma. Synthesis of cholesterol was enhanced at the stage of conversion of B-hydroxy-β-methyl glutaryl-coenzyme A to mevalonate, probably by increasing the activity of the enzyme concerned. Thyroid hormone action on the elimination of cholesterol was influenced by an increase in both the fecal excretion of cholesterol and its conversion to bile acids. All these are reversed in hypothyroidism.

2.2.4.1.2.3.2 Alterations in total lipids

Koppers and Palumbo (1972) observed that a large series of patients with myxedema were hyperlipedemic.

Bierman and Glomset (1981) found that excessive lipid accumulation in plasma could result from either a defective removal of it from plasma or their excessive endogenous production or both. These abnormalities may originate as

primary conditions or may occur as a secondary result of endocrine disorders like diabetes or hypothyroidism.

Loireau et al. (1987) reported that serum concentrations of total lipids were higher in both lean and obese Zucker rats after thyroidectomy. The finding suggested that hypothyroidism could partly be responsible for the altered lipid metabolism.

2.2.4.1.2.4 Alterations in haemoglobin

Muldowney et al. (1957) observed that the deficiency of thyroid hormone chiefly lowered the red cell and haemoglobin ceiling which might be due to slower regeneration of red cells and haemoglobin.

Donati et al. (1965) also suggested that the red blood cell production might be augmented by thyroid hormone in addition to its calorigenic effect.

Reddy (1982) observed hypochromic anaemia due to reduction in erythrocyte count, haemoglobin level and packed cell volume as a result of decreased erythropoietin level in hypothyroidism induced in kids.

2.2.5 Nucleic acids

Since Bovin et al. (1948) established that there was a

constant average amount of deoxyribonucleic acid (DNA) per nucleus for any animal tissues, amount of DNA had become a useful standard of reference for revealing the changes in cell number and cell composition

Because DNA was located in the nucleus and was present in constant amount in each diploid cell Chargaff and Davidson (1955) postulated that the concentration of DNA-P per unit of fresh weight could be high in tissues with little cytoplasm and extra cellular material and low when the proportion of chromosomal material in the cells was reduced. The tissues which were biologically active in synthesis of components, mechanical effects, or nervous activity all had low concentrations of DNA-P (50 ug or less) and relatively large amounts of cytoplasm eg., pancreas of rats which contained 48 ug DNA/100 mg tissue whereas brain and skeletal muscle contained only 10 ug or less

Means et al. (1963) showed that ribonucleic acid (RNA) of the thyroid varied directly with the activity of the gland but the DNA varied much less. This was because the RNA was cytoplasmic in location and was related to synthesis of protein and nucleo-protein, whereas the DNA which remained intra nuclear, occurred in a uniform amount in each cell nucleus and was therefore, directly related to cell population.

Spiegel et al. (1993ab) designed experiments to study the effects of dietary rapeseed presscake meal (15%) on thyroid activity in pigs. Thyroid wet weights were five to six times higher in groups fed rapeseed presscake meal (RPM) than in controls. DNA per gram of dry matter and DNA per total thyroid were 1.5 to 1.7 and 4 to 10 times higher respectively in pigs fed RPM compared to controls.

2.2.6 Effect of cassava and protein malnutrition on performance of rats

Lenzen et al. (1976) demonstrated that hypothyroidism was characterized by reduced body weight gain in male albino Wistar rats.

Annamma Mathew (1979) observed a pronounced weight loss in a group of male albino rats fed a diet containing 85 per cent tapioca (120 µg cyanide/g tuber) and 1 per cent protein. The major factor contributed to the reduction in body weight in this group of rats was loss of appetite caused by protein deficiency. Moreover, the body weight gain in the group maintained on higher levels of protein (27%) and cyanide containing tapioca (59%) was also significantly lower than the control group. The pronounced body weight loss in protein deficient tapioca diet and a significantly less weight gain in

protein sufficient tapioca diet was probably due to the growth inhibiting factor, cyanide present in the diets.

Tewe and Maner (1980, 1981) observed poor performance of albino rats maintained on cyanide containing rations. The feed consumption of albino rats in the experimental groups was found to be inversely proportional to the dietary level of cyanide.

Gomez (1982) fed balanced diets to growing rats throughout a 28-day experimental period. The diet consisted of cassava meal (40-42%), soyabean meal (37-39%), cellulose (5%), corn oil (10%) and mineral-vitamin premixes (5%) supplying 20 per cent crude protein. The cassava meals used were based on two varieties containing 30, 16, and 182, 122 mg cyanide per kg dry matter under solar and artificial drying system respectively for each variety. The results of the study indicated that growth rate and feed consumption were similar for both varieties tested. The difference in the cyanide levels did not seem to be significantly affecting the performance of rats.

Tewe (1984) studied the effect of four cassava-based diets varying in cyanide content (0, 597, 150 and 110 mg/kg), in African giant rats in a 16-week trial. Weight gain, feed conversion ratio and protein efficiency were poorer with

increasing dietary level of cyanide. The reduction in the feed efficiency and protein efficiency ratio was attributed partly to the utilization of sulfur containing amino acids for detoxification of cyanide and calling-forth additional supplementation of such diets with adequate sulfur sources.

2.2.7 Cardiac anamolies

There are no concrete evidences pertaining to cardiac anomalies due to cassava ingestion. However, cardiovascular manifestations in association with thyroid hypofunction have been reported.

Douglas and Jacobson (1957) showed that cardiovascular manifestations reflected to some extent a direct deleterious effect of thyroid hormone deficiency on the heart.

Sobel and Braunwald (1971) also suggested that the cardiovascular manifestations of hypothyroidism might be due to the direct effects of thyroid deficiency on the heart or due to indirect effects of reduced oxidative metabolism in tissue leading to depressed protein synthesis resulting in depressed myocardial contractility.

Recently Shenoy et al. (1993) postulated that chronic cyanide toxicity due to consumption of cassava without adequate intake of protein could lead to myocardial injury

(endomyocardial fibrosis) among young people in Kerala and claimed that biochemical and histomorphological evidence to support the hypothesis were found in experiments they had conducted on rabbits. In group of rabbits fed on low protein cassava diet, they observed impairment of cytochrome oxidase, the enzyme connected with oxygen metabolism leading to impairment of energetics of the myocardial tissue, damage of the hypoxic tissue and occurrence of fibrosis. It was also pointed out that higher intake of proteins could prove to be an effective method to counter the ill-effects of cassava toxicity.

2.2.8 Diabetogenic effect of cassava

Clinical observations suggested an association between pancreatic damage and consumption of cassava (Pitchumoni, 1973).

McMillan and Geevarghese (1979) analysed the geographic distribution of malnutrition diabetes and conducted animal experiments to elucidate the effect of cyanide and malnutrition on this particular type of diabetes. Both the above studies supported the concept that protein-calorie malnutrition, combined with ingestion of cyanide or a cyanide precursor like cassava might cause juvenile diabetes due both to exocrine and endocrine damage to the pancreas. This was

found to be the situation in Kerala. The explanation put forward was that during feeding with the cassava-based diet thiocyanate was produced by cyanide detoxification at the cost of sulfur containing amino acids. Therefore, they assumed that in case of protein malnutrition thiocyanate could not be formed and the cyanide which had not been detoxified due to non-availability of sulfur containing amino acids might be capable of producing pancreatic damage and diabetes. They further noted that goitre and diabetes never coexisted in the same patient in Kerala but they were found together in Eastern Zaire.

Epidemiological data in support of cassava acting as a causative agent for the production of tropical calcifying pancreatitis (TCP) showed that this disease was most prevalent in countries where cassava formed a staple food and where the incidence of cassava induced endemic goitre was more common (Geevarghese, 1982).

Geevarghese (1986) investigated the dietary factors in cassava that could produce pancreatic injury in subjects with protein deficiency. It was assumed that cassava-based diets would only aggravate the protein deficiency further because sulfur containing amino acids (methionine and cysteine) already deficient in the diet are required for detoxification of cyanide. He suggested that protein deficiency in early

child hood would have predisposed the pancreas to toxins like HCN in cassava. As a result diabetes makes its incidence felt in adolescence, a strongly protein-anabolic period. He pointed out in support of this fact that in some parts of Uganda, where the staple food was low-protein-high cyanogenic glycoside cassava, the disease was more common than in Kenya where the staple food consists mainly of maize.

On the other hand, Teuscher et al. (1987) surveyed the incidence of diabetes in West African villages and suggested that a high cassava intake (84% of a mean daily supply of 1916 calories) combined with a low protein consumption (8% of caloric supply) did not cause diabetes. This finding did not support, as far as West African rural population is concerned, the World Health Organization hypothesis that malnutrition related diabetes existed.

Swai et al. (1992) also believed that a high dietary cyanide exposure due to cassava consumption was not a major etiological factor in the production of fibrocalculous pancreatic diabetes or protein-deficient pancreatic diabetes, atleast in an undernourished population as that seen in Tanzania in East Africa.

2.2.9 Insulin secretion

Elgee and Williams (1955) and Cohen (1957) found that slower degradation of insulin in hypothyroid rats could also reduce the demands for insulin by prolonging its biological persistence.

Cohn et al. (1968) suggested that the requirement for insulin might be reduced in hypothyroidism. Since secretion of insulin was proportional to the calories ingested, the diminution might simply reflect the concurrent hypophagia that occurs as a consequence to hypothyroidism.

Similar were the views of Metzger and Freinkel (1971) who reported that some reduction in the requirement for insulin was likely to occur in diabetes occurring alongwith hypothyroidism.

Lenzen et al. (1976) demonstrated in rats that the amount of insulin available for immediate release from the pancreas was apparently dependent on the glucose level in plasma. This very close relationship that existed between plasma glucose level and insulin secretion indicated that pancreatic beta cells were very sensitive to the variations in actual plasma glucose concentrations.

2.2.10 Histopathological alterations

2.2.10.1 Pancreas

Annamma Mathew (1979) reported considerable reduction in the size of the acini and the total cell mass. Granularity of the acinar cells of pancreas was also reduced remarkably in male albino rats. These rats were fed on a diet made up of 85 per cent tapioca with a cyanide content of 120 µg/g tuber and a mere 1 per cent protein for 2 months. The islets and stroma of pancreas of these rats showed no change at all. The changes observed were typical of protein depletion occurred due to tapioca in the diet.

Experimental evidence in support of cassava-based diet as a cause of tropical calcifying pancreatitis (TCP) was provided by Pushpa (1980). The rats were maintained for 18 months on a diet made up of 22.8 per cent cassava having 73 µg cyanide/g tuber. Pancreatic changes such as dilated ductules, papillary infoldings, eosinophilic material in ductular lumen and round cell infiltration were observed. These changes were similar to those seen in early stages of TCP.

Similarly Geevarghese (1982) also noticed that cassava-based diets were related to the development of tropical calcifying pancreatitis (TCP) and pancreatic diabetes (PD). Histopathological examination revealed dilatation of

pancreatic ducts and ductules containing proteinaceous calcified material, acinar atrophy and islet cell destruction in the pancreas.

Takama and Kishino (1985) maintained two groups of rats with 20 and 5 per cent casein in their diets. It was found that the pancreatic structure was well preserved with swollen acinar cells rich in zymogen granules and distinct perinuclear basophilia in rats fed on 20 per cent casein diet. On the other hand, the acinar cells were essentially small (atrophied) and had small amounts of zymogen granules. There was considerable reduction of perinuclear basophilia, but no nuclear change was evident in all the rats of the 5 per cent casein group. They suggested that a protein deficient diet might induce changes in the structure and stability of cellular membranes and also in protein metabolism in the endoplasmic reticulum. This could easily potentiate cellular structure to the toxic action of chemicals.

2.2.10.2 Liver

Tasker (1962) reported moderate degree of parenchymal changes and fatty infiltration in the liver of rats fed on a maize-tapioca diet.

Rao (1964) reported that rats fed on tapioca diet

showed moderate degree of cytoplasmic vacuolation with periportal fatty infiltration.

Ericsson et al. (1966) studied the livers of dogs maintained on a protein-deficient diet for 4 weeks. The study indicated that abnormal accumulations of glycogen occur in hepatic parenchymal cells as a result of protein deficiency.

Geevarghese (1968) postulated that a toxic factor present in tapioca could cause cirrhosis of liver.

Keele and Neil (1971) stated that a high liver glycogen content (Glycogen infiltration) might favour detoxification and protect the liver against the toxic effects of many poisons.

Annamma Mathew (1979) observed irregularly shaped, ragged empty spaces in the cytoplasm of liver cells in most of the rats of all groups fed with or without tapioca irrespective of the levels of protein in their diets. But these cytoplasmic vacuolation were more prominent in rats fed on higher levels of tapioca (85%) having 120 µg cyanide/g tuber and a meagre amount of protein (1%). It was indicated that the glycogen which was not stained with hematoxylin and eosin appeared as translucent deposits in the form of empty spaces in the cytoplasm (Glycogen infiltration). The glycogen infiltration was suggested to be an adaptive response of the

liver to tone up the mechanisms involved in detoxification of cyanide in the absence of adequate protein. It was further indicated that the rats fed with tapioca and adequate amounts of protein did not show marked vacuolar changes in the liver.

2.2.10.3 Heart

Microscopic changes in the myocardium itself were commonly found in both experimental and spontaneous myxoedema. The changes observed were interstitial edema, basophilic degeneration, myofibrilar swelling, loss of striations and fibrosis (Douglas and Jacobson, 1957).

2.3 Effect of cyanogenic glycosides in ruminants

Reports on feeding of tapioca and its associated effects in goats are conspicuously lacking from the available literature. The indirect evidences available on feeding of cyanogenic glycoside-containing rations in ruminants have also been largely controversial.

Flux et al. (1956) found no clear evidence for goitrogenic effect of feeding cyanogenic glycosides to sheep. On the contrary, Nestel (1973), Clarke and Clarke (1975), Kingsbury (1975) and Allison (1978) suggested that breakdown of cyanogenic substances was more probable in ruminants than in non-ruminants. Under the action of rumen micro-organisms

cyanide was believed to be liberated rapidly subject to the availability of suitable conditions in the rumen. At the same time ruminants were found to have a higher tolerance to HCN than the other mammals tested.

Blood et al. (1979) suggested that cyanides ingested in small amounts might be important in the production of clinical goitre in lambs when intakes of iodine was marginal.

Okeke and Oji (1987) evaluated the nutritive value of an ensiled diet (containing grass, cassava peel and poultry excreta in the ratio of 60:20:20) containing 52.5 mg cyanide/kg against the maize silage in West African dwarf bucks. Both the diets were isonitrogenous (14% CP). There was no significant difference in the dry matter intake on the two diets. The blood glucose levels determined at 0, 1, 2, 4 and 8 h after feeding grass ensiled diet were 19.1, 28.5, 32.5, 41.3 and 41.8 mg/dl as against 21.1, 27.4, 33.4, 38.8 and 41.4 mg/dl in bucks given maize silage. The values were not found to be affected by dietary treatment. The post prandial rise in blood glucose was attributed to early increase in the propionic acid at the expense of acetic acid, due to the availability of readily fermentable carbohydrates in both the diets.

Aregheore (1992) maintained two groups of West Africant crossbred ram lambs on isonitrogenous diets containing 40 per cent cassava peel with 7.5 per cent palm kernel meal or 1.65 per cent urea as the protein source. Average daily weight gain and feed conversion efficiency were not different between diets.

2.3.1 Serum thyroxine concentration in goats

Rajan (1989) studied the incidence and nature of hypothyroidism in goats. Among the goats (71) screened 15.4 per cent had low thyroxine levels (below 3.4 µg/dl). The observed values ranged from 2.4 to 6.2 µg/dl.

2.3.2 Insulin secretion in ruminants

Bassett (1975) observed that insulin remained at the centre of metabolic regulation in ruminants, as in other species. Although the blood glucose concentration was usually considered to be the principal metabolic substrate regulating insulin release by the beta cells of most animal species, the plasma glucose concentration itself might not be a major determinant of insulin secretion in sheep under physiological conditions. Other metabolites, such as volatile fatty acids, had been suggested to be effective stimulants for insulin secretion in ruminants in which volatile fatty acids were

produced as end products of rumen fermentation (Sasaki et al., 1977; Sasaki et al., 1984).

Barry and Manley (1985) determined the plasma concentration of insulin on 4 days during a week in two groups of castrated Romney Sheep. The sheep fed on chopped forms of Kale and supplemented with iodine to counteract Kale goitrogens had 15.2 μU/ml as against 15.9 μU/ml insulin in the sheep fed on ryegrass clover diet.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Determination of cyanogenic glycoside content of fresh and processed tapioca of different varieties

3.1.1 Sampling

In the present study tubers of 'Karkidakkan' 'H-165', 'M-4', 'Sree Sahya' (H-2304), 'Sree Visakh' (H-1687), 'Sree Prakash' (S-856), and 'Thottakolly' varieties of tapioca were used for the determination of cyanogenic glycoside (CNG) content. Fresh tubers of the variety 'M-4' were procured from Horticulture College, Kerala Agricultural University, Vellanikkara (Trichur). Tubers of the variety 'Karkidakkan' were obtained by courtesy of Mr. Ibrahim of Modhapallur (Trichur) while tubers of other varieties were obtained by courtesy of late Dr. Sadanandan, Ashtamichra (Trichur). Tubers of the same age (10-12 months of cultivation) and size were plucked from a particular variety grown at different locations of the same plot.

3.1.2 Processing of cassava

Owing to the difference in having higher CNG content in the rind and comparatively lower content of the same in the

cortex, the rind was peeled off and samples were taken from the neck (end attached to the stem), tip and the mid portion of tuber cortex. They were sliced transversally into pieces of 2-3 mm thickness. Each slice was then cut radially into small pieces 6-8 mm and mixed thoroughly. Representative samples of 10 g each were used immediately, one for extraction of CNG as such, another after boiling in 50 ml of water for 30 min and a third sample after drying in the sun for 24 h.

3.1.3 Extraction of cyanogenic glycosides

Each sample of tapioca was separately extracted for 10 min twice with 25 ml boiling ethanol (80%) under reflex. The two extracts obtained from the same sample were pooled and centrifuged for 10 min at 4000 G. The volume of pooled supernatant was made up to 50 ml with 80 per cent ethanol.

3.1.4 Preparation of linamarase

Linamarase was prepared from tapioca rind, by the method of Cooke *et al*. (1978), as this portion contained highest concentration of the enzyme.

3.1.5 Determination of CNG in extracts

CNG content in fresh, boiled and sun dried samples of tapioca in terms of cyanide (HCN) was estimated by the spectrophotometric method of Nambisan and Sundaresan (1984).

3.1.6 Statistical analysis

Data were subjected to analysis of variance test (Snedecor and Cochran, 1967).

3.2 Experiment in rats

A total of sixty male albino Wistar rats, aged 30-40 d, were randomly selected from a lot maintained at Small Animal Breeding Station, Mannuthy. The rats were divided into six groups of 10 each, almost identical in weight (average weight 44 g). The rats were kept individually in (40x22x23 cm) galvanized iron cages.

3.2.1 Experimental diets

During the 12 weeks trial, the rats in six groups received the following diets.

Group No.	Diet
I	Protein = 15%; Tapioca = Nil; Iodine = Nil
II	Protein = 15%; Tapioca = 30%; Iodine = Nil
III	Protein = 7.5%; Tapioca = 30%; Iodine = Nil
IV	Protein = 7.5%; Tapioca without HCN = 30%; Iodine = Nil
V	Protein = 15%; Tapioca = 30%; Iodine = 0.17 mg/kg
VI	Protein = 22.5%; Tapioca = 30%; Iodine = 0.17 mg/kg

The composition of experimental diets is shown in Table 1. Crude protein and dry matter content of the diet is shown in Table 2. The diets were made isocaloric with groundnut oil throughout the experimental period. The diets were fed from the first day of the experiment until they were sacrificed at 10th, 11th and 12th week of the experiment. Tapioca tubers of bitter variety (H-165) were used throughout the study as one of the ingredient for diets II, III, V and VI.

Tapioca tubers were procured at weekly intervals and stored at refrigerated temperature until they were used. After hydrolysis their cyanide (HCN) content was found to be 182.6 µg/g fresh tuber (Table 4) with a dry matter content of 33 to 40 per cent.

The tapioca flour mixed with diet IV was prepared from non bitter variety (M-4). For this the tapioca tubers were cleaned, peeled and cut into pieces. These pieces were then soaked in water for 2 h with the water changing, three times. This was done to solubilize as much cyanogenic glycosides as possible. The tapioca pieces were then crushed and dried under sun for 24 h. This technique brought about complete rupture of cells so that maximum contact between endogenous enzyme and cyanogenic glycosides (CNG) could be established which in turn efficiently hydrolysed the maximum amount of

Table 1. Experimental diets fed to rats

Ingredients	Diet (%)					
	I	II	III	IV	V	VI
Maize	25	14	28	28	14	4
Black gram	11	15	5	5	15	5
Groundnut cake	-	-	-	-	-	41
Gingelly cake	14	25	-	-	25	-
Wheat flour	23	8	30	30	8	-
Wheat bran	25	6	5	5	6	18
Tapioca flour (HCN free)	-	-	-	30	-	-
Tapioca tuber[1]	-	30	30	-	30	30
Mineral & vitamin mix[2]	2	2	2	2	2	2
Iodine (mg/kg)	-	-	-	-	0.17	0.17
Total ME (Kcal/kg)	3600	3600	3600	3600	3600	3600

1. Considering the dry matter content of tapioca tuber as 33-40%, the proportion of tapioca incorporated in diets for Groups II, III, V and VI was made upto 30% on dry matter basis

2. Mineral and vitamin mixture added to the diets was prepared in the laboratory by using analar grade chemicals

Table 2. Dry matter and crude protein content (%) of experimental diets fed to rats

Diet	DM	CP
I	90.18	15.30
II	90.84	15.43
III	90.03	7.65
IV	90.03	7.65
V	90.84	15.43
VI	90.68	22.90

CNG. Removal of 96-99 per cent CNG was reported to be achieved by crushing and sun drying procedure (Nambisan and Sundaresan, 1991). Sun dried tapioca was dried again at 50°C in an oven for 12 h and was then milled before incorporation into the diet of Group IV. The cyanide content of this tapioca diet was found to be negligible (1.35 ug/g).

3.2.2 Experimental procedure

3.2.2.1 Feeding technique

Tapioca tubers of bitter variety (H-165) were peeled, cut into small pieces and fed fresh to Groups II, III, V and VI, in the morning, throughout the period of the study. The quantity of tapioca fed to these groups was according to the body weight (considering the feed consumption as 10 per cent of body weight) and depending upon the required proportion of tapioca to be mixed with each of these experimental diets. After major portion of tapioca was consumed, the feeding trough containing the mixed feed was placed in the cages. Water was provided ad libitum to each rat.

3.2.2.2 Feed consumption (on dry matter basis)

The quantity of feed consumed by each rat in each group was calculated on dry matter basis from the quantity provided and that left over at weekly intervals. This was

done by weighing the mixed feed left by each rat in each group at every week end. Similarly, the cassava chips (left over, if any) were collected, dried and weighed individually and separately for the groups fed fresh tapioca tubers (II, III, V and VI). The quantity of tapioca thus consumed was also calculated on dry matter basis. Total feed consumed by each rat in each group till it was sacrificed, was calculated from weekly feed consumption.

3.2.2.3 Body growth by weight gain

For the purpose of assessing growth trend of six groups of rats, periodical changes in their body weight were studied. Body weight of rats were recorded at the beginning of the experiment and subsequently at weekly intervals until they were sacrificed at 10th, 11th and 12th week of the experiment. The rats were weighed on an 'Avery' counter scale of one gram sensitivity. Total body weight gain was calculated from final and initial body weight of rats in each group.

3.2.2.4 Digestibility of diets

The digestibility coefficient of total dry matter (DM) was determined using the quantitative faecal collection method of Crampton and Llyod (1959). After a period of seven weeks from the beginning of the experimental feeding, faecal

collections were carried out over a period of 24 h in each individual case of five rats in each group. The coefficient of digestibility of dry matter was calculated from values of feed intake and faecal excretion.

3.2.2.5 Slaughtering technique and blood collection

At the tenth and eleventh week of the experiment three rats and at the twelfth week, four rats from each group were sacrificed by exsanguination through cardiac puncture under diethylether anaesthesia. Cardiac puncture was done by hypodermic needle (23 gauge) and blood was drawn into a dry glass syringe (10 ml). The blood samples were immediately transferred to clean, dry and sterilized tubes, and kept in slanting position. It was allowed to stand for 1 h at room temperature and then kept for 4-6 h under refrigerated temperature for separation of serum. The sera were separated by centrifugation in a refrigerated centrifuge maintained at 4°C and centrifuged for 20 min at 3000 G. The clear samples of sera were then divided into aliquots of 0.5-1.0 ml and stored in polypropylene storage vials at -20°C until the analyses for hormones and other metabolites were carried out. About 0.5 ml samples of blood were also collected from each rat into small glass vials on sodium flouride-oxalate mixture for determination of blood glucose and haemoglobin concentrations.

3.2.2.6 Tissue analyses

3.2.2.6.1 Tissue collection

Immediately after sacrifice, the pancreas and thyroid were excised devoid of all loose fat and fascia, rinsed in ice cold normal saline, blotted dry and weighed. A small portion of pancreas was dissected out and fixed in 10 per cent formal saline for histopathological studies, and the remaining portion of pancreas was weighed again. Individual pancreas and thyroid tissues were wrapped in aluminium foil and kept at freezing temperature for determination of protein and DNA content.

3.2.2.6.2 Homogenization

Frozen samples of weighed thyroid tissue were pooled and homogenized in a glass homogenizer. The homogenate was suspended in 0.5 ml cold saline and transferred to a centrifuge tube. The homogenizer was rinsed twice with 0.5 ml cold saline and the contents were transferred to the same centrifuge tube. Similarly, the frozen pancreatic tissue was also homogenized. The centrifuge tubes containing thyroid and pancreatic homogenate were capped and kept at -20°C and analysed for DNA and protein contents as indicators of cell number, density and size of the gland.

3.2.2.6.3 DNA and proteins

DNA and proteins were purified by removing acid soluble compounds and lipids from the homogenized samples according to the method of Schneider (1957).

3.2.2.6.4 DNA extraction

DNA was extracted twice with 5 per cent trichloroacetic acid at 90°C for 15 min (Schneider, 1957). The combined supernatants were used to quantify DNA.

3.2.2.6.5 DNA determination

DNA was estimated by the method of Burton (1956).

3.2.2.6.6 Protein determination

After the extraction of DNA, the precipitate remained in the centrifuge tube was suspended in 0.5 ml distilled water and the protein contents were determined according to the method of Lowry et al. (1951).

3.2.3 Histopathological studies

The liver, heart and pancreatic tissues were dissected out and fixed in 10 per cent formal saline immediately after sacrifice. The tissues were processed by routine paraffin embedding technique (Armed Forces Institute of Pathology,

3.2.4 Blood analyses

3.2.4.1 Haemoglobin

Haemoglobin concentration in blood was determined by the cyanmethemoglobin method (Miale, 1967).

3.2.4.2 Blood glucose

Blood glucose levels were determined by the method of Asatoor and King as detailed by King and Wootton (1959).

3.2.4.3 Serum total protein

Serum total protein concentration was determined by the method of Lowry et al. (1951).

3.2.4.4 Serum total cholesterol

Serum total cholesterol was estimated by the method of Zak (1957).

3.2.4.5 Serum total lipids

Serum total lipid concentration was determined by the phosphovanillin method as adapted by Span Diagnostics Pvt. Ltd. (Udhana), India (Kit No.25926).

3.2.4.6 Serum insulin

Serum insulin was measured by double antibody RIA technique using an antiserum that was raised in guinea pig against porcine insulin. Radioimmunoassay Kit (Code No.RIA K1) were obtained from Board of Radiation and Isotope Technology (BRIT), Bombay. Porcine insulin was used as the reference preparation. The standard curve was linear between 7.5-200 μU/ml reference preparation (insulin standard). The sensitivity of the assay was 2.25 μU/ml on 98% B/B_0 intercept. Sample values were determined by interpolation on the standard curve (prepared on the logit-log paper by plotting % B/B_0 on logit scale against concentration of insulin in μU/ml on a log scale).

3.2.4.7 serum thyroxin (T_4)

Radioimmunoassay of T_4 was performed by using RIA kit (Code No. RIA k5) supplied by BRIT, Bombay. The sensitivity of this assay was 0.5 μg/dl serum on 90% B/B_0 intercept. The standard curve was linear between 2.5-20 ng/ml reference preparation (T_4 standard). T_4 concentration of the sample was expressed in μg/dl by interpolating the value (% B/Bo) from the standard curve.

3.2.5 Statistical analysis

Analysis of variance procedures (Snedecor and Cochran, 1967) were used for evaluating differences between treatments.

3.3 Experiment with kids

Thirty alpine-Malabari crossbred kids of both sexes in the age group of 2½ to 3 months were selected at random from Kerala Agricultural University goat farm and maintained on standard farm conditions. The kids were dewormed before the beginning of the experiment. These kids were divided into three groups (Groups I, II and III) of ten (5 males and 5 females) kids each as uniformly as possible with regard to age and weight.

3.3.1 Experimental diets

During the three month trial, kids in the three groups received the following diets:

Group No.	Diet
I	Protein = 15%, Tapioca = Nil, Iodine = Nil
II	Protein = 15%, Tapioca = 30%, Iodine = 2 mg/kg
III	Protein = 25%, Tapioca = 30%, Iodine = 2 mg/kg

The composition of these three experimental diets is given in Table 3. Tapioca tubers of bitter variety

Table 3. Experimental diets (concentrate mixture) fed to kids

Ingredients	Diet (%)		
	I	II	III
Maize	29.5	8	5
Black gram	16	20	10
Gingelly cake	6	10	35
Casein	1	3	10
Wheat bran	45	26.5	5.5
Tapioca tuber	-	30	30
Fish meal	-	-	2
Mineral mix	2	2	2
Salt	0.5	0.5	0.5
Iodine (mg/kg)	-	2	2
Nutrient			
Dry matter	89.95	89.53	90.18
Crude protein	15.40	15.38	24.72
Calculated TDN (%)	69.15	69.59	71.08

'Karkidakkan' were used throughout the study as one of the ingredient incorporated in the diets for Groups II and III. Tapioca whole tubers were procured at weekly intervals and stored in pits until they were used. The cyanide (HCN) content of tapioca tubers used was 186.3 mg/kg fresh tuber with a dry matter content of 38.9 to 40.5 per cent.

3.3.2 Experimental procedure

3.3.2.1 Feeding technique

The diets were fed from the first day till the end of the experiment (3 months). All the kids were fed individually in separate feeders. The kids in all the three groups were offered concentrate mixture in such a way that it formed about 50 per cent of the total dry matter intake. Remaining 50 per cent of the total dry matter was provided through roughage. For this purpose hybrid Napier grass was offered to the kids. Total dry matter intake was considered to be 4.25 per cent of the body weight (ICAR, 1985). Initially the kids in all the three groups were given 150 g of the concentrate mixture. After every fortnight, the concentrate allowance was increased taking into consideration the increased nutrient needs of the kids commensurate with advancing growth. The tapioca incorporated in the diet of Groups II and III was fed fresh throughout the study in such a way that it formed about 30 per

cent of the total dry matter intake (concentrate and roughage together). Clean drinking water was provided ad libitum to each kid.

3.3.2.2 Feed consumption (on dry matter basis)

Individual records of daily feed intake and left over were maintained. The quantity of feed consumed was calculated from the quantity of feed (concentrate, tapioca and grass) provided and that left over daily by weighing the residual feed ingredients individually. Calculations were made on dry matter basis.

The dry matter intake, kg per 100 kg body weight was calculated taking into account the weight of the animals recorded at fortnightly intervals and the average daily consumption of dry matter (kg) during that fortnight.

3.3.2.3 Body growth by weight gain

Kids were weighed at the beginning of the experiment and at fortnightly intervals during the entire period of the experiment to monitor their growth pattern. Total weight gain was calculated from the difference between the final and initial body weight and expressed in kg.

Average daily weight gain was calculated by dividing the total weight gain by the number of experimental days (90) and expressed in grams.

3.3.2.4 Feed efficiency

Feed efficiency in terms of intake by gain was calculated from the total dry matter consumed divided by the total gain in body weight at the end of the experiment.

3.3.3 Blood parameters

The effect of inclusion of tapioca and iodine in the ration of kids on certain endocrinological and biochemical parameters was studied.

3.3.3.1 Blood collection

Five ml of blood was collected from each animal prior to the start of the experiment and subsequently at every fortnightly intervals, in dry and sterilized tubes for separation of serum. About 1 ml samples of blood were also collected from each kid into small glass vials on sodium fluoride-oxalate mixture for determination of blood glucose and haemoglobin.

3.3.3.2 Haemoglobin

The cyanmethemoglobin method of Miale (1967) was followed for the determination of haemoglobin.

3.3.3.3 Blood glucose

Blood glucose levels were determined by the method of Asatoor and King as detailed by King and Wootton (1959).

3.3.3.4 Serum total protein

Serum total protein was estimated by the method of Lowry et al. (1951).

3.3.3.5 Serum total cholesterol

Serum total cholesterol was measured by the method of Zak (1957).

3.3.3.6 Serum total lipid

Serum total lipid concentration was determined by the phosphovanillin method as adapted by Span Diagnostics Pvt. Ltd. (Udhana), India (Kit No.25926).

3.3.3.7 Serum insulin

Serum insulin was measured by double antibody RIA technique. Radioimmunoassay Kit (Code No. RIA K1) were

obtained from BRIT, Bombay, India. 3.3.3.8 Serum thyroxin (T_4)

Radioimmunoassay of T_4 was carried out by using RIA Kit (Code No. RIA K5) obtained from BRIT, Bombay, India.

3.3.4 Statistical analysis

Analysis of variance procedures (Snedecor and Cochran, 1967) were used for evaluating differences between treatments.

RESULTS

CHAPTER 4

RESULTS

4.1 Determination of cyanogenic glycoside (CNG) content of fresh and processed tapioca of different varieties

Seven varieties of tapioca viz. 'M-4', 'Sree Visakh' (H-1687), 'Thottakolly', 'Sree Prakash' (S-856), 'Sree Sahya' (H-2304), 'H-165' and 'Karkidakkan' were studied. Fresh as well as boiled and sun dried samples of the above varieties of tapioca were analysed for the cyanogenic glycoside (CNG) content in the cortex of the tubers.

4.1.1 Variation in CNG content in fresh samples of different varieties of tapioca

CNG contents of fresh samples of different varieties of tapioca are presented in Table 4. The average CNG content in terms of cyanide in fresh tubers of 'M-4', 'Sree Visakh', 'Thottakolly', 'Sree Prakash', 'Sree Sahya', 'H-165', and 'Karkidakkan' was 40.86 $\pm$ 2.79, 58.02 $\pm$ 4.5, 64.02 $\pm$ 5.15, 69.19 $\pm$ 3.13, 85.19 $\pm$ 3.32, 182.60 $\pm$ 10.0, and 186.31 $\pm$ 5.3 μg cyanide/g tuber respectively.

Results showed a wide variation in CNG levels among the varieties of tapioca studied. The CNG levels were found

to be significantly ($P<0.05$) higher in 'Karkidakkan' and 'H-165' compared to those in other varieties under study. The CNG concentration was lowest in 'M-4' and differed significantly ($P<0.05$) from rest of the varaieties studied. Similarly the CNG concentration recorded for 'Sree Visakh' and 'Thottakolly' was found to be significantly ($P<0.05$) lower than those in 'Sree Sahya' (Table 5).

4.1.2 Effect of boiling on CNG content of tapioca

The effect of boiling on CNG content in seven varieties of tapioca studied is shown in Table 4. The average CNG content in terms of cyanide in the boiled tubers of these varieties was found to be 22.70 ± 2.11, 28.65 ± 3.5, 22.97 ± 3.0, 35.03 ± 2.2, 42.81 ± 3.0, 74.13 ± 6.02 and 88.54 ± 6.1 µg cyanide/g fresh weight of tubers for 'M-4', 'Sree Visakh', 'Thottakolly', 'Sree Prakash', 'Sree Sahya', 'H-165', and 'Karkidakkan' respectively.

It was observed that about half of the CNG was removed during boiling of the samples. The pattern was similar in all the varieties studied except in 'Thottakolly' and 'H-165' varieties where retention of CNG was comparatively lower (35.88 and 40.60% respectively) than the other varieties. Eventhough the CNG levels were reduced more than 50 per cent in 'H-165' and 'Karkidakkan' these two varieties still had

Table 4. Cyanogenic glycoside (CNG) content (before and after boiling and sun drying) of different varieties of tapioca

Sr. No.	Varieties	Cyanogenic glycoside content (µg cyanide/g fresh wt.)			Percentage retention of CNG	
		Fresh	Boiled	Sun dried	After boiling	After sun drying
1.	M-4	40.86±2.79 (10)^{a}	22.70±2.11 (10)^{a}	21.70±2.20 (10)^{a}	55.56	53.11
2.	Sree Visakh (H-1687)	58.02±4.50 (9)^{b}	28.65±3.50 (9)^{ab}	24.86±2.82 (9)^{ab}	49.38	49.38
3.	Thottakolly	64.02±5.15 (10)^{b}	22.97±3.00 (6)^{ab}	29.64±2.39 (10)^{ab}	35.88	46.30
4.	Sree Prakash (S-856)	69.19±3.13 (6)^{bc}	35.03±2.20 (6)^{bc}	32.65±2.10 (6)^{bc}	50.63	47.19
5.	Sree Sahya (H-2304)	85.19±3.32 (6)^{c}	42.81±3.00 (6)^{c}	42.38±2.86 (6)^{c}	50.25	49.75
6.	H-165	182.60±10.00 (10)^{d}	74.13±6.02 (7)^{d}	89.42±6.52 (7)^{d}	40.60	48.97
7.	Karkidakkan	186.31±5.30 (9)^{d}	88.54±6.10 (6)^{e}	89.41±4.85 (6)^{d}	47.52	47.99

Figures in parentheses indicate the number of observations

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 5. Mean squares showing significance in cyanogenic glycoside content (before and after boiling and sun drying) of different varieties of tapioca

Variable	Source of variation	Degrees of freedom	Mean squares
Fresh	Between	6	34341.674**
	Within	53	306.317
Boiled	Between	6	4664.549**
	Within	43	107.715
Sun dried	Between	6	6318.915**
	Within	47	93.422

** (P<0.01)

significantly ($P<0.05$) higher levels of CNG compared to other varieties due to initial high levels of CNG. However, the difference between 'H-165' and 'Karkidakkan' turned out to be significant ($P<0.05$) because of higher loss of CNG in the samples of 'H-165' during the process of boiling (Table 5).

4.1.3 Effect of sun drying on CNG content of tapioca

When the samples of 'M-4', 'Sree Visakh', 'Thottakolly', 'Sree Prakash', 'Sree Sahya', 'H-165', and 'Karkidakkan' varieties of tapioca were subjected to sun drying, the mean CNG concentration in terms of cyanide was found to be 21.70 $\pm$ 2.2, 24.86 $\pm$ 2.82, 29.64 $\pm$ 2.39, 32.65 $\pm$ 2.10, 42.38 $\pm$ 2.86, 89.42 $\pm$ 6.52 and 89.41 $\pm$ 4.85 ug cyanide/g fresh weight of tuber respectively (Table 4).

It was observed that almost 50 per cent of the initial CNG content was lost during the process of sun drying.

4.2 Experiment in rats

Six groups of albino wistar rats were maintained for 12 weeks. Each group comprised of 10 rats (30-40 d old) and was reared on the following experimental diets.

Group No.	Diet
I	Protein = 15%; Tapioca = Nil; Iodine = Nil
II	Protein = 15%; Tapioca = 30%; Iodine = Nil
III	Protein = 7.5%; Tapioca = 30%; Iodine = Nil
IV	Protein = 7.5%; Tapioca without HCN = 30%; Iodine = Nil
V	Protein = 15%; Tapioca = 30%; Iodine = 0.17 mg/kg
VI	Protein = 22.5%; Tapioca = 30%; Iodine = 0.17 mg/kg

Performance of rats on these six experimental diets was evaluated by recording body weight, feed consumption and feed efficiency for a period of 12 weeks. Dry matter digestibility was also determined.

Out of ten rats in each group, used in the present study, a group of three rats each were sacrificed at the 10th and 11th week and the remaining four at the 12th week of the experiment. Blood/serum and tissue samples were collected from these rats and subjected to endocrinological, biochemical and histopathological studies.

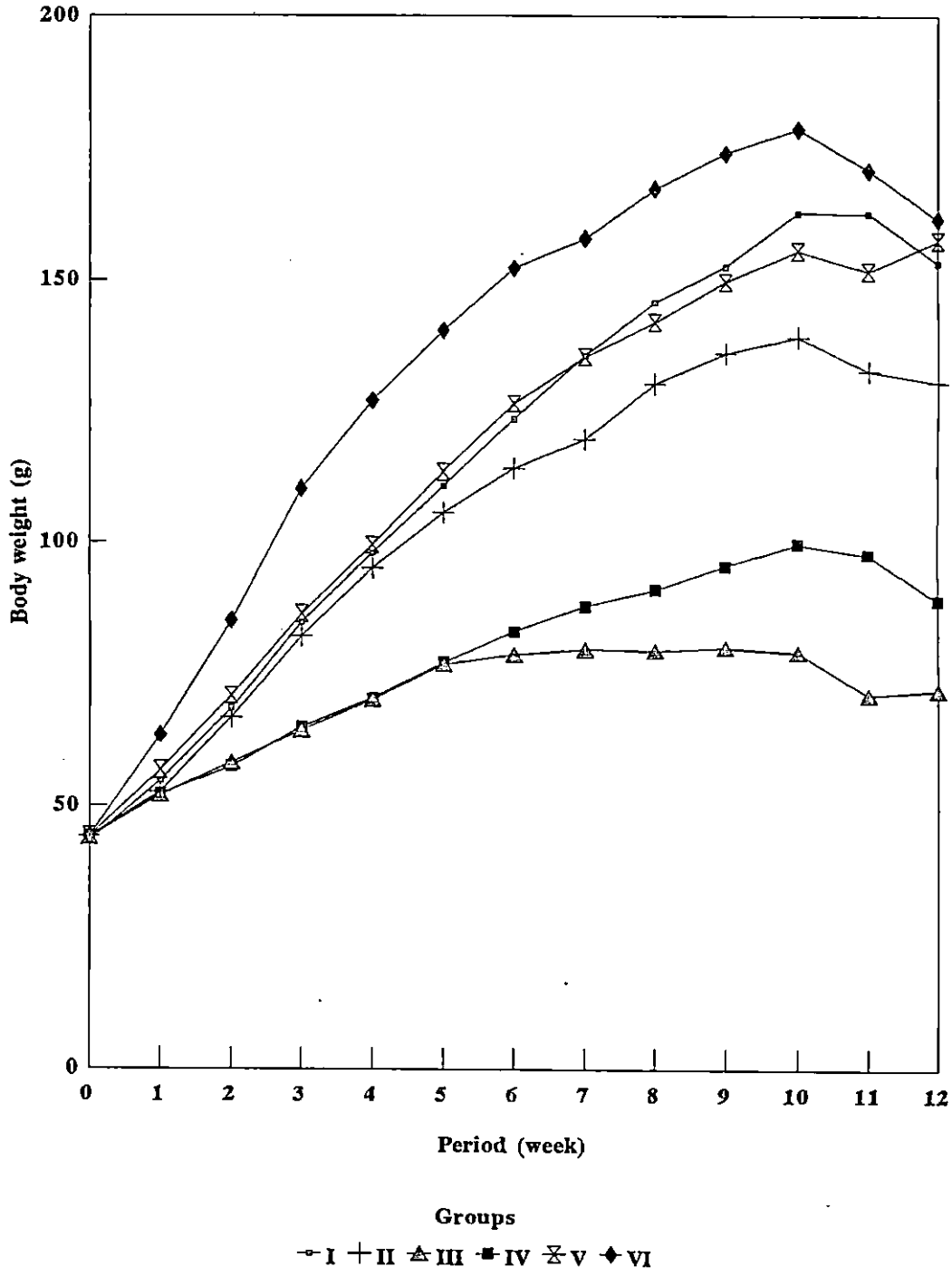
4.2.1 Performance of rats

4.2.1.1 Growth studies

4.2.1.1.1 Body weight gain

Total body weight gain was calculated from final and initial body weight of each rat in each group and the average

Fig.1 INFLUENCE OF DIETARY PROTEIN,TAPIOCA AND IODINE ON BODY WEIGHT OF RATS



total body weight gain is given in Table 6. The weekly changes in body weight are presented in Fig.1.

There was significant ($P<0.05$) difference in total body weight gain in between Groups III and IV. Nevertheless these groups recorded significantly lower total body weight gain compared with the other groups (Table 6 and 7). Group II also recorded significantly ($P<0.05$) lower total body weight gain compared to those in Groups I and V. There was no significant difference in total body weight gain between Groups I and V. Highest total body weight gain was observed in Group VI but did not show significant variation compared to that in Group I (Table 6 and 7).

4.2.1.1.2 Total feed consumption (dry matter)

The average weekly feed intake in different groups of rats is presented in Fig.2.

The average total feed consumption (Table 6) by each rat during a period of 12 weeks of the experiment was lowest in Group III followed by Group IV. The difference between these two groups was significant ($P<0.05$; Table 7). The quantity of feed consumed by rats under Group II was significantly ($P<0.05$) lesser than those in Groups I and V. The total feed consumption recorded in Group I and V was almost similar. The rats in Group VI consumed significantly

Fig.2 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON FEED INTAKE (DRY MATTER) OF RATS

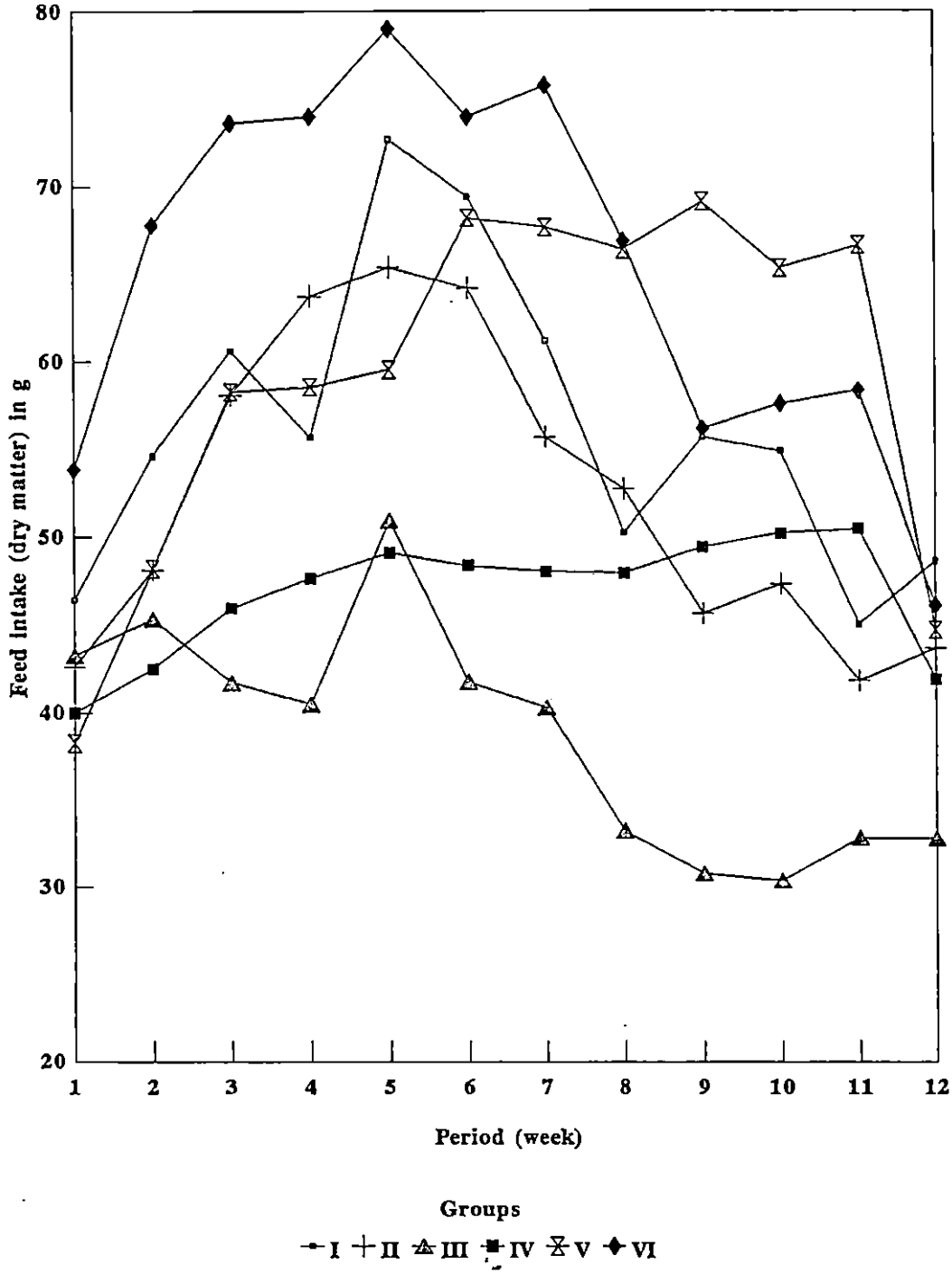


Table 6. Average total weight gain, total feed consumption and feed efficiency under different levels of protein, tapioca and iodine in the diet fed for a period of 12 weeks in rats

Group No.	Diets	Variables		
		Total weight gain (g)	Total feed consumption (g)	Feed efficiency
I	15% protein with nil tapioca	ae 124.90± 6.25	a 642.15± 24.70	a 0.196± 0.011
II	15% protein with 30% tapioca	b 98.00± 3.42	b 584.57± 15.02	b 0.168± 0.006
III	7.5% protein with 30% tapioca	c 33.30± 4.78	c 437.96± 8.53	c 0.076± 0.011
IV	7.5% protein with 30% tapioca without cyanide	d 60.40± 3.69	d 520.39± 12.98	d 0.116± 0.006
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 117.70± 4.58	a 663.76± 16.10	ab 0.178± 0.007
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	e 139.90± 11.33	e 738.24± 25.13	a 0.188± 0.110

Values are average of 10 observations ± S.E.

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 7. Mean squares showing the significance in total weight gain, total feed consumption and feed efficiency of rats

Source of variation	Degrees of freedom	Mean squares		
		Total weight gain	Total feed consumption	Feed efficiency
Between	5	16870.840**	115394.740**	0.0224**
Within	54	393.970	3279.842	0.0008

** (P<0.01)

($P<0.05$) greater quantity of feed compared with the other groups under study (Table 6 and 7).

4.2.1.1.3 Feed efficiency

The mean values of feed efficiency (weight gain per gram of feed) calculated over the entire experimental period of 12 weeks are given in Table 6.

The feed efficiency value recorded for Group IV was found to be significantly ($P<0.05$) higher than Group III. Nevertheless, it was significantly lower than the other groups under study (Table 6 and 7). The feed efficiency value observed in Group II was significantly ($P<0.05$) lower than those in Groups I and VI. There was no significant difference between Groups II and V (Table 7). The feed efficiency value recorded for Group VI did not differ significantly compared to those in Groups I and V. The difference in feed efficiency between Groups I and V was also not significant.

4.2.1.2 Digestibility coefficient of dry matter

Digestibility coefficient of dry matter was determined with respect to feeding six experimental diets to rats and the results are presented in Table 8.

Among the groups studied Group VI recorded the lowest digestibility coefficient ($76.85 \pm 0.36\%$) of dry matter and

Table 8. Digestibility coefficient of dry matter of different diets fed to rats

Group No.	Diets	Digestibility coefficient
I	15% protein with nil tapioca	$78.53^{A} \pm 0.34$
II	15% protein with 30% tapioca	$81.22^{B} \pm 0.39$
III	7.5% protein with 30% tapioca	$83.14^{C} \pm 0.34$
IV	7.5% protein with 30% tapioca without cyanide	$82.20^{BC} \pm 0.46$
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	$78.67^{A} \pm 0.51$
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	$76.85^{D} \pm 0.36$

Values are average of 5 observations $\pm$ S.E.

Means with same superscripts are not significantly different at $P<0.01$

Table 9. Mean squares showing significance in digestibility coefficient of dry matter of different diets fed to rats

Source of variation	Degrees of freedom	Mean squares
Between	5	29.813**
Within	24	0.823

** ($P<0.01$)

showed a highly significant ($P<0.01$) difference from the rest of the experimental groups. Group I and V showed significantly ($P<0.01$) lower digestibility coefficients than in Groups II, III and IV (Table 9). The highest digestibility coefficient (83.14 $\pm$ 0.34%) was in Group III and was significantly ($P<0.01$) higher than the other groups except Group IV.

4.2.2 Endocrinological studies

4.2.2.1 Thyroid weight

Among the groups studied, the relative weight of the thyroid was found to be highest in Group III (Table 10). While in Group IV it was similar to those in Groups I, V and VI. Group II also showed a significant ($P<0.01$) increase in the relative weight of the thyroid as compared to Groups I, IV, V and VI. However, it was significantly ($P<0.01$) lower than that of Group III (Table 10 and 11).

4.2.2.2 DNA content of thyroid

DNA content of thyroid was found to be significantly ($P<0.05$) higher in Group III than those observed in other experimental groups (Table 10 and 11). A marginal increase was also observed in the DNA content of thyroid in Groups II and IV as compared to those in Groups I, V and VI (Table 10).

Table 10. Effect of different levels of protein, tapioca and iodine on the relative weight and protein and DNA contents of thyroid of rats*

Group No.	Diets	Variables		
		Relative weight (mg/100 g B.wt.)(8)	Protein content (µg/100 mg) (4)	DNA content (µg/100 mg) (4)
I	15% protein with nil tapioca	A 7.29± 0.31	a 1131.70± 42.02	a 11.58± 0.86
II	15% protein with 30% tapioca	B 10.51± 0.52	ac 1037.96± 63.00	a 12.77± 1.28
III	7.5% protein with 30% tapioca	C 17.33± 0.42	b 533.45± 45.08	b 17.38± 1.96
IV	7.5% protein with 30% tapioca without cyanide	A 7.90± 0.27	c 887.88± 54.74	a 13.17± 1.11
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	A 7.50± 0.31	ac 1042.85± 23.04	a 11.68± 1.39
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	A 6.87± 0.25	a 1160.09± 140.11	a 11.12± 1.27

Figures in parentheses indicate number of observations

Means in columns with same superscripts (A,B or C) are not significantly different at P<0.01
Means in columns with same superscripts (a, b or c) are not significantly different at P<0.05

* Means are averages of pooled samples collected at different intervals

Table 11. Mean squares showing the significance in relative weight and protein and DNA contents of thyroid of rats fed different levels of protein, tapioca and iodine in the diet

Source of variation	Degrees of freedom	Mean squares		
		Relative weight	Protein content	DNA content
Between	5	129.177**	-	-
Within	42	1.228	-	-
Between	5	-	215532.400**	20.210*
Within	18	-	20616.778	7.302

* (P<0.05) ** (P<0.01)

4.2.2.3 Protein content of thyroid

The protein content of thyroid was 1131.70 ± 42.02, 1037.96 ± 63.00, 1042.85 ± 23.04 and 1160.09 ± 140.11 µg/100 mg in Groups I, II, V and VI respectively (Table 10). Analysis of variance did not reveal significant variation between these groups (Table 11). The protein content of thyroid observed in Group IV (887.88 ± 54.74 ug/100 mg) also did not vary significantly from those in Groups II and V but it was significantly ($P<0.05$) higher than that of Group III (533.45 ± 45.08 µg/100 mg) which was found to be the lowest among the groups studied (Table 10 and 11).

4.2.2.4 Serum thyroxine

At the 10th week of the study, serum thyroxin (T_4) concentration was highest in Group I (Table 12). The concentration of T_4 recorded in Group II was significantly ($P<0.05$) lower compared to those in Group I, V and VI (Table 12 and 13) but was similar to that in Group IV. Among the groups studied, the lowest concentration of T_4 was observed in Group III (Table 12 and 13). Almost similar trend was observed in T_4 concentration at the 11th week of the study (Tables 12 and 13) between the groups.

At the 12th week of the study also the concentration of T_4 was found to be significantly ($P<0.05$) lower in Group II

Table 12. Influence of different levels of protein, tapioca and iodine on serum thyroxine concentration (µg/dl) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	a 8.00± 0.53	A 6.80± 0.50	a 6.20± 0.35	A 6.92± 0.34
II	15% protein with 30% tapioca	b 5.40± 0.40	B 4.70± 0.15	b 4.00± 0.28	B 4.63± 0.25
III	7.5% protein with 30% tapioca	c 2.52± 0.15	C 2.37± 0.19	d 2.13± 0.13	C 2.32± 0.09
IV	7.5% protein with 30% tapioca without cyanide	b 5.33± 0.49	B 5.00± 0.12	bc 5.09± 0.38	B 5.14± 0.20
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 7.27± 0.59	A 6.10± 0.27	ac 6.08± 0.71	A 6.42± 0.36
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 7.20± 0.53	A 6.87± 0.24	a 6.43± 0.76	A 6.79± 0.33

Figures in parentheses indicate number of observations

Means in columns with same superscripts (A,B or C) are not significantly different at P<0.01

Means in columns with same superscripts (a,b,c or d) are not significantly different at P<0.05

Table 13. Mean squares showing the significance in serum thyroxine concentration at the 10-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	11.982**
	Within	12	0.667
11	Between	5	8.578**
	Within	12	0.229
12	Between	5	11.119**
	Within	18	0.959
Entire	Between	5	30.915**
	Within	54	0.770

** ($P<0.01$)

compared to those in Groups I, V and VI. Group IV manifested no change in T_4 concentration and did not vary significantly from those in Groups II and V. The levels of T_4 recorded in Group III was the lowest among the groups under study (Tables 12 and 13).

The overall concentration of T_4 was highest in Group I followed by Group VI and V evincing no significant difference between themselves (Table 12). Group II showed significantly ($P<0.01$) lower concentration of T_4 than those in the above groups (Table 12 and 13). There was no significant difference in T_4 concentration between the Groups II and IV. The T_4 concentration remained to be the lowest in Group III than all the other groups under study (Table 12 and 13).

4.2.2.5 DNA content of pancreas

At the 10th week of the experiment DNA content (µg/100 mg) of pancreas was found to be almost similar in all the experimental groups studied (Table 14 and 15). Similar was the trend for the 11th and 12th week of the experiment (Table 14 and 15). However, considering the entire period of the experiment, the DNA content of pancreas was significantly ($P<0.05$) higher in Group III than those in Groups I, II, V and VI (Table 14 and 15). The DNA content of pancreas

Table 14. Influence of different levels of protein, tapioca and iodine on DNA content of pancreas (µg/100 mg) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	a 38.86± 0.76	a 38.08± 0.53	a 39.00± 1.13	a 38.69± 0.50
II	15% protein with 30% tapioca	a 40.08± 0.89	a 40.43± 1.10	a 39.46± 0.94	ac 39.87± 0.50
III	7.5% protein with 30% tapioca	a 42.45± 3.38	a 41.93± 2.51	a 42.14± 2.51	b 42.17± 1.42
IV	7.5% protein with 30% tapioca without cyanide	a 41.18± 1.38	a 40.61± 1.70	a 41.00± 1.58	bc 40.93± 1.24
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 39.15± 1.30	a 38.47± 1.10	a 39.42± 1.33	ac 39.06± 0.86
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 38.50± 0.58	a 38.03± 0.61	a 38.69± 1.02	a 38.44± 0.44

Figures in parentheses indicate number of observations

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 15. Mean squares showing the significance in DNA content of pancreas at the 10th-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	6.980 NS
	Within	12	8.376
11	Between	5	8.175 NS
	Within	12	6.101
12	Between	5	6.305 NS
	Within	18	9.198
Entire	Between	5	21.070*
	Within	54	6.275

NS (Non-significant)

* ($P<0.05$)

recorded in Group IV was also significantly ($P<0.05$) higher as compared to Groups I and VI (Table 14 and 15).

4.2.2.6 Protein content of pancreas

At the 10th week of the study, the protein content of pancreas was found to be lowest in Group III (Table 16) but did not differ significantly compared to that of Group IV (Table 17). Although the protein content of pancreas was lower in Group IV it was not significantly different as compared to Groups I, II and V (Table 16 and 17). Similarly no significant difference was evident between Groups I, II, V and VI (Table 16 and 17).

At the 11th week of the study, Groups I, II, V and VI showed significantly ($P<0.05$) higher protein content of pancreas than those in Groups III and IV (Table 16 and 17).

The protein content of pancreas at the 12th week of the experiment followed the same trend as observed at the 10th week of the experiment (Table 16 and 17).

During the entire period of the study, Group III continued to show the lowest protein content of pancreas (Table 16) and differed significantly ($P<0.05$) from those in other groups except Group IV (Table 17). The difference in protein content of pancreas in between Groups I, II and V was

Table 16. Influence of different levels of protein, tapioca and iodine on protein content of pancreas (µg/100 mg) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	ac 450.27± 56.78	a 462.56± 31.96	ac 403.96± 15.00	ac 435.43± 19.72
II	15% protein with 30% tapioca	ac 415.27± 37.00	a 412.80± 43.48	ac 363.19± 53.75	c 393.69± 25.91
III	7.5% protein with 30% tapioca	b 287.08± 50.43	b 255.97± 23.73	b 263.42± 19.25	b 268.28± 16.56
IV	7.5% protein with 30% tapioca without cyanide	bc 340.54± 41.53	b 303.43± 42.45	bc 333.17± 14.73	b 326.46± 17.04
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	ac 433.99± 7.47	a 419.44± 28.76	ac 364.59± 32.10	ac 401.87± 17.40
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 494.51± 12.43	a 483.61± 37.51	a 426.39± 34.23	a 463.99± 19.15

Figures in parentheses indicate number of observations

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 17. Mean squares showing the significance in protein content of pancreas at the 10-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	17433.930*
	Within	12	4533.480
11	Between	5	24524.740**
	Within	12	3755.083
12	Between	5	13131.460*
	Within	18	3934.931
Entire	Between	5	52250.460**
	Within	54	3824.139

* (P<0.05)

** (P<0.01)

not significant. However, there appeared to be a significant difference in between Groups II and VI (Table 16 and 17).

4.2.2.7 Serum insulin

Highest level of serum insulin was observed in Group II (Table 18) and showed significant ($P<0.05$) differences as compared to Group III, IV and VI (Table 19). However, there was no significant difference between Groups I, II and V. The serum insulin level observed in Group VI also did not vary significantly from those in Groups I, III, IV and V at the 10th week of study (Table 18 and 19).

Although the serum insulin levels were found to be altered at the 11th and 12th week of the study, the differences were not significant between the groups studied (Table 18 and 19).

The overall serum insulin level recorded in Groups I and VI during the entire period of the study (Table 18) did not show significant variation from those groups showing either higher (Groups II and V) or lower (Groups III and IV) levels of serum insulin (Table 18 and 19).

4.2.3 Biochemical studies

4.2.3.1 Blood glucose

Significantly ($P<0.05$) lower levels of blood glucose

Table 18. Influence of different levels of protein, tapioca and iodine on serum insulin concentration (μU/ml) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	ab 26.14± 2.24	a 31.48± 7.04	a 29.58± 4.49	ab 29.12± 2.61
II	15% protein with 30% tapioca	a 35.00± 4.38	a 40.02± 4.23	a 34.43± 8.70	a 36.28± 3.64
III	7.5% protein with 30% tapioca	b 20.75± 2.61	a 24.35± 9.09	a 21.47± 3.02	b 22.12± 2.73
IV	7.5% protein with 30% tapioca without cyanide	b 22.17± 2.29	a 28.95± 5.13	a 24.99± 4.11	b 25.33± 2.26
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	ac 31.53± 3.33	a 36.52± 5.60	a 34.10± 4.68	a 34.06± 2.48
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	bc 23.68± 2.38	a 33.00± 6.02	a 31.29± 6.75	ab 29.52± 3.27

Figures in parentheses indicate number of observations

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 19. Mean squares showing the significance in serum insulin concentration at the 10th-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	94.484*
	Within	12	27.644
11	Between	5	91.752 NS
	Within	12	122.068
12	Between	5	106.573 NS
	Within	18	126.115
Entire	Between	5	277.263*
	Within	54	82.440

NS (Non-significant)

* (P<0.05)

Table 20. Summarised data on serum insulin, blood glucose and insulin-to-glucose ratio in rats fed on different levels of protein, tapioca and iodine in the diet

Group No.	Diets	Variables		
		Serum insulin (μU/ml)	Blood glucose (mg/100 ml)	Insulin-to glucose ratio (μU/mg)
I	15% protein with nil tapioca	ab 29.12± 2.61	ac 86.45± 1.78	a 33.68± 2.71
II	15% protein with 30% tapioca	a 36.28± 3.64	b 107.24± 8.08	a 33.83± 3.97
III	7.5% protein with 30% tapioca	b 22.12± 2.73	a 74.60± 3.25	a 29.65± 2.48
IV	7.5% protein with 30% tapioca without cyanide	b 25.33± 2.26	a 79.20± 2.14	a 32.00± 2.00
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 34.06± 2.48	c 92.29± 2.86	a 36.90± 2.78
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	ab 29.52± 3.27	ac 86.24± 4.00	a 34.23± 2.86

Values are average of 10 observations ± S.E

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 21. Mean squares showing the significance in serum insulin, blood glucose and insulin-to-glucose ratio in rats

Source of variation	Degrees of freedom	Mean squares		
		Serum insulin	Blood glucose	Insulin-to glucose ratio
Between	5	277.263*	1304.046**	78.496 NS
Within	54	82.440	179.801	81.929

NS (Non-significant)

* (P<0.05)

** (P<0.01)

were observed in Groups III and IV (Table 22 and 23) as compared to those in Groups I, II and V but Group VI did not show significant variation from Groups III and IV at the 10th week of the study (Table 23).

Although highest blood glucose level was recorded in Group II at the 11th week of the study, no significant difference was evident between the groups studied (Table 22 and 23).

At the 12th week of the study, Group II continued to show highest concentration of blood glucose (Table 22) and varied significantly ($P<0.05$) among all the groups studied (Table 23).

The overall levels of blood glucose during the entire period of the study remained to be significantly ($P<0.05$) higher in Group II as compared to other groups (Table 22 and 23). The groups (III and IV) which recorded lower levels of blood glucose did not show significant variation from those in Groups I and VI. Similarly there was no significant difference in between Groups I, V and VI (Table 23).

4.2.3.2 Serum total protein

Among the groups studied those in Groups I,II, V and VI showed almost similar concentrations of serum total protein

Table 22. Influence of different levels of protein, tapioca and iodine on blood glucose concentration (mg/dl) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	a 90.93± 2.32	a 83.93± 3.03	a 84.99± 3.02	ac 86.45± 1.78
II	15% protein with 30% tapioca	a 94.89± 5.15	a 118.22± 25.21	b 108.19± 9.50	b 107.24± 8.08
III	7.5% protein with 30% tapioca	b 71.78± 4.06	a 78.08± 6.22	a 74.10± 6.83	a 74.60± 3.25
IV	7.5% protein with 30% tapioca without cyanide	b 74.22± 1.49	a 84.59± 2.86	a 78.69± 3.97	a 79.20± 2.14
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 89.71± 6.69	a 98.80± 5.73	a 89.35± 2.74	c 92.29± 2.86
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	ab 81.58± 3.43	a 95.96± 12.11	a 82.46± 2.68	ac 86.24± 4.00

Figures in parentheses indicate number of observations

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 23. Mean squares showing the significance in blood glucose concentration at the 10th-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	269.863*
	Within	12	53.534
11	Between	5	632.071 NS
	Within	12	435.334
12	Between	5	569.367**
	Within	18	117.574
Entire	Between	5	1304.046**
	Within	54	179.801

NS (Non-significant)

* ($P<0.05$)

** ($P<0.01$)

at the 10th week of the experiment (Table 24). The concentration of serum total protein was significantly ($P<0.05$) lower in Group IV (Table 25) compared to all the groups. Conversely, Group III showed highest concentration of serum total protein but did not differ significantly from those in Groups I and II.

At the 11th week of the study serum total protein concentration was found to be significantly ($P<0.05$) lower in Group IV than those in other groups under study (Table 24 and 25).

At the 12th week of the study also, as in the case of all the other time intervals, the serum total protein level was the lowest ($P<0.05$) in Group IV than rest of the groups (Table 24 and 25). The highest concentration observed in Group III did not differ significantly from those in Groups II, V and VI (Table 25).

The overall concentration of serum total protein during the entire period of the study was lowest ($P<0.05$) in Group IV while the maximum concentration was recorded in Group III as compared to other groups under study (Table 24 and 25). No significant difference was observed between Groups I, II, V and VI.

Table 24. Influence of different levels of protein, tapioca and iodine on serum total protein concentration (g/dl) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	ab 8.06±0.10	a 7.87±0.43	a 7.39±0.41	a 7.73±0.21
II	15% protein with 30% tapioca	ab 8.01±0.31	a 8.00±0.23	ab 8.08±0.18	a 8.03±0.12
III	7.5% protein with 30% tapioca	b 9.23±0.57	a 7.91±0.21	b 8.90±0.62	b 8.70±0.33
IV	7.5% protein with 30% tapioca without cyanide	c 5.78±0.53	b 6.14±0.59	c 6.22±0.33	c 6.07±0.25
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 7.57±0.61	a 7.59±0.49	ab 7.93±0.34	a 7.72±0.24
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 7.81±0.44	a 8.11±0.30	ab 7.83±0.36	a 7.91±0.19

Figures in parentheses indicate number of observations

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 25. Mean squares showing the significance in serum total protein concentration at the 10th-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	3.771**
	Within	12	0.635
11	Between	5	1.623*
	Within	12	0.486
12	Between	5	3.149**
	Within	18	0.619
Entire	Between	5	7.671**
	Within	54	0.540

* ($P<0.05$)

** ($P<0.01$)

4.2.3.3 Serum total cholesterol

The serum total cholesterol concentration was found to be significantly ($P<0.05$) higher in Group III than all the other groups (Table 26 and 27) at the 10th week of the study. An increase in serum total cholesterol level was apparent in Group II as compared to Groups I, IV, V and VI (Table 26).

A similar trend was evident for the subsequent periods (11th and 12th week) of the study (Table 26 and 27).

The overall concentration of serum total cholesterol remained to be significantly ($P<0.05$) lower in Group IV as compared to Group III which had highest concentration among the groups studied. On the other hand, Group II also showed significantly ($P<0.05$) higher levels of serum total cholesterol than those in Groups I, IV, V and VI (Table 26 and 27).

4.2.3.4 Serum total lipid

Among the groups studied, the concentration of serum total lipid was found to be highest in Group III at all stages of the experiment (Table 28) with no significant difference noticed between the groups studied (Table 29).

In the overall level of serum total lipid significantly ($P<0.01$) higher level was noticed in Group III

Table 26. Influence of different levels of protein, tapioca and iodine on serum total cholesterol concentration (mg/dl) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	$45.93^{a} \pm 5.34$	$47.85^{a} \pm 2.88$	$50.72^{a} \pm 1.29$	$48.42^{a} \pm 1.76$
II	15% protein with 30% tapioca	$54.85^{a} \pm 1.80$	$58.33^{a} \pm 1.03$	$60.00^{a} \pm 0.91$	$57.96^{c} \pm 0.95$
III	7.5% protein with 30% tapioca	$68.29^{b} \pm 2.25$	$75.85^{b} \pm 6.65$	$79.45^{b} \pm 3.77$	$75.02^{b} \pm 2.75$
IV	7.5% protein with 30% tapioca without cyanide	$48.59^{a} \pm 5.81$	$48.81^{a} \pm 3.87$	$52.45^{a} \pm 5.98$	$50.20^{a} \pm 2.90$
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	$49.78^{a} \pm 1.33$	$52.89^{a} \pm 3.00$	$52.80^{a} \pm 2.04$	$51.74^{a} \pm 1.18$
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	$48.17^{a} \pm 4.62$	$52.31^{a} \pm 2.81$	$51.78^{a} \pm 3.99$	$50.85^{a} \pm 2.10$

Figures in parentheses indicate number of observations

Means in columns with same superscripts are not significantly different at $P<0.05$

Table 27. Mean squares showing the significance in serum total cholesterol concentration at the 10th-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	203.742*
	Within	12	46.824
11	Between	5	326.891**
	Within	12	42.682
12	Between	5	490.650**
	Within	18	47.952
Entire	Between	5	1001.487**
	Within	54	43.006

* (P<0.05)

** (P<0.01)

Table 28. Influence of different levels of protein, tapioca and iodine on serum total lipid concentration (mg/dl) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	221.54± 24.53	220.77± 28.46	235.00± 5.08	226.62± 10.15 A
II	15% protein with 30% tapioca	231.15± 8.55	236.41± 28.70	253.85± 41.06	247.54± 17.06 A
III	7.5% protein with 30% tapioca	308.69± 12.42	318.98± 14.36	334.62± 48.00	322.15± 18.56 B
IV	7.5% protein with 30% tapioca without cyanide	241.49± 26.78	238.45± 27.01	242.96± 20.78	241.17± 12.42 A
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	237.44± 33.76	226.67± 12.60	231.96± 10.57	232.01± 10.17 A
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	220.00± 33.15	218.97± 15.29	228.08± 13.30	222.92± 10.70 A

Figures in parentheses indicate number of observations

Means in column with same superscripts are not significantly different at $P<0.01$

Table 29. Mean squares showing the significance in serum total lipid concentration at the 10th-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	3286.609 NS
	Within	12	1892.625
11	Between	5	4304.384 NS
	Within	12	1472.266
12	Between	5	6510.928 NS
	Within	18	3156.650
Entire	Between	5	13768.338**
	Within	54	1851.326

NS (Non-significant)

** (P<0.01)

(Table 28 and 29) compared to rest of the groups. The serum total lipid concentration was higher in Group II compared to Group I but the difference was not significant.

4.2.3.5 Haemoglobin

Among the groups studied the level of haemoglobin was found to be significantly ($P<0.05$) lower in Group III at the 10th week of the experiment (Table 30 and 31). Group II also showed significantly ($P<0.05$) lower concentration of haemoglobin than those in Groups I, V and VI but was significantly ($P<0.05$) higher compared to that of Group IV.

At the 11th week of the study, the concentration of haemoglobin recorded in Group III was found to be significantly ($P<0.05$) lower among all the groups studied (Table 30 and 31). Group II did not show any significant difference compared to those in Groups IV and V. However, the haemoglobin concentration was significantly ($P<0.05$) lower in Group II than those in Groups I and VI.

At the 12th week of the study the trend in haemoglobin concentration among different groups was similar to that observed at the 11th week of the study except that the concentration of haemoglobin observed in Group IV was significantly ($P<0.05$) lower than that of Group II (Table 30 and 31).

Table 30. Influence of different levels of protein, tapioca and iodine on haemoglobin concentration (g/dl) at the 10th-12th week of the study in rats

Group No.	Diets	Period (week)			
		10(3)	11(3)	12(4)	Entire(10)
I	15% protein with nil tapioca	a 11.65±0.39	a 11.04±0.33	a 10.81±0.54	A 11.13±0.27
II	15% protein with 30% tapioca	b 10.15±0.20	bd 9.33±0.40	b 9.42±0.20	B 9.61±0.18
III	7.5% protein with 30% tapioca	c 6.89±0.22	c 6.69±0.37	c 6.55±0.37	C 6.69±0.18
IV	7.5% protein with 30% tapioca without cyanide	d 8.81±0.28	d 8.35±0.57	d 8.02±0.24	D 8.36±0.22
V	15% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 11.38±0.55	ab 10.37±0.38	ab 10.29±0.42	A 10.64±0.28
VI	22.5% protein with 30% tapioca and iodine @ 0.17 mg/kg	a 11.47±0.34	a 11.25±0.64	a 10.73±0.44	A 11.11±0.27

Figures in parentheses indicate number of observations

Means in columns with same superscripts (A,B,C or D) are not significantly different at $P<0.01$

Means in columns with same superscripts (a,b,c or d) are not significantly different at $P<0.05$

Table 31. Mean squares showing the significance in haemoglobin concentration at the 10th-12th week of the study in rats

Period (week)	Source of variation	Degrees of freedom	Mean squares
10	Between	5	10.726**
	Within	12	0.362
11	Between	5	9.262**
	Within	12	0.644
12	Between	5	11.632**
	Within	18	0.599
Entire	Between	5	31.405**
	Within	54	0.555

** (P<0.01)

During the entire period of the study the haemoglobin concentration in Group III remained to be significantly ($P<0.01$) lower among all the groups studied. The overall concentration of haemoglobin observed in Group II was also found to be significantly ($P<0.01$) lower than those in Groups I, V and VI but it was significantly higher than that in Group IV (Table 30 and 31).

4.2.4 Histopathological studies

Histopathological evaluation of liver, pancreas and heart from rats of all experimental groups was made at 10th, 11th and 12th week of the experiment.

Intensity of pathological changes was more severe in Group III even though there was not much variation at the different time intervals. The hepatic cells showed marked diffuse parenchymatous changes (Plate VII) with occasional individual cell necrosis. Most of the cells had a clear vacuolated cytoplasm with vesicular and condensed nucleus. The space of disse was not prominent. The exocrine cells of the pancreas showed vacuolar changes and a few cells had undergone necrosis. Many cells had eosinophilic granular cytoplasm. There was interstitial oedema which could be seen separating the lobules. In general, the islet cells were intact eventhough an occasional beta cell showed degenerative

changes (Plate VIII). The cardiac muscles showed interstitial oedema and occasional degenerative changes. In some cases the sarcolemma appeared fragmented and sarcoplasm swollen (Plate IX).

In Group IV the liver showed moderate vacuolar changes. Some of the hepatic cells were swollen with condensed nuclei (Plate X). The pancreatic cells, both the exocrine and endocrine did not reveal any degenerative changes (Plate XI). There was distension in the interlobular space. There was interstitial oedema in the myocardium. No other significant change in the myocardium was noticed (Plate XII).

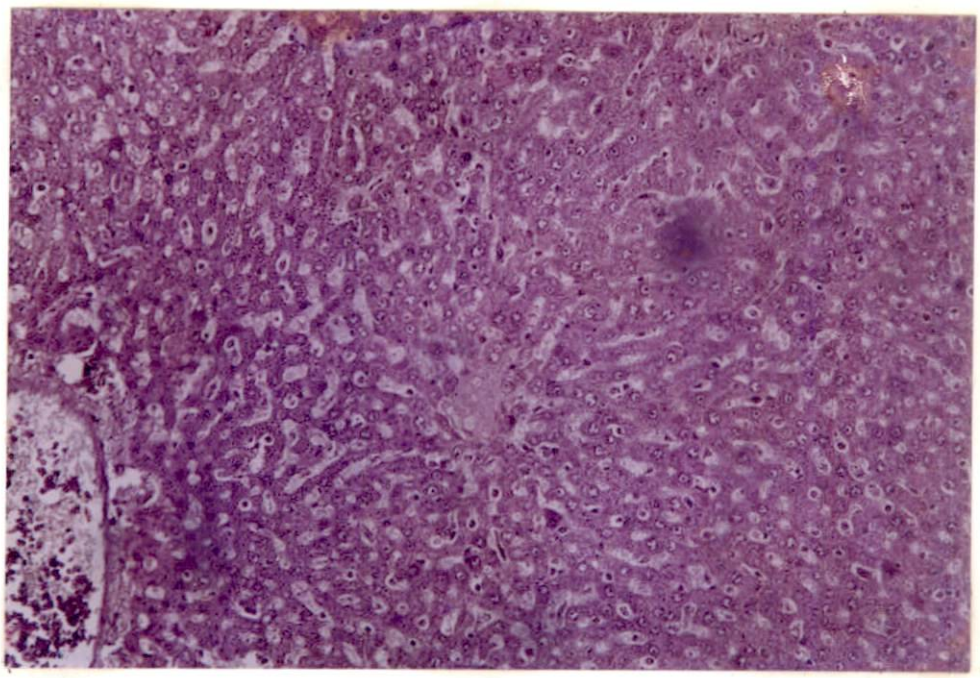
The hepatic cells appeared slightly swollen with occasional cells showing vacuolar changes in Group II (Plate IV). The pancreas showed slight oedema and the exocrine cells showed occasional degenerative changes. The islet cells appeared normal (Plate V). There was slight interstitial oedema in the myocardium (Plate VI).

In Group V, a few of the hepatic cells appeared swollen with a granular cytoplasm (Plate XIII). Pancreatic cells did not exhibit pathological alterations (Plate XIV). The myocardium appeared normal (Plate XV).

The hepatic cells in Group VI did not show significant

Plate I Group I - Normal liver histology - H&E x 250

Plate II Group I - Normal pancreas histology - H&E x 250



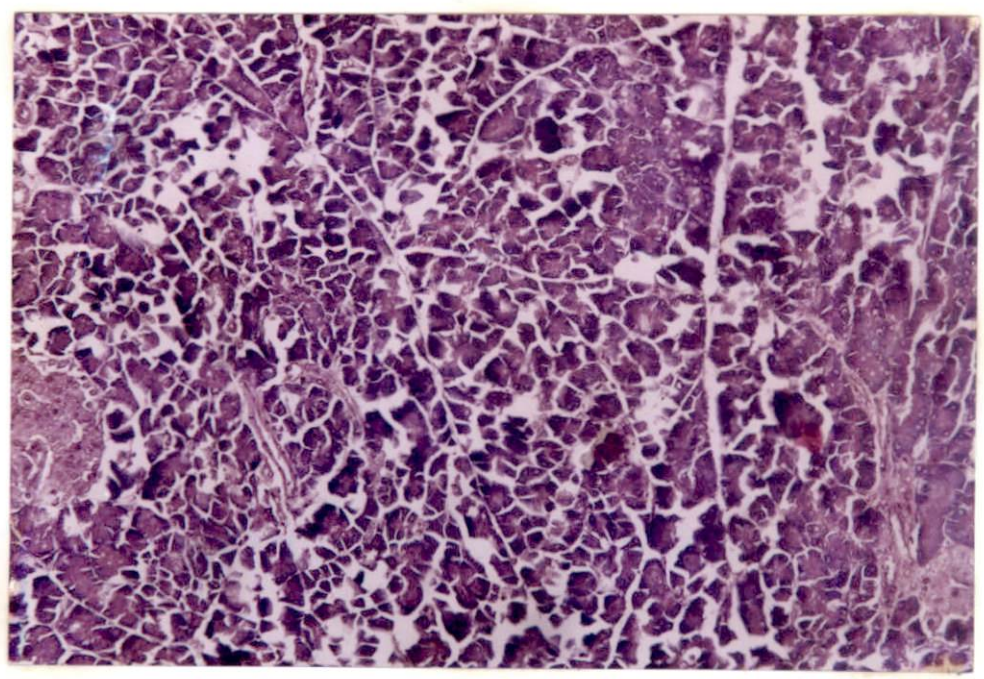
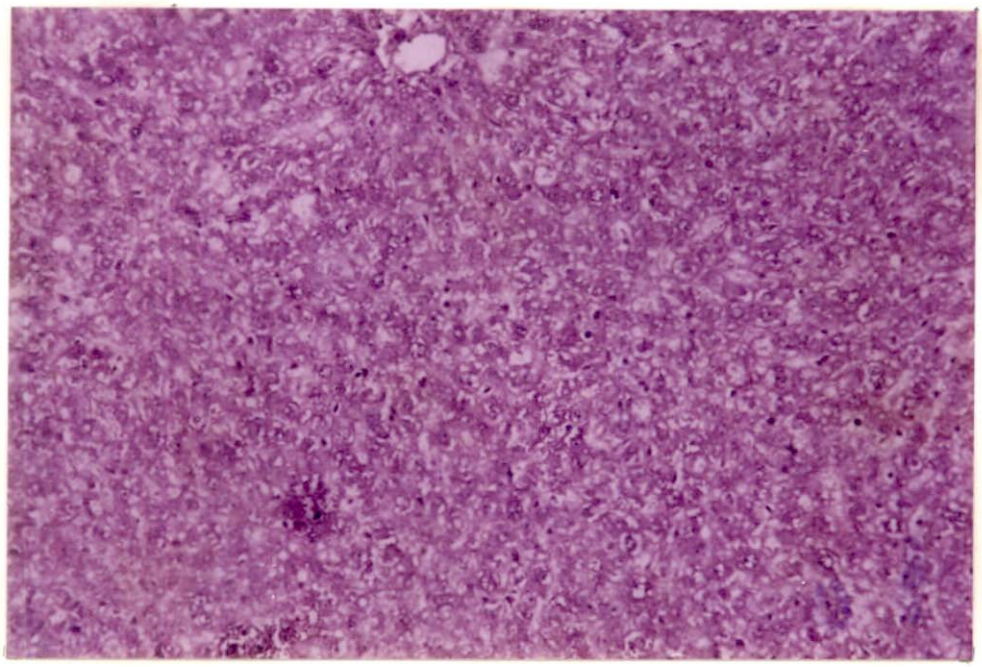


Plate III Group I - Normal myocardium histology - H&E x 250

Plate IV Group II - Liver with swollen hepatic cells, some cell showing vacuolar changes - H&E x 150



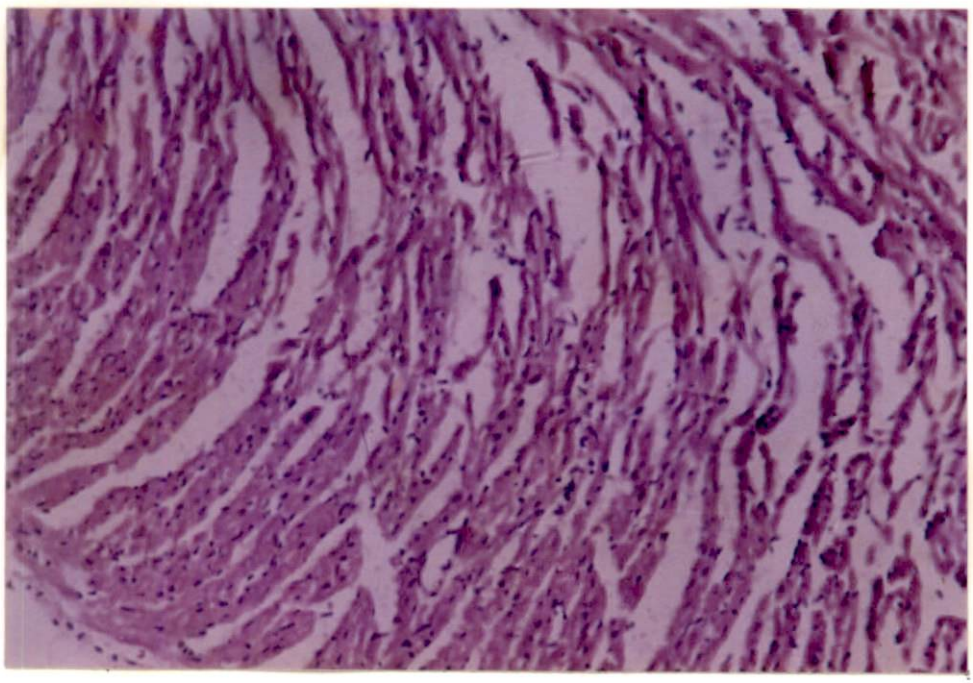
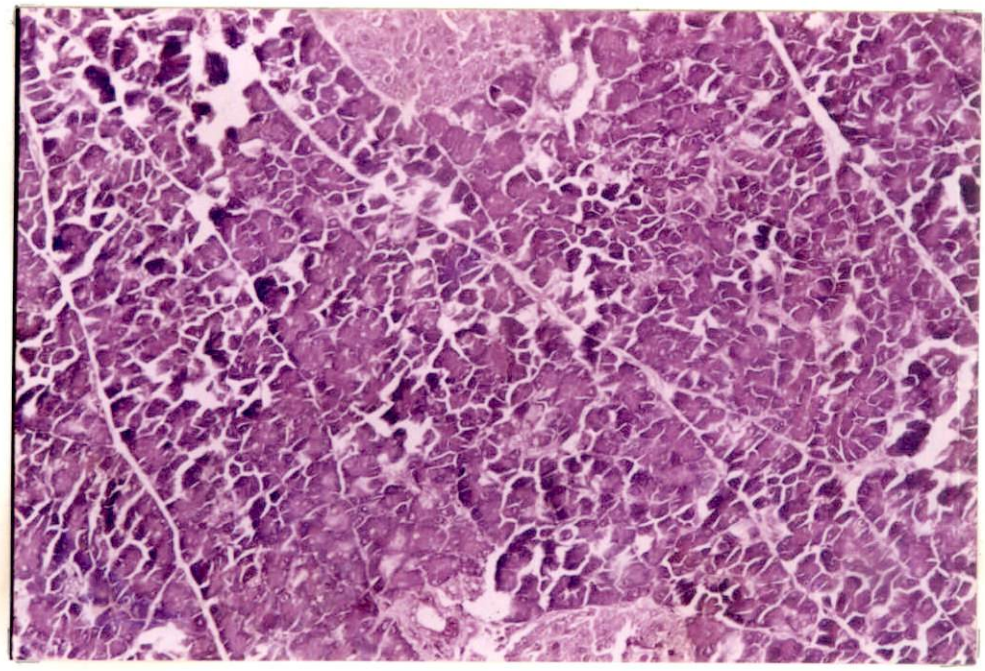


Plate V Group II - Pancreas with slight interstitial oedema and occasional degenerative changes of the exocrine cells -H&E x 200

Plate VI Group II - Myocardium with slight interstitial oedema - H&E x 200



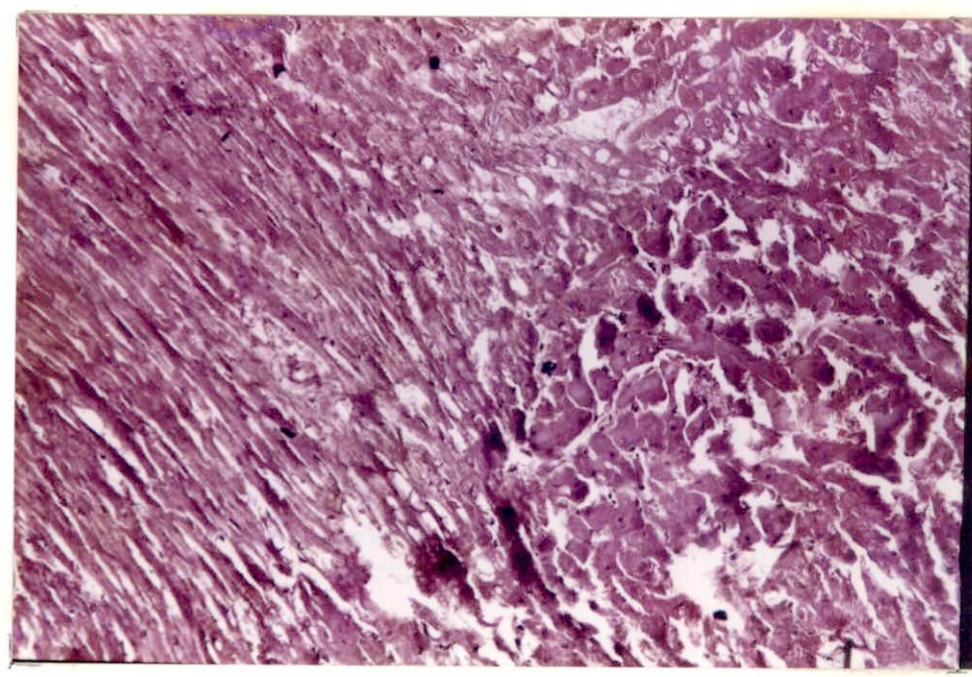
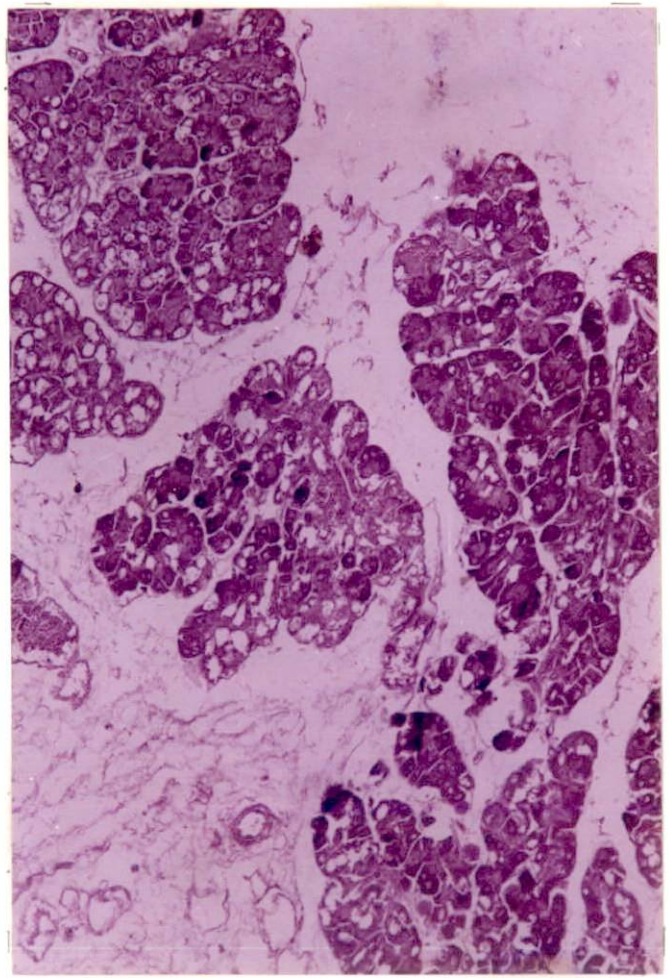


Plate VII Group III - Liver cells with marked vacuolar changes. Nuclei either condensed or vesicular - H&E x 250

Plate VIII Group III - Pancreatic exocrine cells showing degenerative and necrotic changes. Many cells with a vacuolar appearance. Prominent interstitial oedema - H&E x 400



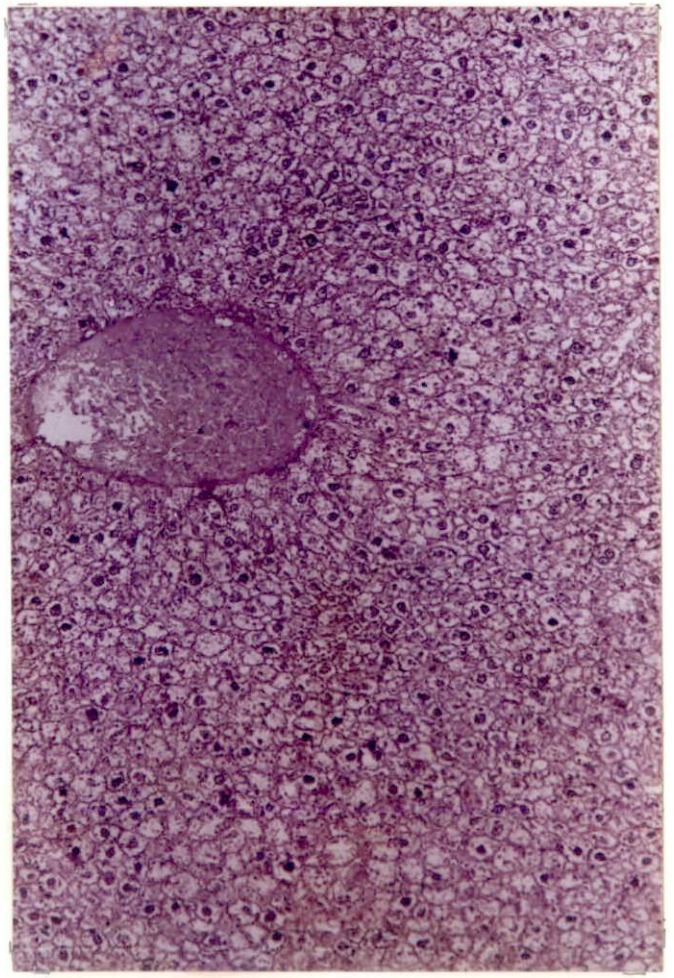
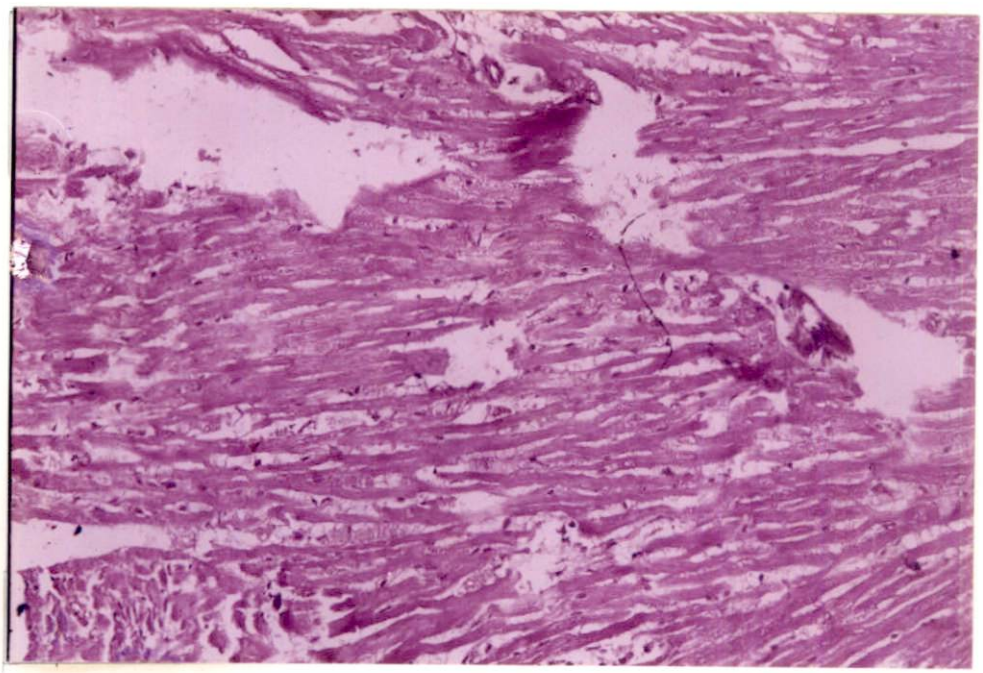


Plate IX Group III - Myocardium showing interstitial oedema, sarcoplasm swollen and sarcolemma fragmented - H&E x 200

Plate X Group IV - Liver showing moderate vacuolar changes - H&E x 400



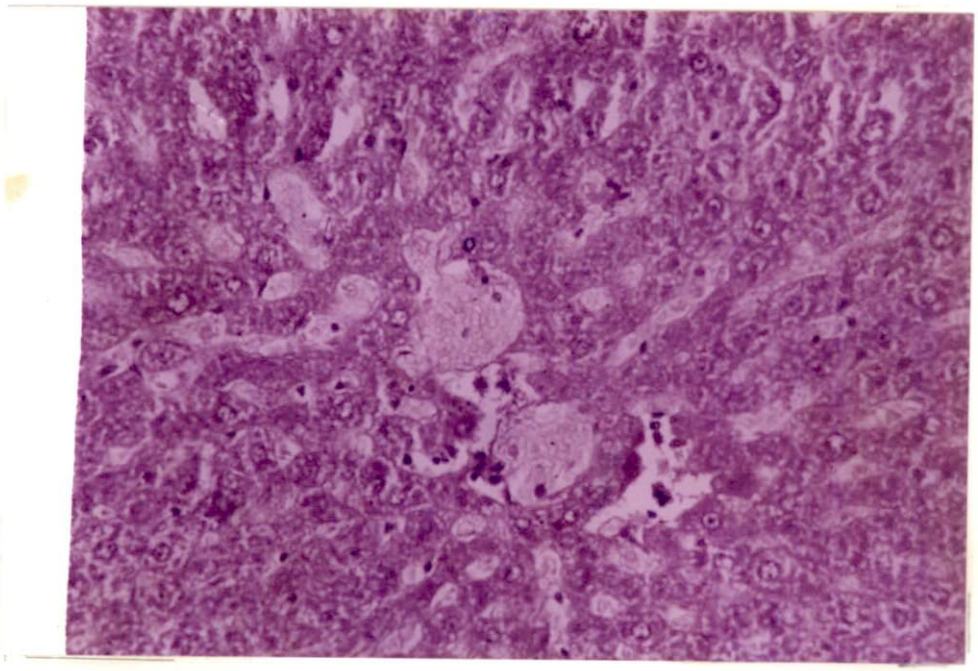
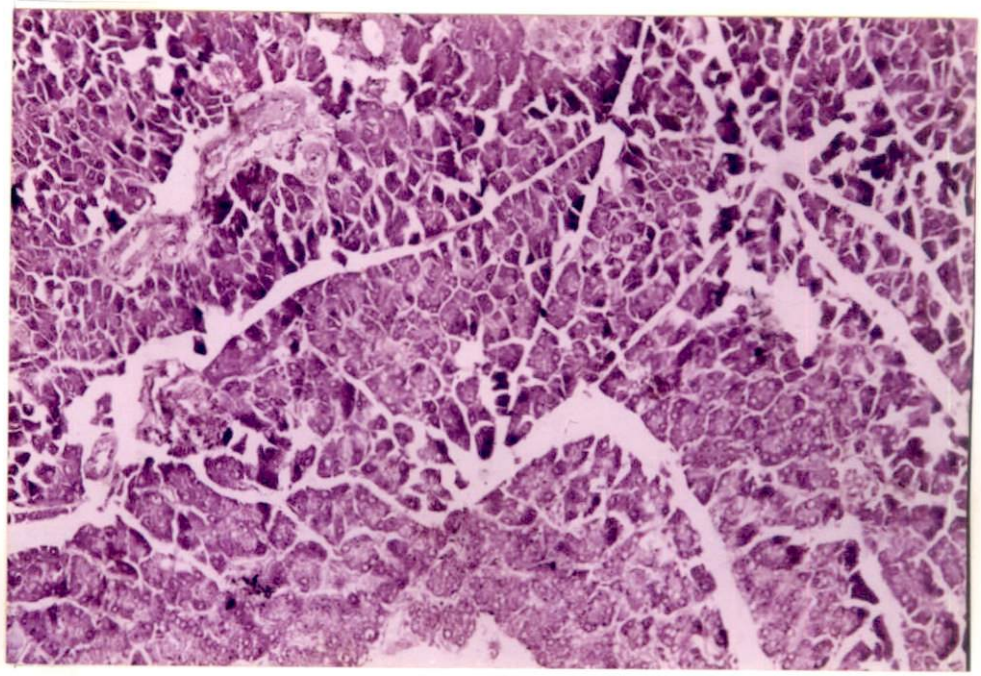


Plate XI Group IV - Pancreas showing distention of interlobular space - H&E x 200

Plate XII Group IV - Myocardium with interstitial oedema - H&E x 400



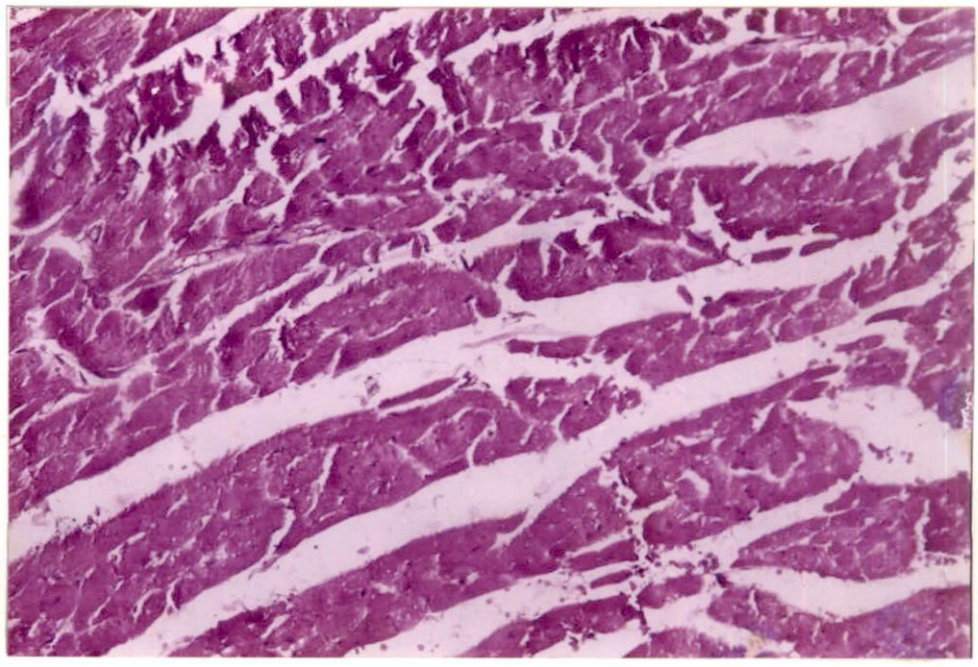
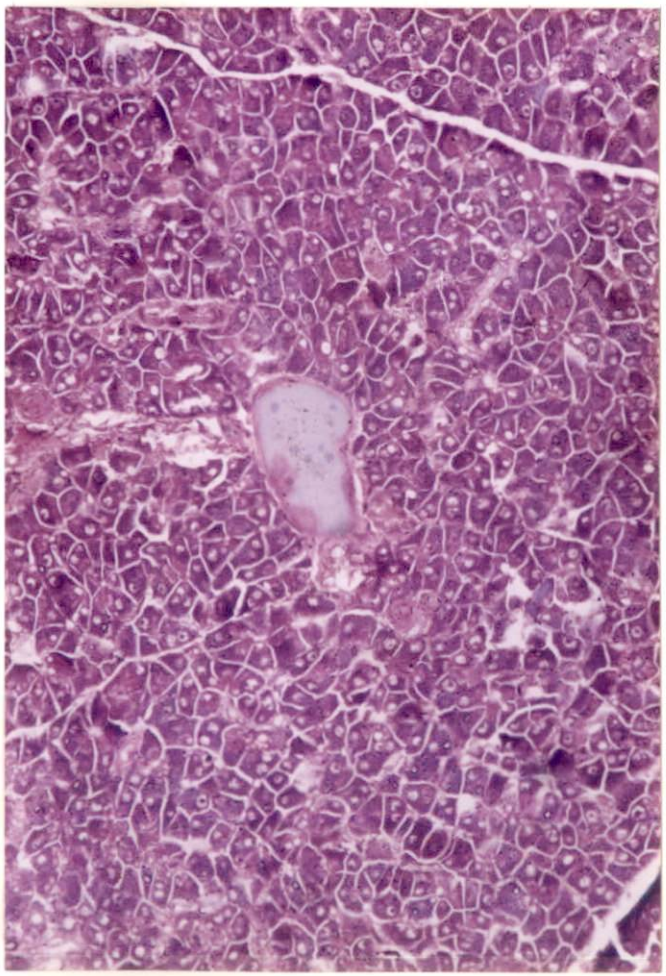


Plate XIII Group V - Granular cytoplasm in a few of the hepatic cells - H&E x 150

Plate XIV Group V - Exocrine part of the pancreas with no cytological alteration - H&E x 250



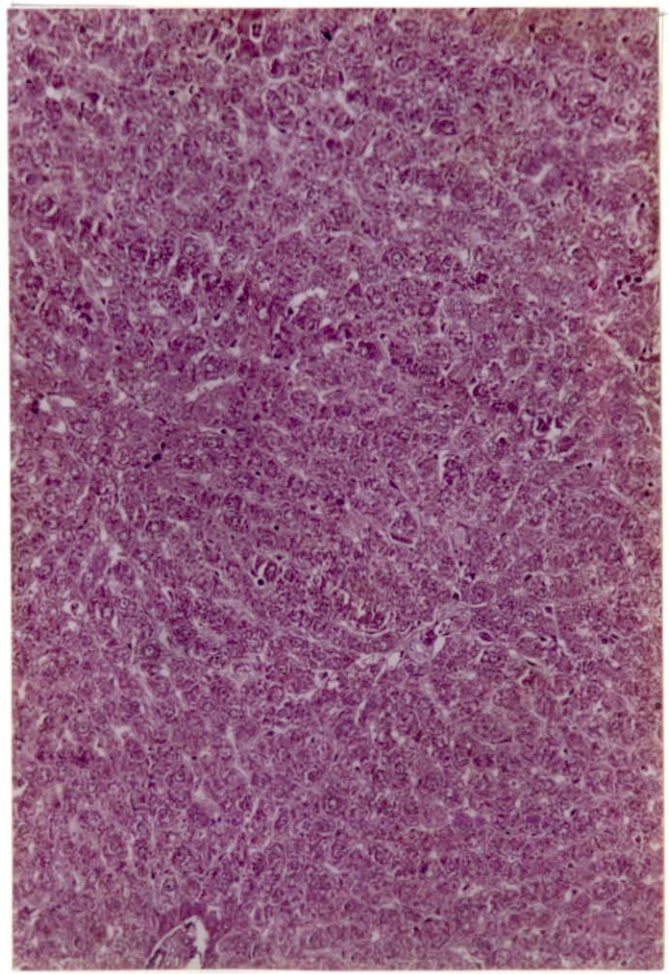
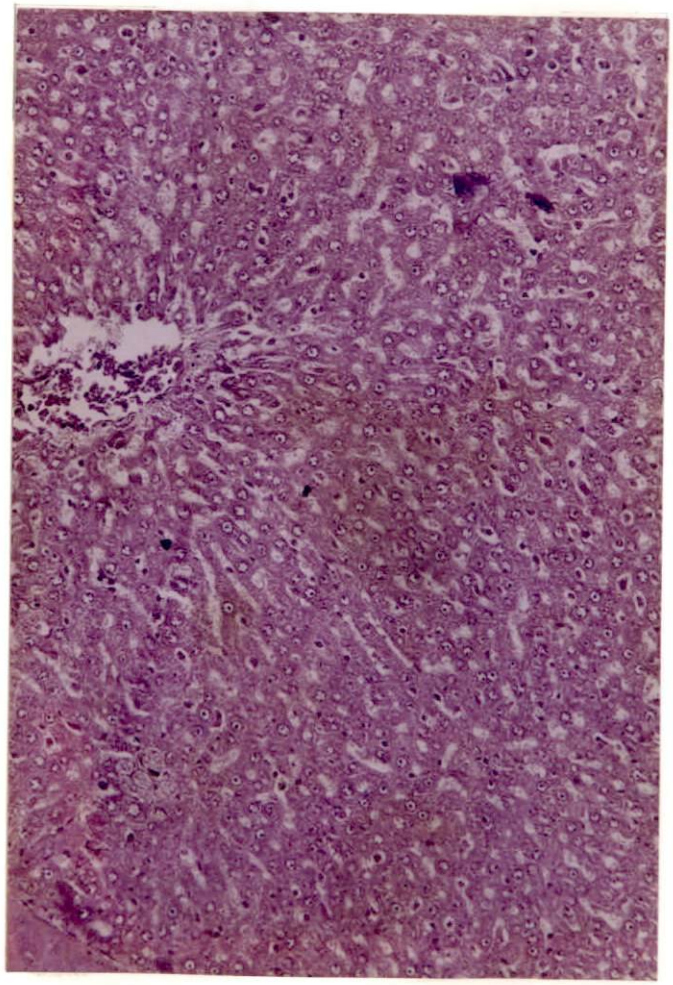


Plate XV Group V - Myocardium with no significant cytological alteration - H&E x 250

Plate XVI Group VI - Normal hepatic cells - H&E x 150



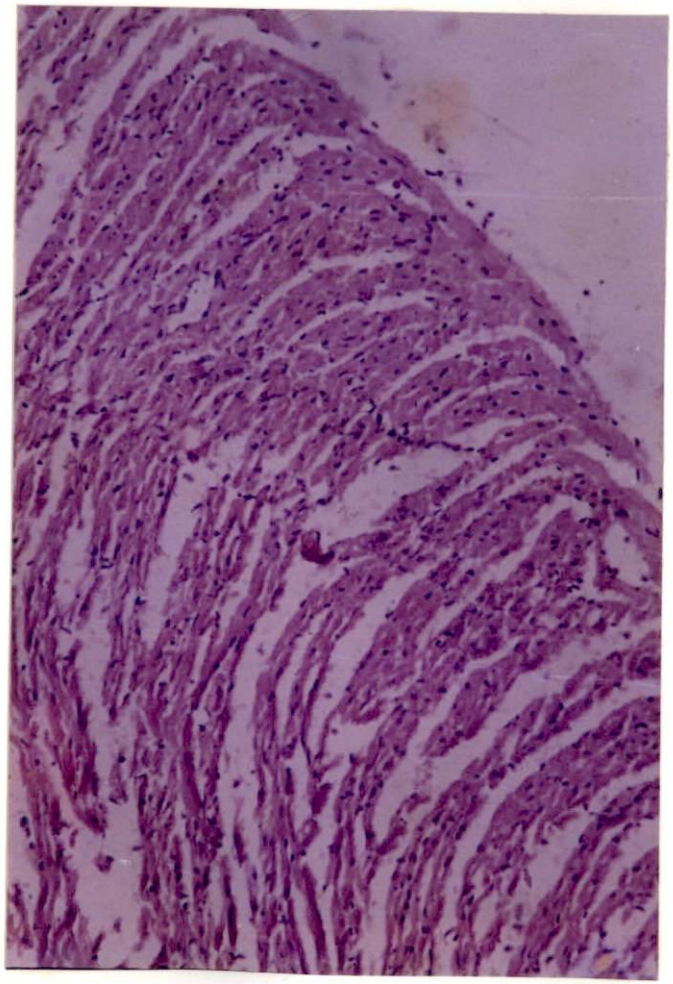
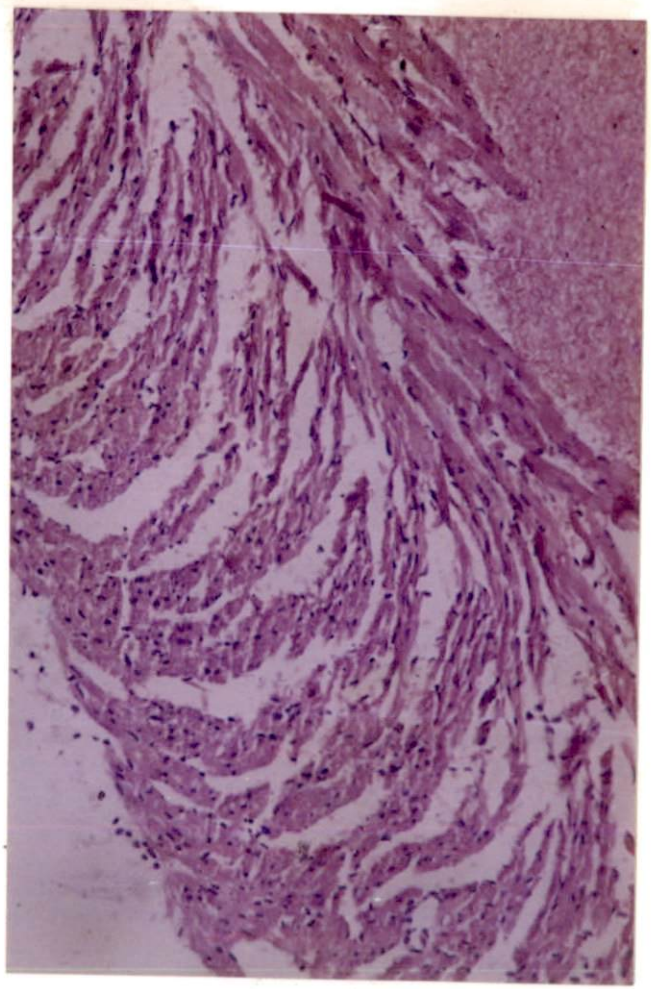
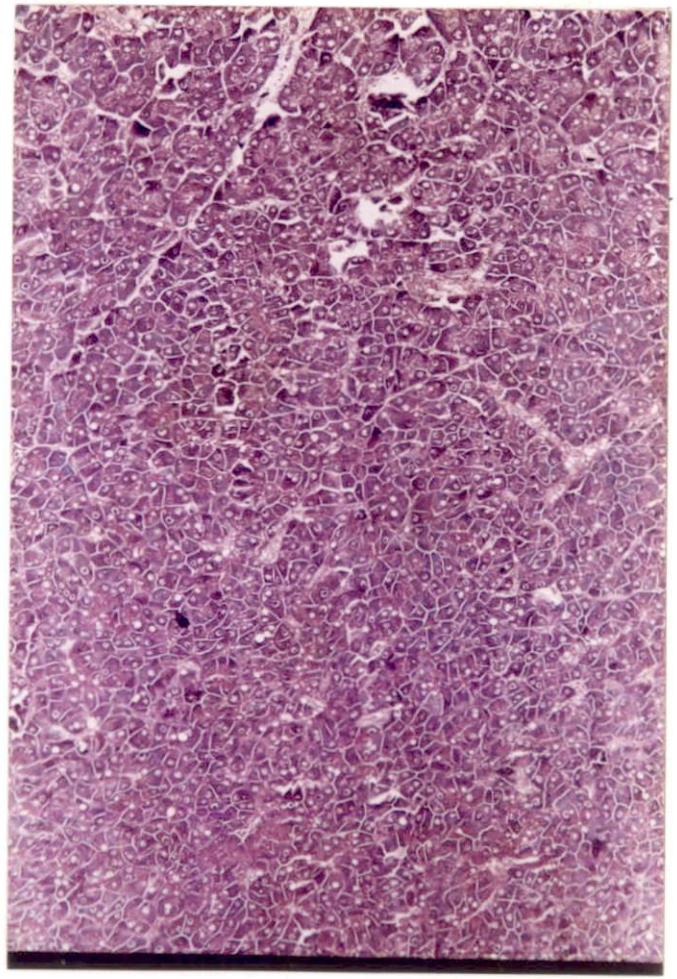


Plate XVII Group V - Normal exocrine region of the pancreas - H&E x 150

Plate XVIII Group V - Myocardial fibres with no alteration - H&E x 250





alterations (Plate XVI). The pancreas (Plate XVII) and myocardium appeared histologically normal (Plate XVIII).

The organs in Group I did not show any significant pathologic changes (Plates I, II and III).

4.3 Experiment in kids

Performance of kids on two levels of protein in a tapioca-based diet supplemented with iodine was evaluated against a control group maintined with normal diet without tapioca. The study was conducted on 3 groups of kids as given below.

Group No.	Diet
I	Protein = 15%; Tapioca = Nil; Iodine = Nil
II	Protein = 15%; Tapioca = 30%; Iodine = 2 mg/kg
III	Protein = 25%; Tapioca = 30%; Iodine = 2 mg/kg

4.3.1 Growth studies

4.3.1.1 Body weight gain

The development in body weight at fortnightly intervals in the three groups of kids is given in Table 32 and is presented in Fig.3.

Fig.3 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON BODY WEIGHT OF KIDS

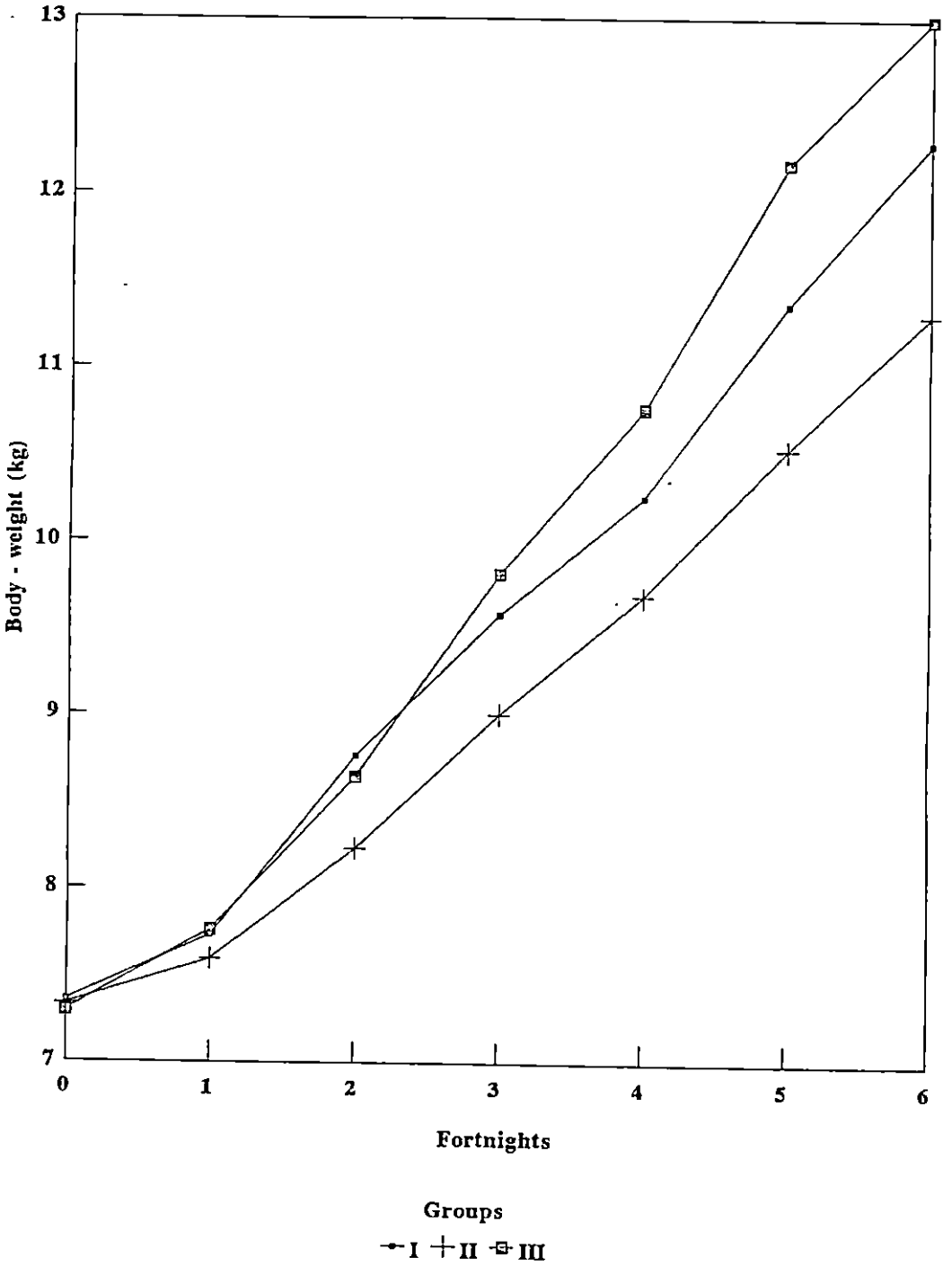


Table 32. Influence of different levels of protein, tapioca and iodine on body weight recorded at fortnightly interval in kids

Group No.	Diets	Period (Fortnight)						
		0	1	2	3	4	5	6
I	15% protein with nil tapioca	7.35± 0.48	7.73± 0.51	8.76± 0.53	9.57± 0.56	10.25± 0.60	11.36± 0.61	12.29± 0.68
II	15% protein with 30% tapioca + iodine @ 2 mg/kg feed	7.33± 0.50	7.59± 0.55	8.23± 0.60	9.00± 0.62	9.68± 0.65	10.53± 0.71	11.30± 0.78
III	25% protein with 30% tapioca + iodine @ 2 mg/kg feed	7.29± 0.40	7.76± 0.45	8.64± 0.50	9.81± 0.54	10.76± 0.62	12.17± 0.67	13.00± 0.79

Values are average of 10 observations ± S.E

The mean total weight gain calculated over the entire experimental period was 4.94 ± 0.31 kg in Group I which did not differ significantly from the mean total weight gain of Group II (3.97 ± 0.45 kg) and Group III (5.71 ± 0.61 kg). However, the mean total weight gain recorded in Group III was significantly ($P<0.05$) higher than Group II (Table 34 and 35).

4.3.1.2 Dry matter intake

The average daily dry matter intake during a fortnight of trial period is given in Table 33 and Fig.4.

The dry matter intake calculated over the entire period of the experiment was 3.56 ± 0.12, 3.72 ± 0.16 and 3.71 ± 0.09 kg per 100 kg body weight for kids in Groups I, II and III respectively evincing absence of any significant difference between themselves (Table 34 and 35).

4.3.1.3 Feed efficiency

The value of feed by gain (kg dry matter per kg live weight gain) calculated over the entire period was increased in Group II but it did not differ significantly from the other groups (Tables 34 and 35).

Fig.4 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON DAILY DRY MATTER INTAKE (kg/100kg BODY WEIGHT) OF KIDS

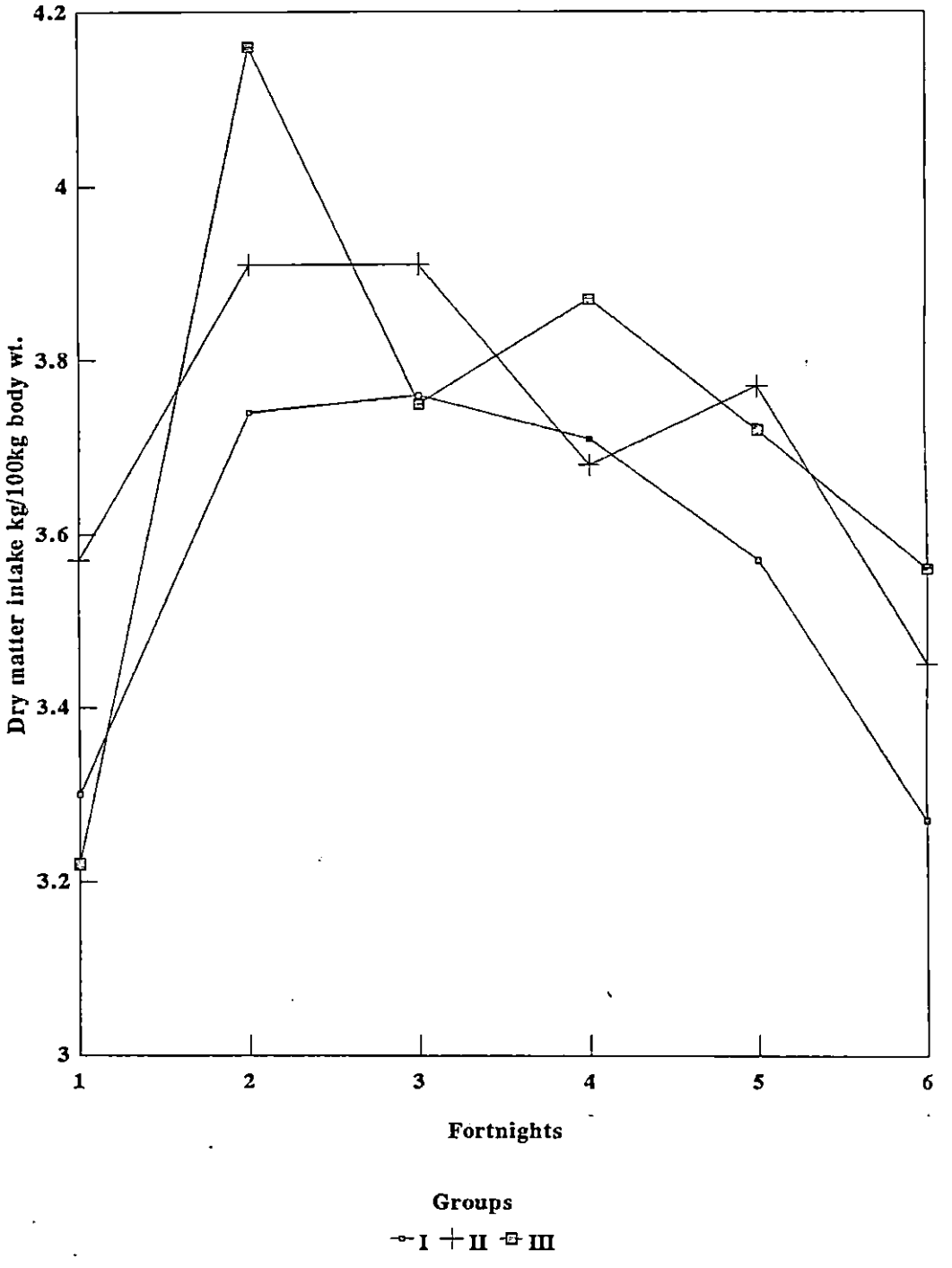


Table 33. Influence of levels of protein, tapioca and iodine on daily dry matter intake (kg/100 kg body weight) of kids during a fortnight

Group No.	Diets	Period (Fortnight)						
		1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	3.30± 0.13	3.74± 0.17	3.76± 0.12	3.71± 0.16	3.57± 0.13	3.27± 0.12	3.56± 0.12
II	15% protein with 30% tapioca and iodine @ 2 mg/kg	3.57± 0.20	3.91± 0.18	3.91± 0.15	3.68± 0.15	3.77± 0.19	3.45± 0.16	3.72± 0.16
III	25% protein with 30% tapioca and iodine @ 2 mg/kg	3.22± 0.21	4.16± 0.09	3.75± 0.14	3.87± 0.15	3.72± 0.17	3.56± 0.12	3.71± 0.09

Values are average of 10 observations ± S.E

Table 34. Average body weight (initial and final), total weight gain, daily weight gain, dry matter intake and feed gain ratio of kids under different levels of protein, tapioca and iodine in the diet

Group No.	Diets	Initial body weight (kg)	Final body weight (kg)	Total weight gain (kg)	Average daily weight gain (g)	Dry matter intake (kg/100 kg body weight	Feed-gain ratio
I	15% protein with nil tapioca	7.35± 0.48	12.29± 0.68	4.94± 0.31 ab	54.89± 0.45 ab	3.56± 0.12	6.55± 0.39
II	15% protein with 30% tapioca and iodine @ 2 mg/kg	7.33± 0.50	11.30± 0.78	3.97± 0.45 b	44.11± 5.01 b	3.72± 0.16	8.48± 0.88
III	25% protein with 30% tapioca and iodine @ 2 mg/kg	7.29± 0.40	13.00± 0.79	5.71± 0.61 a	63.44± 6.79 a	3.71± 0.09	6.73± 0.87

Values are average of 10 observations ± S.E

Means in columns with same superscripts are not significantly different at P<0.05

Table 35. Mean squares showing the significance in average body weight (initial and final), total weight gain, daily weight gain, dry matter intake and feed-gain ratio in kids

Source of variation	Degrees of freedom	Mean squares					
		Variables					
		Initial body weight	Final body weight	Total weight gain	Average daily weight gain	Dry matter intake	Feed-gain ratio
Between	2	NS 0.008	NS 7.380	* 7.561	* 933.383	NS 0.083	NS 11.374
Within	27	2.134	5.699	2.245	277.058	0.155	5.612

NS (Non significant) * ($P<0.05$)

4.3.2 Endocrinological studies

4.3.2.1 Serum thyroxine (T_4)

The mean serum thyroxine values of the three groups of kids at fortnightly intervals are shown in Table 36 and Fig.5.

Over the entire experimental period the mean levels of T_4 were 5.21 $\pm$ 0.30, 4.75 $\pm$ 0.29 and 5.26 $\pm$ 0.38 µg/dl respectively in Groups I, II and III (Table 43) with no significant difference between the groups (Table 44).

4.3.2.2 Serum insulin

The mean serum insulin levels in the three groups of kids at fortnightly intervals are given in Table 37 and presented in Fig.6.

There was no significant difference in the serum insulin levels over the entire trial period (Table 44). The overall serum insulin levels were 10.30 $\pm$ 0.52, 11.28 $\pm$ 0.72 and 11.05 $\pm$ 0.98 µU/ml in Groups I, II and III respectively (Table 43).

4.3.3 Biochemical studies

4.3.3.1 Blood glucose

The data on blood glucose levels during the experimental period are shown in Table 38 and Fig.7.

Fig.5 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON SERUM THYROXINE LEVEL IN KIDS

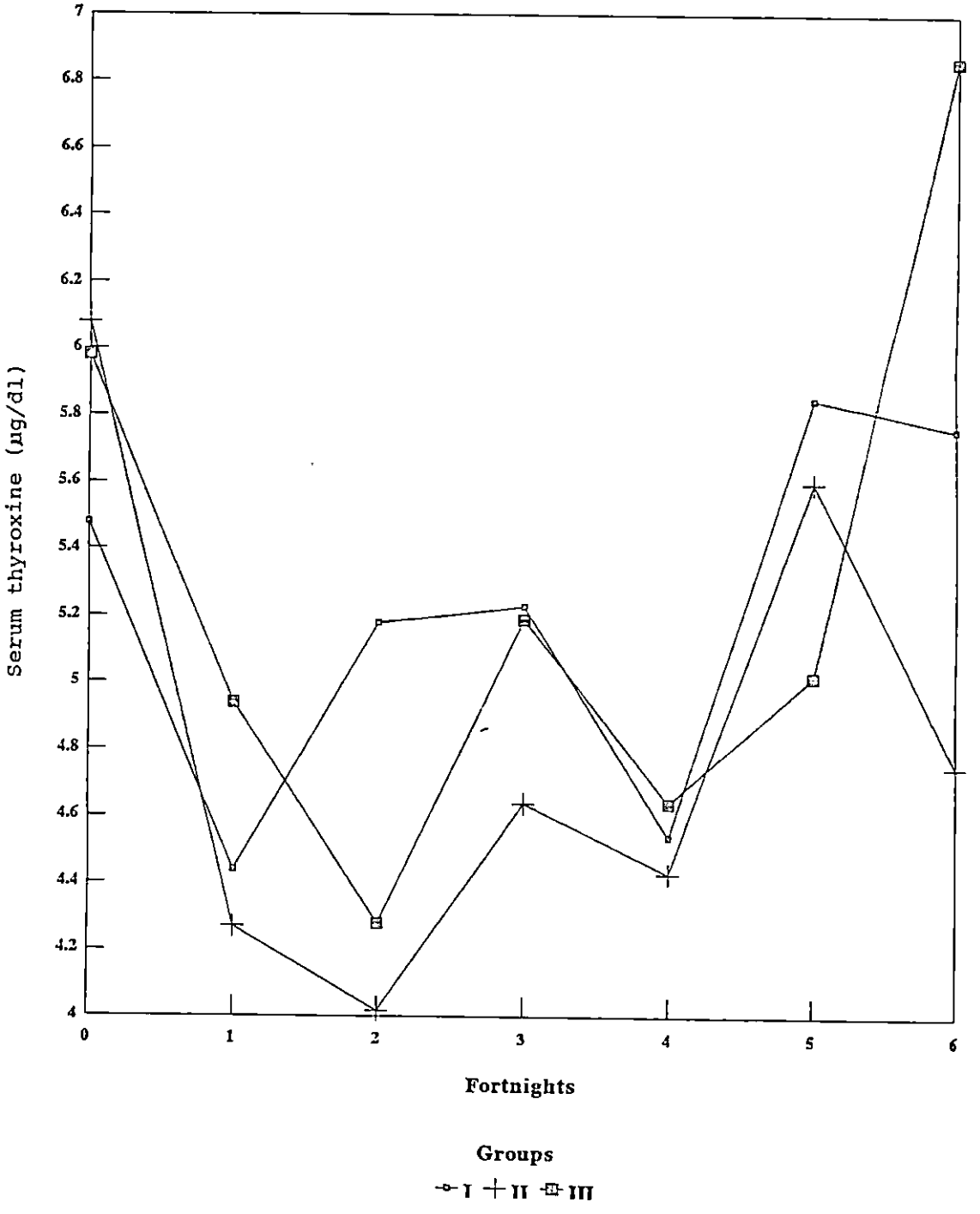


Table 36. Influence of different levels of protein, tapioca and iodine on serum thyroxine concentration (µg/dl) at fortnightly interval in kids

Group No.	Diets	Period (Fortnight)							
		0	1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	5.48± 0.37	4.44± 0.27	5.18± 0.52	5.23± 0.49	4.54± 0.56	5.85± 0.47	5.76± 0.41	5.21± 0.30
II	15% protein with 30% tapioca + iodine @ 2 mg/kg feed	6.08± 0.55	4.27± 0.84	4.02± 0.46	4.64± 0.16	4.43± 0.36	5.60± 0.53	4.75± 0.54	4.75± 0.29
III	25% protein with 30% tapioca + iodine @ 2 mg/kg feed	5.98± 0.91	4.94± 0.54	4.28± 0.30	5.19± 0.41	4.64± 0.60	5.02± 0.41	6.86± 1.03	5.26± 0.38

Values are average of 10 observations ± S.E

Fig.6 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON SERUM INSULIN LEVEL IN KIDS

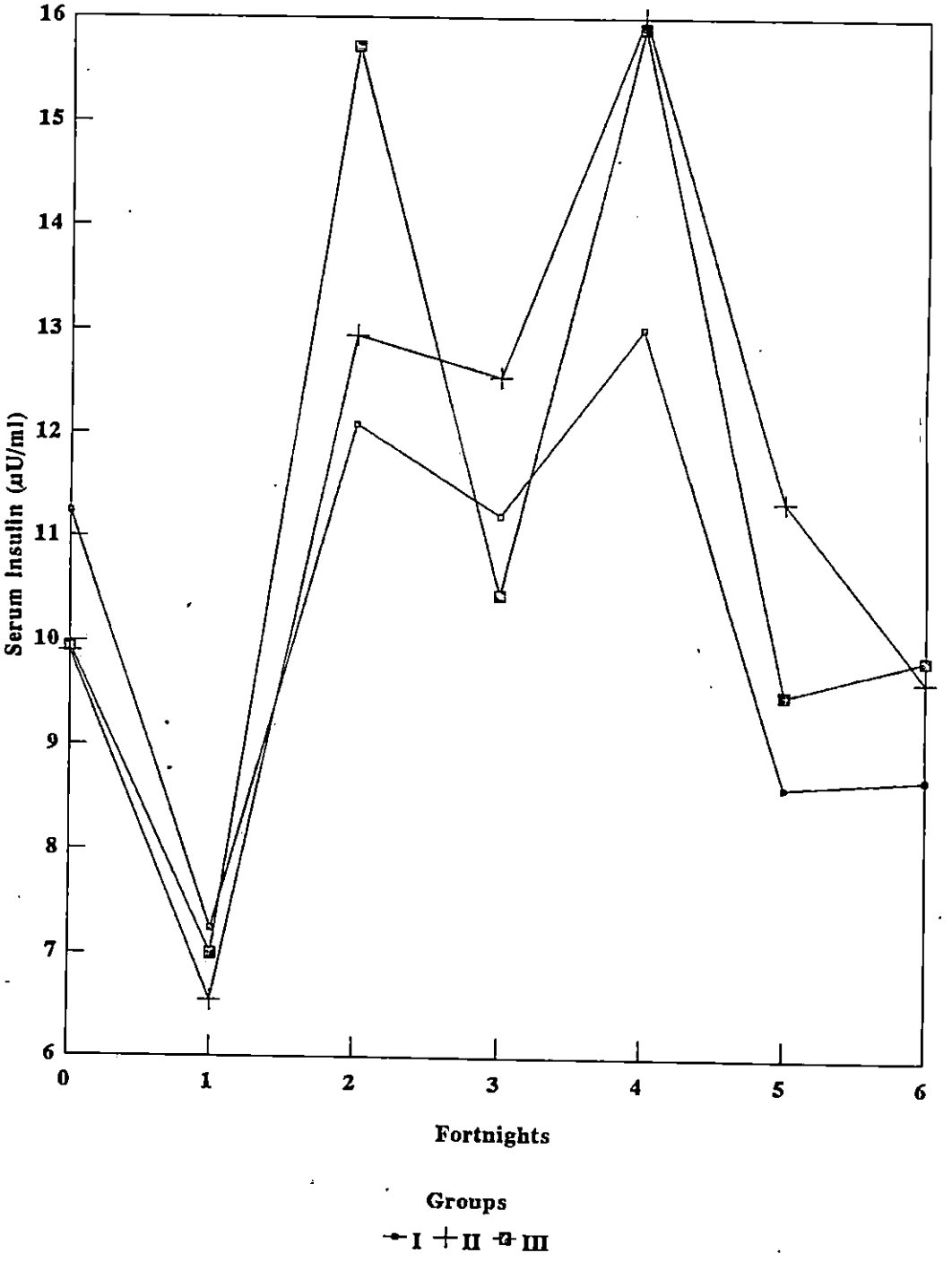


Table 37. Influence of different levels of protein, tapioca and iodine on serum insulin concentration (μU/ml) at fortnightly interval in kids

Group No.	Diets	Period (Fortnight)							
		0	1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	11.24± 1.02	7.24± 0.91	12.09± 0.97	11.21± 1.01	13.02± 0.82	8.61± 0.73	8.70± 0.78	10.30± 0.52
II	15% protein with 30% tapiocn and iodine @ 2 mg/kg	9.90± 0.95	6.55± 1.19	12.95± 1.57	12.55± 1.55	16.00± 1.44	11.35± 1.67	9.64± 0.99	11.28± 0.72
III	25% protein with 30% tapioca and iodine @ 2 mg/kg	9.94± 1.77	7.00± 1.46	15.72± 3.74	10.45± 0.92	15.90± 2.73	9.50± 0.79	9.85± 2.15	11.05± 0.98

Values are average of 10 observations ± S.E

Fig.7 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON BLOOD GLUCOSE LEVEL IN KIDS

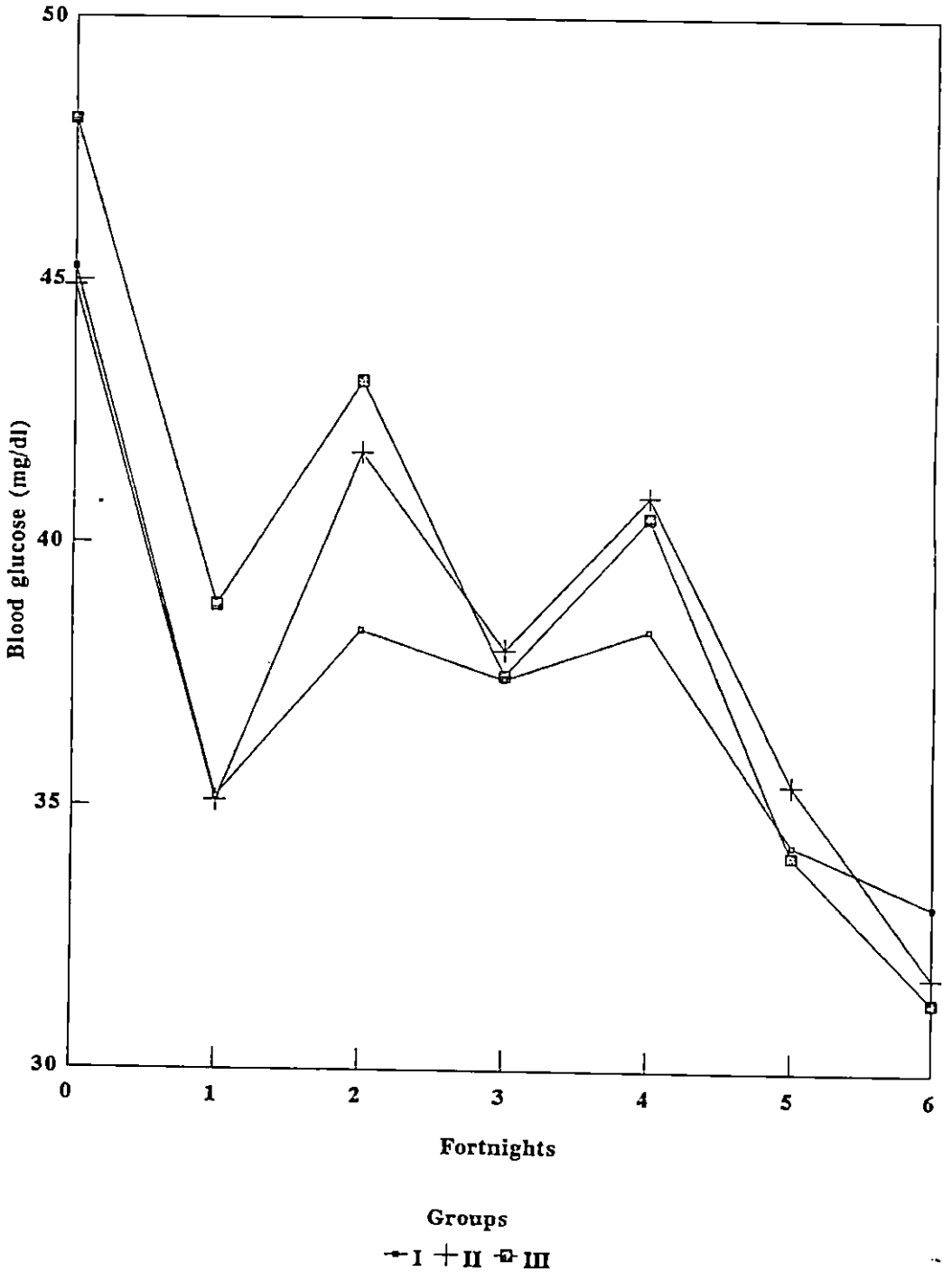


Table 38. Influence of different levels of protein, tapioca and iodine on blood glucose concentration (mg/dl) at fortnightly interval in kids

Group No.	Diets	Period (Fortnight)							
		0	1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	45.25± 1.78	35.18± 1.21	38.35± 2.45	37.43± 1.77	38.36± 2.19	34.20± 2.82	33.16± 2.14	37.42± 0.93
II	15% protein with 30% tapioca and iodine @ 2 mg/kg	44.91± 1.71	35.10± 2.48	41.73± 2.45	37.98± 1.65	40.90± 1.59	35.44± 2.72	31.81± 1.98	38.27± 1.16
III	25% protein with 30% tapioca and iodine @ 2 mg/kg	48.05± 3.81	38.82± 2.48	43.10± 1.84	37.50± 1.95	40.50± 2.84	34.10± 1.30	31.35± 1.40	39.05± 0.95

Values are average of 10 observations ± S.E

There was no significant difference in the overall blood glucose concentration in the groups of kids studied (Tables 43 and 44).

4.3.3.2 Serum total protein

The serum total protein values recorded for the three groups of kids at fortnightly intervals are set out in Table 39 and is shown in Fig.8.

Over the entire trial period the mean levels of serum total protein were 7.03 ± 0.07, 6.92 ± 0.13 and 7.27 ± 0.11 g/dl in Groups I, II and III respectively (Table 43) evincing no significant difference between the groups (Table 44).

4.3.3.3 Serum total cholesterol

The mean serum total cholesterol concentrations recorded at fortnightly intervals in three groups of kids are given in Table 40 and presented in Fig.9.

The mean serum total cholesterol levels over the entire experimental period were almost similar in the groups studied (Table 43). The levels recorded were 113.98 ± 3.02 (Group I), 118.00 ± 4.60 (Group II) and 112.11 ± 2.55 mg/dl (Group III).

Fig.8 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON SERUM TOTAL PROTEIN LEVEL IN KIDS

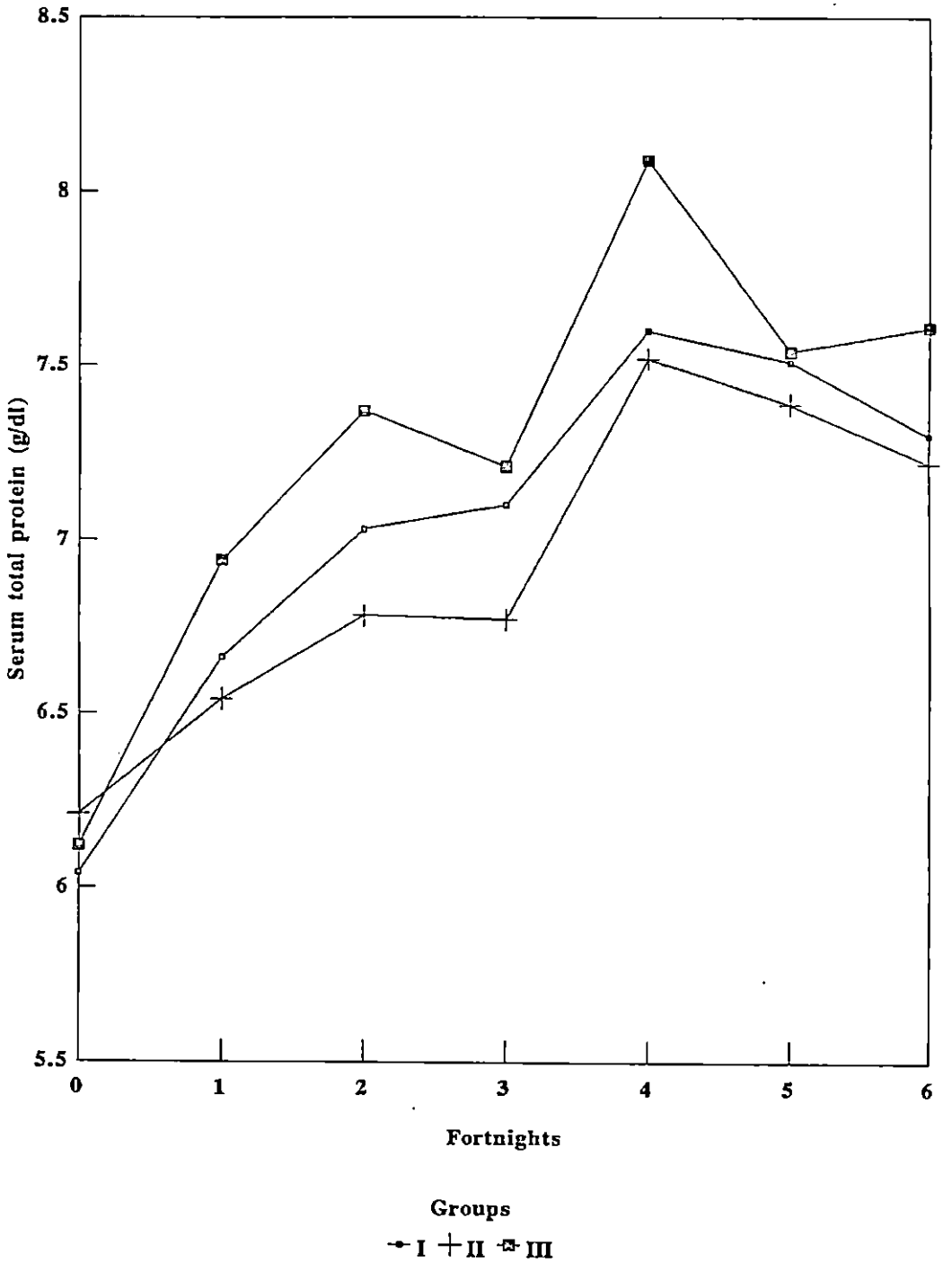


Table 39. Influence of different levels of protein, tapioca and iodine on serum total protein concentration (g/dl) at fortnightly intervals in kids

Group No.	Diets	Period (Fortnight)							
		0	1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	6.04± 0.12	6.66± 0.21	7.03± 0.19	7.10± 0.13	7.60± 0.19	7.51± 0.14	7.30± 0.16	7.03± 0.07
II	15% protein + 30% tapioca and iodine @ 2 mg/kg	6.21± 0.20	6.54± 0.26	6.78± 0.25	6.77± 0.22	7.52± 0.22	7.39± 0.21	7.22± 0.22	6.92± 0.13
III	25% protein + 30% tapioca and iodine @ 2 mg/kg	6.12± 0.24	6.94± 0.26	7.37± 0.27	7.21± 0.28	8.09± 0.21	7.54± 0.18	7.61± 0.15	7.27± 0.11

Values are average of 10 observations ± S.E

Fig.9 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON SERUM TOTAL CHOLESTEROL LEVEL IN KIDS

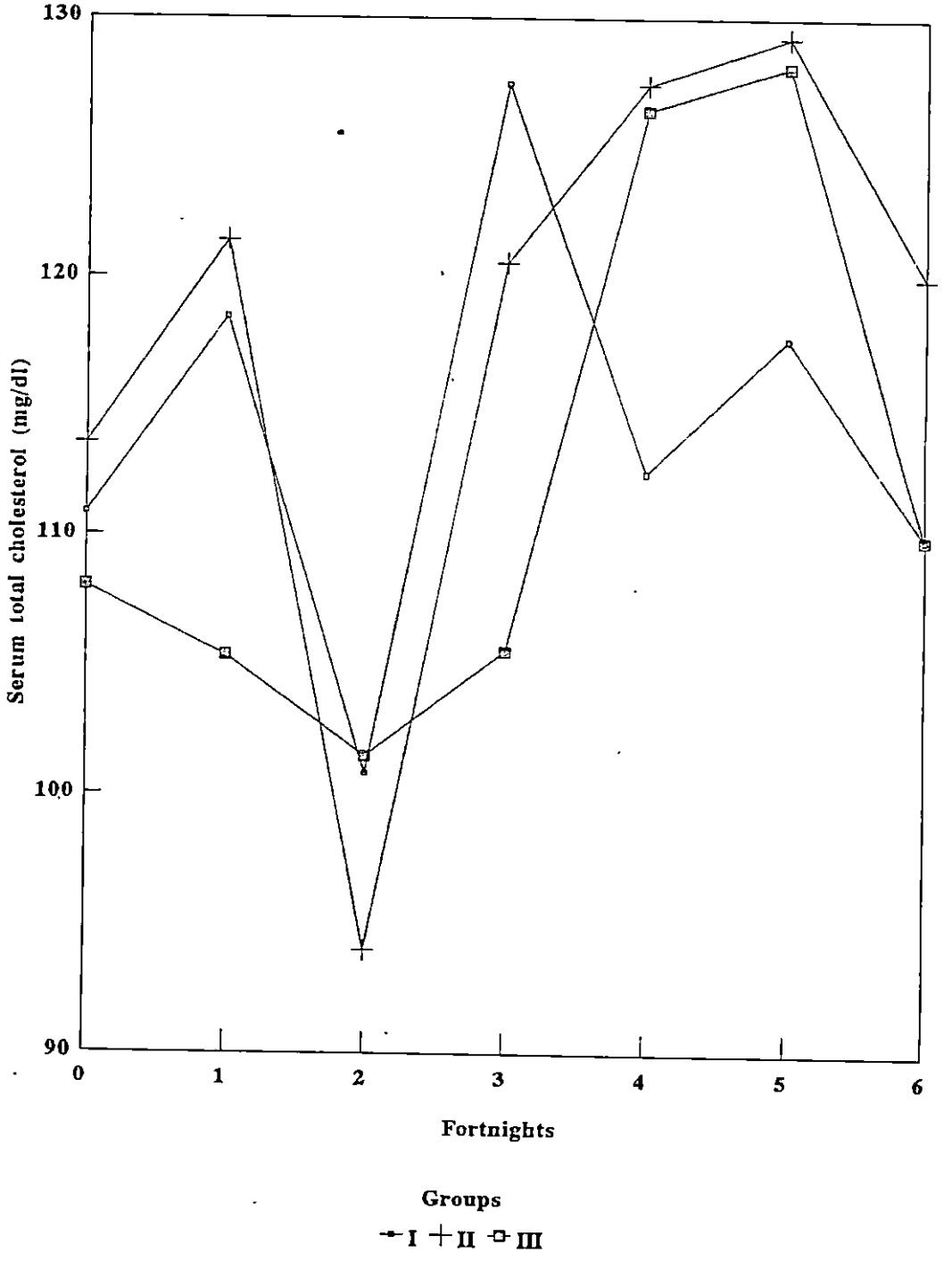


Table 40. Influence of different levels of protein, tapioca and iodine on serum total cholesterol concentration (mg/dl) at fortnightly interval in kids

Group No.	Diets	Period (Fortnight)							
		0	1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	110.85± 4.44	118.44± 7.94	100.82± 6.64	127.45± 3.32	112.43± 2.08	117.62± 5.79	109.89± 7.84	113.98± 3.02
II	15% protein with 30% tapioca + iodine @ 2 mg/kg feed	113.56± 3.49	121.39± 5.27	93.95± 3.05	120.53± 5.66	127.41± 10.02	129.23± 6.21	119.97± 6.57	118.00± 4.60
III	25% protein with 30% tapioca + iodine @ 2 mg/kg feed	108.00± 2.92	105.35± 4.20	101.48± 6.88	105.51± 4.27	126.44± 3.67	128.11± 3.71	109.88± 3.99	112.11± 2.55

Values are average of 10 observations ± S.E

4.3.3.4 Serum total lipid

The mean group values of serum total lipid at fortnightly intervals in three groups of kids are shown in Table 41 and Fig.10.

The three groups of kids under study showed no significant variation in their serum total lipid throughout the experimental period. The mean overall levels of serum total lipid in Groups I, II and III were 208.07 $\pm$ 7.43, 211.07 $\pm$ 7.24 and 209.33 $\pm$ 3.87 mg/dl respectively (Tables 43 and 44).

4.3.3.5 Haemoglobin

The mean groups values for haemoglobin recorded at fortnightly intervals in kids are shown in Table 42 and Fig.11.

The mean overall haemoglobin levels in the three groups of kids were 8.64 $\pm$ 0.14 (Group I), 8.60 $\pm$ 0.20 (Group II) and 8.71 $\pm$ 0.17 (Group III) and the differences between the groups were not significant (Tables 43 and 44).

Fig.10 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON SERUM TOTAL LIPID LEVEL IN KIDS

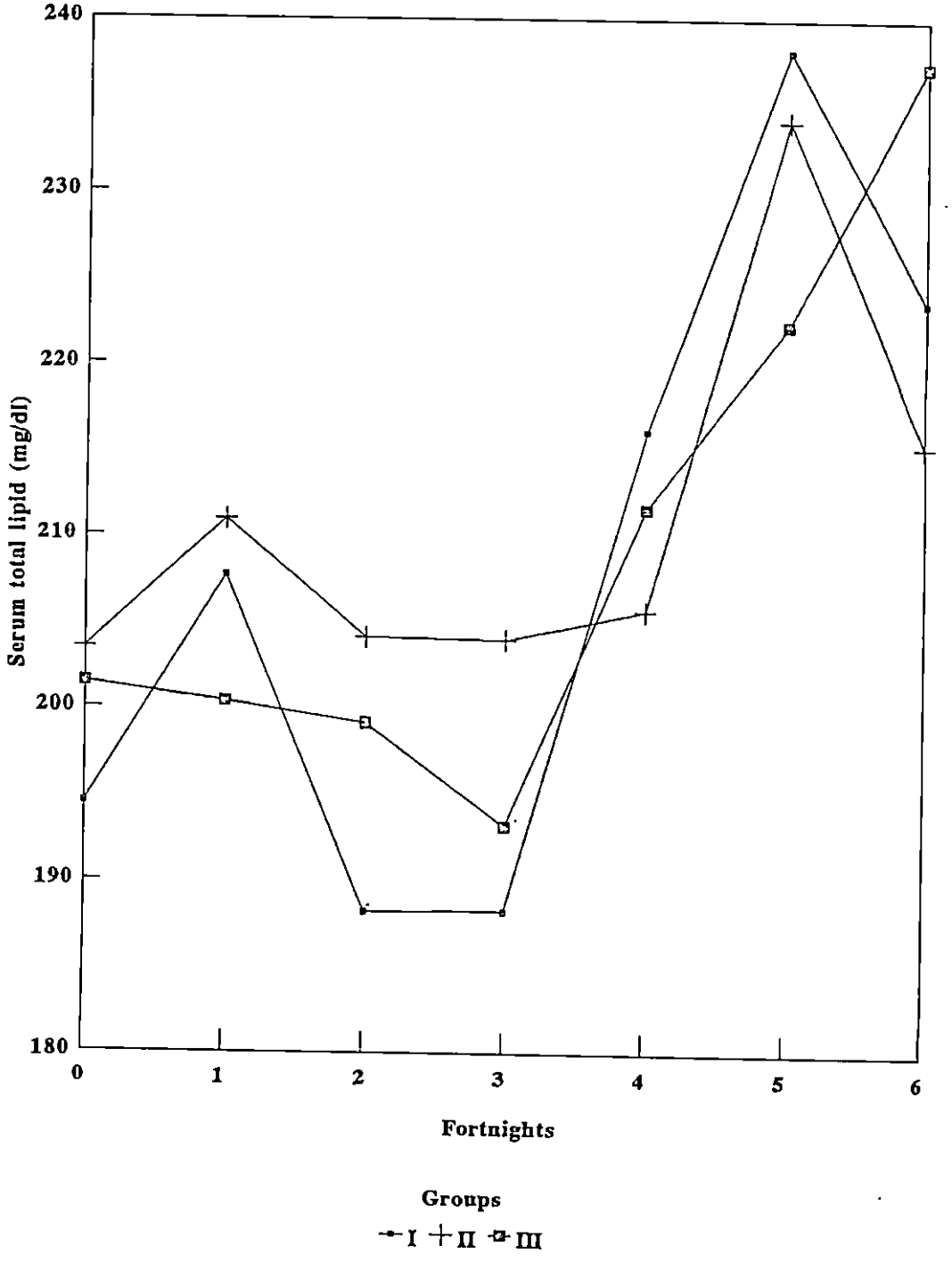


Table 41. Influence of different levels of protein, tapioca and iodine on serum total lipid concentration (mg/dl) at fortnightly interval in kids

Group No.	Diets	Period (Fortnight)							
		0	1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	194.49± 8.86	207.66± 10.91	188.22± 5.89	188.27± 4.85	216.12± 13.47	238.15± 11.32	223.62± 10.69	208.07± 7.43
II	15% protein with 30% tapioca + iodine @ 2 mg/kg	203.46± 9.46	210.93± 15.20	204.10± 9.13	203.96± 10.93	205.67± 10.32	234.11± 7.62	215.30± 10.47	211.07± 7.24
III	25% protein with 30% tapioca + iodine @ 2 mg/kg	201.46± 4.53	200.35± 5.62	199.11± 5.70	193.17± 5.83	211.63± 5.18	222.28± 5.54	237.32± 8.41	209.33± 3.87

Values are average of 10 observations ± S.E

Fig.11 INFLUENCE OF DIETARY PROTEIN, TAPIOCA AND IODINE ON HAEMOGLOBIN LEVEL IN KIDS

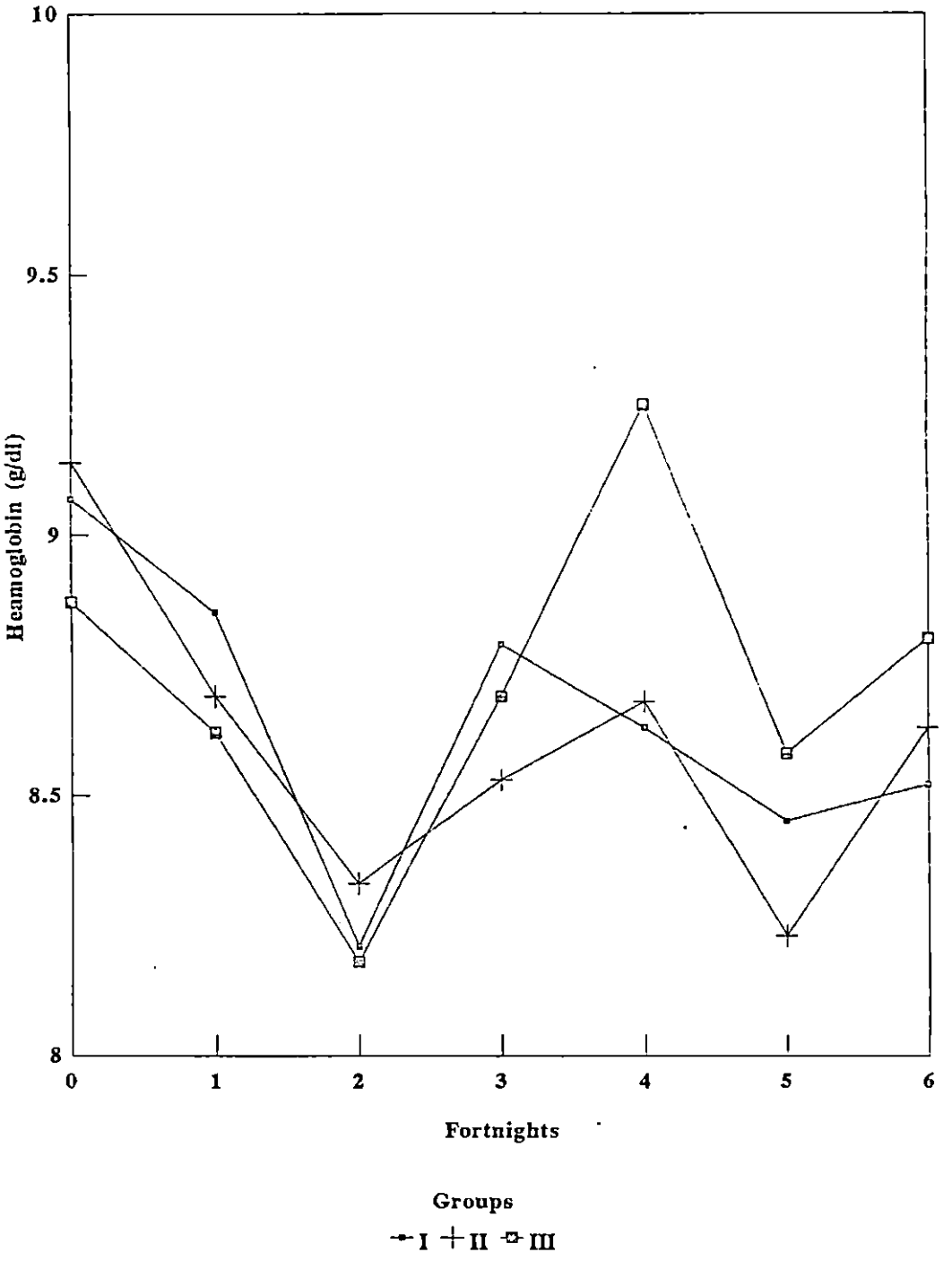


Table 42. Influence of different levels of protein, tapioca and iodine on haemoglobin concentration (g/dl) at fortnightly interval in kids

Group No.	Diets	Period (Fortnight)							
		0	1	2	3	4	5	6	Entire
I	15% protein with nil tapioca	9.07± 0.28	8.85± 0.27	8.21± 0.11	8.79± 0.11	8.63± 0.17	8.45± 0.17	8.52± 0.13	8.64± 0.14
II	15% protein with 30% tapioca + iodine @ 2 mg/kg feed	9.14± 0.32	8.69± 0.34	8.33± 0.39	8.53± 0.31	8.68± 0.23	8.23± 0.18	8.63± 0.28	8.60± 0.20
III	25% protein with 30% tapioca + iodine @ 2 mg/kg feed	8.87± 0.31	8.62± 0.26	8.18± 0.24	8.69± 0.25	9.25± 0.17	8.58± 0.23	8.80± 0.14	8.71± 0.17

Values are average of 10 observations ± S.E

Table 43. Summarised data on blood metabolites in kids fed different levels of protein, tapioca and iodine (mean group values calculated over the entire experimental period)

Group No.	Diets	Serum thyroxine (µg/dl)	Serum insulin (µU/ml)	Blood glucose (mg/dl)	Serum total protein (g/dl)	Serum total cholesterol (mg/dl)	Serum total lipid (mg/dl)	Haemo-globin (g/dl)
I	15% protein with nil tapioca	5.21± 0.30	10.30± 0.52	37.42± 0.93	7.03± 0.07	113.98± 3.02	208.07± 7.43	8.64± 0.14
II	15% protein with 30% tapioca and iodine @ 2 mg/kg	4.75± 0.29	11.28± 0.72	38.27± 1.16	6.92± 0.13	118.00± 4.60	211.07± 7.24	8.60± 0.20
III	25% protein with 30% tapioca and iodine @ 2 mg/kg	5.26± 0.38	11.05± 0.98	39.05± 0.95	7.27± 0.11	112.11± 2.55	209.33± 3.87	8.71± 0.17

None of the mean differ significantly

Table 44. Mean squares showing significance in blood metabolites calculated over the entire period of the study in kids

Source of variation	Degrees of freedom	Mean squares (variables)						
		Serum thyroxine	Serum insulin	Blood glucose	Serum total protein	Serum total cholesterol	Serum total lipid	Haemo-globin
Between	2	0.779	2.609	6.670	0.318	90.711	22.804	0.031
Within	27	1.058	5.810	10.452	0.113	122.521	408.361	0.301

None of the mean differ significantly

DISCUSSION

CHAPTER 5

DISCUSSION

5.1 Determination of cyanogenic glycoside (CNG) content of fresh and processed tapioca of different varieties

The information on the CNG content of different varieties of tapioca and the extent of its removal by different methods of processing of the tubers, are scanty.

5.1.1 Variation in CNG content in fresh samples of different varieties of tapioca

A significant variation in CNG content was observed among the different varieties of tapioca studied. Since a positive correlation exists between the degree of bitterness and CNG content of different tapioca varieties they are classified as non bitter, bitter and very bitter according to low, medium or high content of cyanide in their tubers (Nambisan and Sundaresan, 1991). According to them the CNG content varies from 27.5 to 77.5 µg cyanide/g of fresh non bitter tubers, and 100 to 180 µg cyanide/g of fresh bitter tubers and 320-1100 µg cyanide/g of fresh tuber in very bitter varieties. This confirms that among the varieties studied 'H-165' (182.6 $\pm$ 10 µg cyanide/g fresh tuber) and 'Karkidakkan' (186.31 $\pm$ 5.3 µg cyanide/g fresh tuber) fall

under bitter varieties while other varieties (40.86 to 85.19 ug cyanide/g fresh tuber) studied can be regarded as non bitter (Table 4). Although very little data are available on the CNG content of different varieties of tapioca, the CNG content found in the present investigation for 'M-4', 'Sree Visakh' and 'Sree Sahya' are quite comparable with those reported by Nambisan and Sundaresan (1985, 1991). However, the levels reported for 'H-165' (140 to 160 ug cyanide/g tuber) appeared to be lower than that observed in the present study (Table 4). Comparatively higher levels of CNG observed in 'H-165' in the present study may be ascribed to environmental factors such as differences in soil and temperature (DeBruijn, 1973). The information on CNG content of other varieties (Sree Prakash, Thottakolly and Karkidakkan) is lacking from the available literature.

5.1.2 Effect of boiling on CNG content of tapioca

In the present study, when tapioca samples were cooked by boiling in water, on an average 50 per cent retention of CNG was observed in most of the varieties studied except Thottakolly and 'H-165' (Table 4). In both these varieties CNG were lost to the extent of about 64 per cent and 59 per cent respectively. At boiling temperatures, the enzyme linamarase which hydrolyses cyanogenic glycosides is inactivated rapidly and hence in the process of boiling, the

loss of CNG occurs mostly by its solubilization in water. The enzyme degradation of CNG does not contribute significantly during this short period of processing (Nambisan and Sundaresan, 1991). Therefore, it is possible that while boiling, CNG in the samples of 'Thottakolly' and 'H-165' might have got solubilized and lost more than the CNG present in the tubers of other varieties. The extent of retention of CNG observed in other varieties particularly 'M-4', 'Sree Visakh' and 'Sree Sahya' is in agreement with those reported by Nambisan and Sundaresan (1985, 1991).

5.1.3 Effect of sun drying on CNG content of tapioca

Results of the study showed that sun drying could reduce the level of CNG in tapioca to an appreciable extent. Nevertheless, this process also could not remove all the CNG from tapioca samples, but leaves behind almost 50 per cent of their initial levels. The extent of retention of CNG in sun dried samples of tapioca in the present investigation was almost similar to that reported by Nambisan and Sundaresan (1985, 1991). It may be assumed that the loss of CNG which occurred in the process of sun drying was due to enzymic degradation of CNG at environmental temperature. This was due to the slower drying which provided ideal conditions for maximum enzymatic degradation of CNG (Nambisan and Sundaresan, 1991). At lower temperatures, like sun drying endogenous

linamarase remains active for longer period of time (Gomez et al., 1984).

5.2 Experiment in rats

5.2.1 Performance of rats

5.2.1.1 Growth studies

5.2.1.1.1 Body weight gain

The results (Table 6) of the study revealed significant differences in total body weight gain which followed the pattern of availability of dietary protein and the levels of thyroxine in different groups studied.

The rats fed on a protein deficient diet (7.5%) with 30 per cent tapioca containing high amounts of cyanide (Group III) showed retarded growth and their mean total body weight gain (Table 6) was only about 55 per cent of that of the Group IV the tapioca of the diet of which contained no cyanide (30% tapioca without cyanide + 7.5% protein). The possible growth inhibiting effect of cyanide (Yoshida et al., 1966) can partly be attributed to utilization of dietary protein for detoxification of cyanide (Annamma Mathew, 1979). Sulfur containing amino acids are required for detoxification of cyanide, thereby aggravating the protein deficiency further (Geevarghese, 1982). Under the circumstances it may be assumed that the rats in Group III were grossly protein

deficient which resulted in significantly lower weight gain compared to that of Group IV. The better performance observed in the case of Group IV with the same amount (7.5%) of protein was therefore, due to the absence of cyanide of tapioca origin sparing the dietary proteins wholly for growth purpose. This may further substantiate the hypothesis that growth inhibiting effect is present in cyanide containing food (Kamalu, 1991). Further, the depressed growth observed in Group III is evidently due to the extremely low levels of serum thyroxine (Table 12) which would result in the slowing of cellular growth. Lenzen et al. (1976) demonstrated that thyroid insufficiency was associated with reduced body weight gain in male albino Wistar rats. According to Schooley et al. (1966), Burstein et al. (1979) and Coiro et al. (1979) hypothyroidism leads to reduced amounts of growth hormone. It is possible that the extremely reduced levels of serum throxine in Group III would have reduced the growth hormone secretion leading to depressed growth.

The growth inhibiting effect of cyanide and thyroid insufficiency can be evident in the case of Group II also (30% tapioca + 15% protein) even though it was not as marked as in Group III (Table 8) since the level of protein in the diet was higher (15%) than that of Group III. Nevertheless, the rats in Group II did not grow as much as Group I (15% protein

without tapioca) and their mean total body weight gain was about 79 per cent of that of Group I. This observation corroborates with the findings of Annamma Mathew (1979) that 27 per cent protein when added with 59 per cent tapioca in the diet did not result in appreciable growth and the total body weight gain of male albino rats was significantly lower than the control (27% protein diet). Similar were the observations made in albino rats (Tewe and Maner, 1980, 1981) and African giant rats (Tewe, 1984) reared on cyanide containing rations.

Even though the amount of cyanide present in the diet of Group V (30% tapioca + 15% protein + iodine) was equal to that of Group II, the growth inhibiting effect of cyanide was not evident in Group V (Table 6). Actually the Group V showed significantly higher body weight gain than Group II (Table 6 and 7). Since the diet of Group V was supplemented with iodine, the iodine in the diet was able to assist thyroxine production (Green, 1971). With normal metabolic rates established, the rats compensated for the lost proteins (utilized in the process of cyanide detoxification) by consuming significantly more feed than those in Group II (Table 6). Under such conditions, it is likely that with a possible synergistic effect of thyroxine with growth hormone, the net growth rate of Group V was maintained resulting in about 17 per cent more weight gain compared to that of

Group II. It may further be noted that the total body weight gain in Group V was almost similar (94%) to that of Group I. Group I was maintained on a diet containing 15 per cent protein only without tapioca. This diet provided sufficient levels of protein for normal rate of growth.

The role of supplemented iodine and protein in preventing the inhibitory effects of cyanide and thiocyanate on thyroid functions and ultimately in promoting growth can be evident in the case of Group VI. The significantly higher body weight gain observed in Group VI (30% tapioca + 22.5% protein + iodine) compared to that of Group V (Table 6) was due to the additional amount of protein when thyroid functions were maintained normal in the presence of excess iodine.

The study reveals that to ensure normal growth of rats on tapioca containing cyanogenic principles sufficient good quality protein and iodine should be incorporated in the diet to prevent protein deficiency and hypothyroidism.

5.2.1.1.2 Total feed consumption (dry matter)

The total feed consumption in all the six groups of rats reared on six different diets showed significant differences (Table 6 and 7).

The lowest feed consumption recorded in Group III (Table 6) could partly be ascribed to lower amounts of dietary proteins (7.5%) combined with tapioca (30%) which contained cyanide causing further reduction in available protein level. In addition to protein deficiency, the overall depression in metabolic rate, evidently due to deficiency of thyroxine (Table 12), could naturally depress the feed consumption. Further, the rats in Group III showed reduced growth, and their mean total body weight gain was about 55 per cent of that of the Group IV (30% cynide-free tapioca + 7.5% protein). Due to lesser body weight and size, the rats in Group III consumed about 16 per cent lesser feed than those in Group IV.

The total feed consumption was also reduced significantly in Group II (30% tapioca + 15% protein) compared to that in Group I (15% protein without tapioca). The reduction in total feed consumption in Group II was also due to reasons ascribed for Group III.

When a diet similar to that of Group II was fed to Group V with supplementation of iodine (to ward off the inhibitory effects of cyanide and thiocyanate on thyroid functions), the rats grew faster (about 17%) and consumed significantly more quantity (about 12%) of feed than Group II (Table 8). Moreover, since the overall metabolic rates were

maintained, the rats in Group V consumed even more feed (about 3%) than those in Group I (Table 6).

When the metabolic rates were maintained, the effect of dietary protein becomes more evident in case of Group VI (30% tapioca + 22.5% protein + iodine). With the supplementation of 7.5 per cent more protein in the diet the total feed consumption was significantly increased in Group VI (Table 6) compared to that of Group V (by about 10%) and Group I (by about 13%).

Results of the study indicate that the feed consumption is largely a sum total effect of the net amount of dietary proteins available and the overall metabolic rate.

5.2.1.1.3 Feed efficiency

With 7.5 per cent protein in the presence of cyanide containing 30 per cent tapioca, Group III showed poor performance with respect to total body weight gain, feed consumption and hence the feed efficiency compared to that of Group IV (Table 6) maintained on a diet with cyanide-free tapioca. Similarly feed efficiency value recorded for the group fed on 30% tapioca and 15 per cent protein (Group II) was also found to be significantly lower than that of the group fed with tapioca free diet having 15 per cent protein (Group I). The significantly lower feed efficiency values in

Group II and III compared to those in Group I and IV (Tables 6 and 7) may reflect the difference in comparative availability of dietary proteins in the presence or absence of cyanide in their diets. Thomas (1966) had reported that feed efficiency values increased in the order of the protein content of the diets. However, the increased feed efficiency value was not observed in spite of having higher content of protein (22.5%) in the diet of Group VI (30% tapioca + 22.5% protein + iodine). This group presented almost similar value as those in Group I (15% protein without tapioca) and Group V (30% tapioca + 15% protein + iodine). This might probably be due to greater amount (about 13% and 10% more than groups I and V respectively) of feed consumed by rats in Group VI (Table 6). If the quantity of feed consumed is implicated in influencing the feed efficiency, it may further be pointed out that in spite of significantly higher growth rate, the feed efficiency value obtained in Group V was not significantly higher than Group II (Table 6 and 7). It may support the view that the comparatively lower feed efficiency values observed in Group V and VI were the result of more feed consumption disproportionate to their growth rate.

5.2.1.2 Digestibility coefficient of dry matter

The dry matter digestibility coefficients of six different experimental diets showed highly significant

differences in between the groups studied (Tables 8 and 9). The observation made suggests that the digestibility of dry matter in these groups was almost inversely related to the quantity of feed consumed (Table 6), greater the quantity of feed consumed lower was the digestibility. An increase in the quantity of food consumed causes an increase in the rate of passage of food through the gastrointestinal tract so that there may be a reduction in its digestibility (McDonald et al., 1982).

5.2.2 Endocrinological studies

5.2.2.1 Thyroid weight

Among the groups studied the relative weight of the thyroid gland (Table 10) was increased significantly in Group III (30% tapioca + 7.5% protein). The increase in the weight of the thyroid is a manifestation of a compensatory hyperplastic response mediated through thyroid stimulating hormone (TSH) under the influence of low thyroxine level (Green, 1971; Reddy, 1982; Udupa et al., 1983). A significant increase in the weight of the thyroid has been reported in rats (Annamma Mathew, 1979) and in pigs (Tewe, 1982) reared on tapioca-based diets. However, this compensatory response was not evident in the case of Group IV which was given a similar diet but tapioca was devoid of cyanide. It may indicate that

in the absence of cyanide of tapioca origin significantly higher levels of thyroxine were produced compared to that in Group III (Table 12) which prevented the pituitary from releasing TSH and stimulation of thyroid in Group IV (Table 10). As a result the group manifested no alteration in the thyroid weight which was similar to those in Groups I, V and VI.

The effect of cyanide of tapioca origin on thyroid activity become more evident from the finding that the relative weight of the thyroid was significantly increased in Group II (30% tapioca + 15% protein) compared to Group I (15% protein alone). The incorporation of 30 per cent tapioca in the diet of Group II with 15% protein resulted in significant reduction in thyroxine concentration (Table 12) leading to compensatory enlargement of thyroid. However, the extent of enlargement of thyroid was lesser in this group than that observed in Group III. This might possibly be due to difference in the levels of thyroxine observed in these two groups (Table 12).

On the other hand, no significant change in the relative weight of the thyroid was observed in Group V (Table 10) which was maintained on a similar diet as that of Group II but with the supplementation of iodine. With the effect of iodine on thyroid activity (as explained in section 5.2.2.4),

this group presented a normal and almost similar weight of thyroid as those in Group I. Similarly the inhibitory effects of cyanide and thiocyanate due to 30 per cent tapioca in the diet of Group VI seem to have been completely nullified by higher proportion of protein (22.5%) and the beneficial effect of iodine supplementation which resulted in normal thyroid weight.

5.2.2.2 DNA content of thyroid

The significantly increased DNA content of thyroid (Table 10 and 11) in Group III (30% tapioca + 7.5% protein) compared with the other groups shows that the pituitary mediated compensatory hyperplasia had taken place leading to the enlargement of the gland (Table 10). It also suggests that the DNA:protein ratio was increased due to the significant reduction in protein content of thyroid in Group III (Table 10). A significantly higher DNA content per total thyroid has been reported by Spiegel et al. (1993ab) in pigs fed rapeseed presscake meal, a goitrogen. An apparent increase in the DNA content of thyroid as observed in Group IV compared with the other groups (I, V and VI) might be due to comparatively lesser content of protein in the thyroid (Table 10) since the dietary level of protein was lowest (7.5%).

Although the thyroid was also enlarged in Group II (30# tapioca + 15% protein), the DNA content of thyroid was not increased significantly in this group (Table 10). This might possibly be due to the reason that the dietary deficiency of protein was not as marked as in Group III. This may be indicated by protein content of thyroid in Group II (Table 10) compared to those in Group I (15% protein alone), V (30% tapioca + 15% protein + iodine) and VI (30% tapioca + 22.5% protein + iodine).

5.2.2.3 Protein content of thyroid

The protein content of thyroid (in the group fed on 15% protein diet (Group I) was found to be 1131.70 $\pm$ 42.02 as against 1037.96 $\pm$ 63.00 µg/100 mg in Group II (30% tapioca + 15% protein) evincing no significant difference between these two groups (Table 10 and 11). However, an apparent reduction in the protein content in Group II might suggest the effect of tapioca (30%), instrumental in utilizing protein for the elimination of HCN thereby reducing the net amount of protein available for synthesis of tissue protein. It may further be revealed from Table 10 that when a diet similar to that of Group II was fed to Group V with supplementation of iodine the protein content of thyroid remained similar to that of Group II. This indicated a similar effect of tapioca as it was present in Group II. The beneficial effect, if at all it

is present, due to iodine can only be assisting thyroxine production. The protein content of thyroid in Group VI was similar to that in Group I which indicated that the deleterious effect of tapioca on protein utilization might have been eliminated by the increased content of protein (22.5%) leaving sufficient amount of protein for the synthesis of tissue protein in thyroid as in the case of Group I.

On the other hand, when rats were fed on protein (7.5%) deficient tapioca (30%) diet (Group III), the protein deficiency might have been aggravated further. Consequently, the protein content available for tissue protein formation in thyroid was reduced markedly in Group III (Table 10). Protein plays an important role in iodine metabolism since it is involved in the production of the tyrosyl derivatives of thyroid hormones (Tewe, 1982). This is an observation in support of the present finding that the deficiency of protein coupled with depletion of iodine due to thiocyanate could play a vital role in the production of goitrous symptoms in Group III. It may further be noted that the level of protein in the diet fed to Group IV was similar (7.5%) to that of Group III, the tapioca component in the diet of which did not contain cyanide. As a result the availability of dietary protein was comparatively more. This may be reflected by the

presence of significantly higher level of protein in the thyroid of Group IV than those in Group III (Table 10).

5.2.2.4 Serum thyroxine

The data on the levels of serum thyroxine in rats revealed that among the groups studied, the highest concentration of serum thyroxine was recorded in Group I maintained on 15 per cent protein diet with no tapioca or iodine (Table 12). Analysis of variance for different periods of the study (Table 13) revealed a significant difference between Groups I and II. Group II was fed a diet containing 15 per cent protein and 30 per cent tapioca. Large amounts of thiocynate are produced endogeneously as a result of detoxification of cyanide present in tapioca (Ekpechi et al., 1966; Delange et al., 1973 and Ermans et al., 1980). During chronic thiocyanate overloading renal clearance is enhanced so that thiocyanate is eliminated rapidly. However, during its rapid clearance, thiocyanate exerts a competitive action on the renal tubular resorption of iodide. Consequently alongwith thiocyanate, iodide ion is also excreted leading to severe depletion in the iodine stores of the gland (Ermans et al., 1980). In addition to protein, iodine is an essential element for the synthesis of thyroid hormones. Since iodine was not supplemented in the diet of Group II to overcome the inhibitory effects of cyanide and thiocyanate it may be

assumed that eventhough there was no appreciable reduction in thyroid protein (Table 10), the loss of iodine might have caused a significant decrease in the circulating thyroxine in Group II.

A significant observation worth mentioning is that when a diet similar to that of Group II was fed to Group V alongwith supplementation of iodine, no significant effect on serum thyroxine levels was evident throughout the study as compared to Group I (Table 12). This suggests that an adequate amount of iodine when provided alongwith optimum level of protein, even higher proportion of tapioca in the diet could not influence the thyroxine production to a greater extent. A high iodine intake could reverse the goitrogenic effect of thiocyanate (Means et al., 1963) by allowing sufficient iodide to enter by diffusion, despite the presence of iodine transport inhibitors, so that normal rates of hormone synthesis are maintained (Green, 1971). The significantly higher serum thyroxine levels in Group V compared to that of Group II (Table 12) may therefore, be attributed to the beneficial effect of excess iodine in the diet (Delange et al., 1982).

It may be emphasized that with higher levels of dietary protein (22.5%) the deleterious effect of tapioca (30%) could be completely nullified with the incorporation of

excess iodine in the diet (Group VI). This is indicated by more or less similar levels of serum thyroxine observed in Group VI throughout the study (Table 12) compared to those in Group I where diet contained no tapioca (15% protein alone).

Among the experimental groups, lowest levels of serum thyroxine were found in Group III (Table 12). As in the case of Group II, V and VI, the diet of Group III also contained 30 per cent tapioca but was deficient in proteins (7.5%) and no iodine was supplemented. Dietary deficiency of protein was aggravated further due to the presence of tapioca in the diet, consequently, the protein content of thyroid was reduced to a lowest level (Table 10). Extreme protein deficiency coupled with severe depletion of iodine caused extremely low levels of thyroxine in Group III.

The significantly higher levels of serum thyroxine in Group IV (30% cyanide-free tapioca + 7.5% protein) as compared to Group III would again highlight the deleterious effects of cyanide and thiocyanate on thyroid functions (Table 12) as observed in Group II and III.

The study suggested that in addition to protein, iodine is an essential factor in thyroid hormonogenesis. When animals are maintained on tapioca-based diet iodine is lost alongwith the excretion of thiocyanate. Hence it must be

supplemented in sufficient quantity alongwith optimum levels of protein to combat the ill effects of cyanide and thiocyanate on thyroid functions.

5.2.2.5 DNA content of pancreas

DNA content per unit weight of tissue reflects the cell population in that tissue. If a tissue is deficient in protein due to improper growth and development, the number of cells per unit weight of tissue is likely to be more accounting for comparatively more DNA than that in healthy and protein rich tissue. Low levels of dietary proteins may reduce the synthesis of tissue proteins leading to a reduction in cytoplasmic granules and a decrease in cell volume. This results in packing of cells and thereby increasing the number of cells per unit weight of tissue. Consequently, the DNA: protein ratio per unit weight of tissue is increased. Therefore, it may be assumed that the differences observed in the DNA content of pancreas among the groups studied (Table 14 and 15) might possibly be due to variation in the pancreatic development reflecting the relative availability of dietary proteins for tissue protein synthesis in each group.

5.2.2.6 Protein content of pancreas

Protein content of pancreas in six different groups under study revealed significant differences at each stage of

investigation (Table 16 and 17). The results suggested that the observed differences in protein content of pancreas between the groups were primarily due to varying levels of dietary proteins determining the tissue protein synthesis.

5.2.2.7 Serum insulin

In general, the pattern related to insulin levels in serum (Table 18) was found to be in tune with the variations in the levels of blood glucose in different groups at various stages of the investigation (Table 22). This is in accordance with the direct relationship existing between the concentration of glucose in the blood and the release of insulin. Lenzen et al. (1976) demonstrated in rats that the amount of insulin available for immediate release from the pancreas is apparently dependent on the plasma glucose level. This close correlation indicated that pancreatic beta cells are equipped with a very sensitive mechanism for the detection of variations in the levels of plasma glucose at a given time.

The differences observed in the serum insulin levels among the groups studied if explained on the basis of insulin-to-glucose ratio (µU/mg), it will be seen that the amount of insulin (µU) required to dispose off one milligram of glucose was almost similar in the groups studied (Table 20 and 21). However, there was comparatively high requirement of insulin

(36.90 ± 2.78 µU/mg) in rats under Group V (30% tapioca + 15% protein + iodine). On the other hand, the rats under Group III with similar level of tapioca (30%) but lower level of protein (7.5%) without supplementation of iodine in the diet required a little lesser amount of insulin (29.65 ± 2.48 µU/mg). Even the Group II (30% tapioca + 15% protein) which showed maximum blood glucose concentrations throughout the study had a more or less similar insulin-to-glucose ratio (33.83 ± 3.97 µU/mg) compared to those observed in Group I (33.68 ± 2.71 µU/mg), IV (32.00 ± 2.00 µU/mg) and VI (34.23 ± 2.86 µU/mg).

In short, the variations observed in serum insulin levels seem to be due to variations in the blood glucose concentrations at different periods of the study. There was no significant variation in insulin-to-glucose ratio among the groups studied. The study did not reflect the effect of cyanide, if any, on the synthesis and release of insulin from the pancreas.

5.2.3 Biochemical studies

5.2.3.1 Blood glucose

The trend with respect to blood glucose levels among the experimental groups of rats has been evincing no definite

pattern of response (Table 22) at different time intervals of the study.

The groups of rats studied, were given diets containing variable levels of protein with or without supplementation of iodine (Table 1). The diets given to Groups II, III, V and VI were containing 30 per cent tapioca which contained cyanide whereas the tapioca incorporated in the diet of Group IV was devoid of cyanide. The diet fed to Group I did not contain any tapioca (Table 1).

It is fairly well established that one of the major pathways of detoxification of cyanides is conversion of cyanide to thiocyanate which requires sulfur-containing amino acids (Geevarghese, 1982) in the presence of an enzyme rhodanase (Nartey, 1973 and Conn, 1978). It is likely that the requirement of sulfur-containing amino acids is met by dietary sources of protein. Ultimately, a portion of dietary protein is utilized for this purpose. Even if a tapioca based diet is extremely protein deficient the excess amount of HCN (over and above that may be detoxified by available amounts of proteins) may be converted to thiocyanate by drawing on proteins from body reserves. Further, since rats have higher content of rhodanase in the liver compared to other animals (Himwich and Saunders, 1948), the rate of detoxification of cyanide may also be higher.

Under such circumstances, if at all there had been any free cyanide escaping detoxification, it should have been more in case of Group III since it was given lower (7.5%) amount of dietary protein. Consequently, maximum blood glucose concentration should have been observed in Group III. On the contrary, the blood glucose levels in this group were found to be lowest among the groups studied (Table 22) but within the physiological range. The effect of cyanide on blood glucose concentration reported by McMillan and Geevarghese (1979) in rats exposed chronically to potassium cyanide in drinking water might have been due to the absorption of cyanide completely causing mild hyperglycaemia during the initial exposure only. The reason ascribed for the failure of hyperglycaemia was the capability of rats to continuously neutralize cyanide at least 20 times the lethal amount day after day, as a substantial metabolic task. At the same time, Annamma Mathew (1979) observed no alteration in blood glucose level even when 85 per cent tapioca was incorporated with a mere 1 per cent protein in the diet of male albino rats. This indicates that the amount of cyanide escaped detoxification may not be able to bring about market changes in blood glucose levels.

Therefore it seems plausible to assume that occasional or inconsistent increase in blood glucose levels particularly

in Group II (Table 22) was not essentially due to the cyanide present in 30 per cent tapioca of their diet. This might possibly be due to individual variations, as the blood glucose levels observed in all the groups under study were well within the physiological range (50-135 mg%) reported by Harkness and Wagner (1989).

5.2.3.2 Serum total protein

Group IV was fed on a diet containing 7.5 per cent protein alongwith 30 per cent tapioca devoid of cyanide. The serum total protein level in Group IV was significantly ($P<0.05$) lower than those in other groups including Group III (Table 24 and 25). The results indicate that the significantly lower levels of serum total proteins in Group IV were due to the deficiency of dietary protein.

Although the diet fed to Group III was similar to that of Group IV, the tapioca contained cyanide. On this diet, the serum total protein levels were found to be significantly ($P<0.05$) higher than Group IV as well as those fed on higher levels of protein (Groups I, II, V and VI). The elevation in serum total protein concentration in Group III is related mainly to the presence of sublethal levels of cyanide and thiocyanate in the plasma causing alteration in thyroid functions and inhibiting the cellular oxidation and oxidative

phosphorylation. All these factors together would generally produce a decrease in the rate of metabolic activities and consequently a slowing down in the turnover of protein and other cell constituents (Kamalu, 1991). Similar increase in serum total protein levels was reported in hypothyroidism in man (Lamberg and Grasbeck, 1955) and in goats (Reddy, 1982). Besides this, Nangia et al. (1975) also pointed out that the defective utilization of nutrients in the absence of optimum levels of thyroxine causes accumulation of protein. Hence a positive nitrogen balance is usually observed in hypothyroidism (Udupa et al., 1983) due mainly to the decrease in protein catabolism and secondarily to interfering with anabolic processes. As a result amino acids are accumulated and not utilized for protein synthesis and growth (Kamalu, 1991).

A similar phenomenon was observed in Group II (Table 22) although the elevation in serum total protein levels was not as marked as in the case of Group III. This might probably be due to the thyroxine deficiency being not as pronounced as observed in Group III (Table 12).

Since iodine was supplemented in the diets of Group V (30% tapioca + 15% protein + iodine) and VI (30% tapioca + 22.5% protein + iodine) to overcome the inhibitory effects of cyanide and thiocyanate of tapioca origin, the thyroid

activity was maintained at normal level. Consequently, no effect on protein metabolism was evident in these groups and the serum total protein levels remained unaltered.

5.2.3.3 Serum total cholesterol

During all the periods of the present study serum total cholesterol levels were found to be significantly higher in rats under Group III (Table 26 and 27). The increase in the serum total cholesterol in Group III (30% tapioca + 7.5% protein) might be a result of development of hypothyroid condition where plasma cholesterol level is usually raised (Hellman et al., 1959; Meittinen, 1968; Metzger and Freinkel, 1971 and Sobel and Braunwald, 1971). Group III showed significantly reduced concentration of thyroxine at each stage of investigation (Table 12). Since biosynthesis of cholesterol is inversely proportional to the thyroid activity (Mason and Wilkinson, 1973) it may be reasonable to assume that the extreme deficiency of thyroxine ultimately could lead to both decreased faecal excretion of cholesterol and its conversion to bile acids (Fletcher and Myant, 1958 and Ingbar and Woeber, 1981) resulting in hypercholesterolemia.

It is relevant to mention here that in the absence of cyanide and thiocyanate of tapioca origin significantly higher levels of thyroxine were observed in Group IV compared to

Group III (Table 12) as a result there was no appreciable alteration in serum total cholesterol levels (Table 26).

The effect of thyroxine deficiency in influencing the serum cholesterol levels may become further evident in the case of Group II (30% tapioca + 15% protein). The overall serum total cholesterol level was significantly raised (Table 26 and 27) due to thyroid insufficiency (Table 12) compared to that of Group I maintained on 15 per cent protein alone without any tapioca. Peters and Man (1950) suggested that the increase in the serum cholesterol is a specific change in lipid metabolism due to deficiency of thyroxine. This is an observation in support of the present finding in the case of Groups II and III. The finding is pertinent and has to be emphasized that with the supplementation of iodine optimum concentration of thyroxine was maintained in the case of Group V (30% tapioca + 15% protein + iodine) and VI (30% tapioca + 22.5% protein + iodine) and hence serum total cholesterol levels remained unaltered in these groups (Table 26).

5.2.3.4 Serum total lipids

Thyroid hormones are normally related to synthesis, mobilization and degradation of lipids. However, under conditions of hormone deficiency it may influence virtually

all aspects of lipid metabolism especially the degradation of lipids. This may lead to accumulation of lipids in blood plasma (Ingbar and Woeber, 1981). The higher levels of serum total lipids observed in Group III (30% tapioca + 7.5% protein) may therefore, be the result of deficiency of thyroxine (Table 12 and 28). Bierman and Glomset (1981) have also pointed out excessive accumulation of lipid in plasma as a result of either defective removal of lipids from plasma or excessive production of lipids or both due to endocrine disorders like diabletes or hypothyroidism.

Although the degradation of lipids was not hampered significantly in Group II (3% tapioca + 15% protein), the apparent increase in serum total lipids (Table 28) compared to that of Group I (15% protein without tapioca) may also be indicative of thyroxine deficiency (Table 12).

The inhibitory effects of cyanide and thiocyanate on synthesis and release of thyroxine was eliminated following supplementation of iodine in the diets of Group V (30% tapioca + 15% protein + iodine) and VI (30% tapioca + 22.5% protein + iodine). Consequently, stimulatory effect of thyroxine on degradation of lipids was quite evident in these groups. This is an observation which would elucidate the fact that the metabolic reactions of lipids are stimulated by thyroxine.

5.2.3.5 Haemoglobin

Haemoglobin concentration was found to be lowest in Group III as compared to all the other groups (Table 30 and 31). Group III was given a diet containing 30 per cent tapioca along with 7.5 per cent protein only which by itself might have caused the reduction of haemoglobin to the lowest level. In addition, 30 per cent tapioca which contained cyanide possibly further reduced the availability of protein in the body (as explained in section 5.2.3.1 and section 5.2.3.2). Another reason for the marked reduction of haemoglobin might be the thyroid hormone deficiency caused by the ingestion of tapioca (Table 12). According to Muldowney et al. (1957) deficiency of thyroid hormone chiefly lowers the red cell and haemoglobin concentration due to slower regeneration of red cells and haemoglobin from the stem cells. However, rats reared on a similar ration but with tapioca not containing cyanide showed significantly higher haemoglobin concentration highlighting the effect of cyanogenic principles present in tapioca playing a greater role than even a deficiency of dietary protein.

The effect of cyanogenic principles in tapioca becomes all the more pronounced in the case of Group II. A significant reduction of haemoglobin in Group II compared to

that of Group I reflect the deleterious effects of cyanogens in tapioca on thyroid function as described above.

The decisive role of dietary protein in combination with thyroxine on haemoglobin production can further be emphasized by the observation made in the case of Groups V and VI (Table 30). In spite of the presence of 30 per cent tapioca in their diets, the supplementation of iodine maintained the thyroxine to normal levels (Table 12). As a result the haemoglobin concentration was more or less equal to those observed in Group I.

5.2.4 Histopathological studies

The pathological changes seen in Group III (7.5% protein + 30% tapioca) could be due to the combined effect of hypoproteinemia and cyanide present in the tapioca. Vacuolar changes in the hepatic cells of tapioca fed rats have been reported by many workers (Tasker, 1962; Rao, 1964 and Annamma Mathew, 1979).

In addition to the parenchymatous changes seen in the liver, the acinar cells of the pancreas also revealed retrogressive changes in Group III indicating the possibility of the action of cyanide and hypoproteinemia on the pancreatic cells. Takama and Kishino (1985) observed that protein-deficient diet might induce changes in the pancreatic

structure and alter the stability of cellular membranes in rats. The deficiency of protein in the presence of tapioca, therefore, could potentiate the pancreatic cells to the action of cyanide. However, typical calcifying pancreatitis as reported by Pushpa (1980) was not observed in rats of the present investigation. The calcifying pancreatitis observed by her could be due to the exposure of pancreas to cyanide for a longer period of time (18 months) even though the level of cyanide in the diet of rats was lower (73 µg/g tuber).

The toxic action of cyanide is further exemplified by the cytological alterations seen in the myocardial fibres. Interstitial oedema in myocardium along with other changes as observed in Group III have been reported in both experimental and spontaneous myxoedema by Douglas and Jacobson (1957).

In Group IV (7.5% protein + 30% tapioca without HCN) in spite of lesions suggestive of hypoproteinemia in the liver (Errickson et al., 1966), major pathological changes in the pancreatic cells were absent indicating that it was the cyanide liberated through tapioca ingestion that caused cytological alterations seen in Group III. The distension of interlobular space in the pancreas and the interstitial oedema in the myocardium may be attributed to protein deficiency.

In Group II eventhough the level of protein was similar to that of Group I (15%), mild vacuolar changes seen in the liver could be due to the cyanide present in tapioca (30%). The exocrine pancreas showed occasional degenerative changes and heart revealed slight interstitial oedema which could also be attributed to the cyanide toxicity.

In the case of rats in Group V (15% protein + 30% tapioca + iodine), the supplementation of iodine in the presence of 30 per cent tapioca in the diet prevented the major pathological changes leaving only minimal alterations in their organs.

Similarly no histological deviations were seen in Group VI also which was reared on iodine (0.17 mg/kg) supplemented protein (22.5%) rich diet with tapioca (30%).

It may be pertinent to note in this context that Group I which was not provided with tapioca in the diet, all the organs studied in the rats appeared histologically quite normal. These observations indicate that the cytological alterations seen in the experimental groups were due to toxicity of cyanide containing tapioca superimposed by protein deficiency.

5.3 Experiment in kids

5.3.1 Growth studies

5.3.1.1 Body weight gain

There was no significant difference in growth rate (total weight gain and average daily weight gain) in Groups I (15% protein without tapioca) and II (15% protein + 30% tapioca + iodine).

From the available literature and the present study on rats, it is evident that sulfur-containing amino acids are required for the conversion of cyanide of tapioca to thiocynate. As a result a portion of dietary protein is utilized for this purpose reducing the level of dietary protein available to the body. It may explain the apparent differences in growth rate between Groups I and II (Table 34) indicating the reduced availability of protein for growth in Group II due to the presence of tapioca which might have deprived the animals of some amount of the protein presented in the diet. Similar non-significant differences in growth rate (Table 34) were recorded by Areghore (1992) in crossbred ram lambs fed on a diet containing 40 per cent cassava peel with 7.5 per cent palm kernel or 1.65 per cent urea as protein source.

The effect of cyanogenic principles of tapioca in reducing the availability of dietary protein for growth becomes all the more evident in Group III. In spite of the highest level (25%) of dietary protein, tapioca (30%) in the diet of the kids did not produce significantly higher growth compared to that in Group I (Table 34). With 10 per cent more protein in the diet the kids in Group III showed only around 15 per cent higher growth than Group I which was not significantly higher (Table 35).

The significant difference in growth rate observed between Groups II and III (Tables 34 and 35) evidently indicted the significance of difference in protein levels even with the presence of tapioca in their diets.

Mercy et al. (1981a), in growing Alpine-Malabari kids fed on 21.3 per cent protein diet recorded similar results as noticed in Group III (Table 34) of the present investigation. The average daily weight gain observed in Groups I and II was almost similar to that reported by Shyama (1994) in the same breed of goats reared on 16 and 12 per cent protein diet respectively.

5.3.1.2 Dry matter intake

The dry matter intake in the three groups of kids under study evinced no significant difference between

themselves (Table 34 and 35). It may indicate that palatability of tapioca containing diets (Groups II and III) was similar to the one not containing tapioca (Group I) and hence did not alter significantly the dry matter intake of any of the three groups. Similar observation was made by Okeki and Oji (1987) who found that the dry matter intake did not differ in West African dwarf bucks fed on a cassava peel ensiled diet, compared to bucks given the maize silage. The dry matter intake values obtained for the three groups of kids under study are almost similar to those reported by Mercy et al. (1981a) for growing Alpine-Malabari kids.

5.3.1.3 Feed efficiency

The feed by gain ratio was apparently higher (Table 34 and 35) for the group fed on 15 per cent protein + 30 per cent tapioca + iodine (Group II) as against the groups fed on either 25 per cent protein + 30 per cent tapioca + iodine (Group III) or 15 per cent protein without tapioca (Group I). Non-significant differences in feed efficiency have also been reported by Aregheore (1992) in West African Crossbred ram lambs fed on 40 per cent cassava with 7.5 per cent palm kernal meal or 1.65 per cent urea as the protein source. The feed efficiency values recorded in the present study for the three groups of kids are comparable with those reported by Mercy et al. (1981a) for Alpine-Malabari kids.

5.3.2 Endocrinological studies

5.3.2.1 Serum thyroxine

In the investigation in rats it has been clarified that in addition to protein, iodine is an essential factor in thyroid hormonogenesis. When animals are maintained on tapioca-based diets iodine is lost along with the excretion of thiocyanate (produced endogenously due to cyanide detoxification) leading to severe depletion in the iodine stores of the thyroid gland and hence depressing the circulating levels of thyroxine. It was also noted that when tapioca-based diets were fed to rats along with supplementation of iodine and sufficient levels of protein (Groups V and VI), no significant effect on serum thyroxine levels was evident (Table 12, Section 5.2.2.4).

Following supplementation of iodine, it may be assumed that the response of kids to tapioca-based diets (Groups II and III) was similar to that observed in Groups V and VI of rats. The results of the study in kids indicated that there was no significant influence of cyanogenic principles of tapioca on thyroxine synthesis in the presence of iodine (Table 36 and Fig.5). The average levels of serum thyroxine over the entire trial period of the study in Groups II (15% protein + 30% tapioca + iodine) and III (25% protein + 30%

tapioca + iodine) were almost similar (Table 43 and 44) to that of Group I (15% protein without tapioca). The results suggested that if at all kids were susceptible to cyanogenic principles of tapioca it was nullified by the availability of sufficient levels of dietary protein and iodine, in particular, which may be evident from a non-significant difference in thyroxine levels between the groups studied. The values recorded for these groups of kids in the present study corroborate well with the values (3.8-7.77 µg/dl) reported by Jones and Meggarity (1983) in goats fed with lucern; and Rajan (1989) in goats (3.4-6.2 µg/dl).

5.3.2.2 Serum insulin

The results of the present study indicated that the effect of feeding of tapioca based diet on secretion and release of insulin in kids was not significant (Table 43 and 44). In the present study although the trend in the insulin response was in accordance with the changes in the blood glucose concentration (Fig.6 and 7), it was not always proportional to the levels of blood glucose at different time intervals (Table 37 and 38). The blood glucose concentration is usually considered to be the principal metabolic substrate regulating insulin release by the beta cells of most animal species (Sasaki et al., 1984). In the case of ruminants, however, over and above the plasma glucose concentration by

itself, other metabolites such as volatile fatty acids have been implicated as effective regulators of insulin secretion (Sasaki et al., 1977). Thus it seems possible that the insulin levels observed in the present study might have been influenced from time to time not only by the concentration of glucose alone but also by the amounts of volatile fatty acids. The VFAs may be playing an equally major role in determining the insulin response in the kids.

5.3.3 Biochemical studies

5.3.3.1 Blood glucose

No significant effect of cyanide contained in tapioca was evident in kids on their blood glucose levels (Table 44; Fig.7), although tapioca fed to Groups II (15% protein + 30% tapioca + iodine) and III (25% protein + 30% tapioca + iodine) contained higher amounts of cyanide (186.3 mg cyanide/kg). The overall levels of blood glucose in Groups II and III were almost similar to those in Group I (tapioca-free diet). It may be assumed that the cyanide present in tapioca might have been detoxified efficiently by the protein sufficiently incorporated in their diets. Information on feeding of tapioca and its accompanying effects on blood glucose and other aspects of metabolism in goats are conspicuously lacking from the available literature. Okeke and Oji (1987) fed a

grass ensiled diet (Grass 60 : Cassava peel 20 : poultry excreta 20) containing 52.5 mg cyanide/kg to West African dwarf bucks and found no significant effect on blood glucose levels due to cyanide present in the diet compared to the control group fed on maize silage.

The changes in blood glucose levels from the start to the end of the experiment indicated that there was almost a steady decrease in blood glucose concentration in all groups of kids (Table 38; Fig.7). This change could be explained as due to the production of more and more volatile fatty acids as rumen development progressed with age in kids.

5.3.3.2 Serum total protein

The results of the study revealed no significant alteration in serum total protein concentration in Group II (15% protein + 30% tapioca + iodine). It may indicate that the kids in Group II were having sufficient protein supply as in Group I (Table 44; Fig.8). As a result there was no significant difference in growth rate between these two groups (Table 34).

A significant difference in the concentration of serum total protein was also not observed in between Groups II and III (Table 43 and 44) despite the significantly higher

growth rate recorded in Group III (25% protein + 30% tapioca + iodine) due to higher levels of dietary protein.

The serum total protein levels recorded, in the three groups of kids in the present study were within the normal range. This suggested that inspite of incorporating 30 per cent tapioca in the diets of Groups II and III, no untoward effect was produced and the results were at par with Group I which was not receiving any tapioca. Almost similar concentrations of serum total protein have been reported by Mercy et al. (1981a) for Alpine-Malabari Kids. The results are indicative of the fact that the goitrogenic effects of tapioca can effectively be corrected by 15 per cent protein by itself when supplemented with sufficient quantities of iodine. Following supplementation of iodine, it may be assumed that iodine counteracted the antithyroid effects of tapioca in the same way as in rats (section 5.2.3.2) in the present investigation.

5.3.3.3 Serum total cholesterol

In the present study in kids serum total cholesterol levels were found to be unaltered (Table 40). The effect of thyroxine in regulating normal serum cholesterol level was evident from the studies on rats in the present investigation (as explained in section 5.2.3.3). It is possible that with

the supplementation of iodine the inhibitory effects of cyanide and thiocyanate of tapioca origin on thyroid functions were eliminated in the groups of kids fed with tapioca. As a result Group II (15% protein + 30% tapioca + iodine) and Group III (25% protein + 30% tapioca + iodine) presented almost similar levels of serum total cholesterol (Table 43 and 44) compared to that of Group I (15% protein with no tapioca). The levels of serum total cholesterol in these groups fall in the range (80-130 mg/dl) given by Benjamin (1978).

5.3.3.4 Serum total lipid

The results of the study indicated that the serum total lipid levels were not influenced appreciably by feeding regimen (Table 41 and 44) throughout the study. The phenomenon of accumulation of lipid as observed in the case of rats (Table 28; Section 5.2.3.4) due to ingestion of tapioca and consequent thyroid insufficiency was not evident in kids. This might have been due to the supplementation of iodine in the diets of Groups II and III. Following incorporation of iodine with tapioca diets, almost normal levels of thyroxine were observed in these groups (Table 36). The normal levels of thyroxine could have maintained a normal serum lipid concentration as in the case of Group I which was not fed with tapioca. However, the elevation in serum total lipid in all

the groups in the later half of the experiment might be an age dependent phenomenon (Fig.10).

5.3.3.5 Haemoglobin

The effect of cyanogenic principles of tapioca in reducing the concentration of haemoglobin in Groups II and III of rats (Table 30; Section 5.2.3.5) was not evident in kids fed on tapioca based diets (Table 42). Following supplementation of iodine, the response of kids to cyanide and thiocyanate of tapioca origin was similar to that observed in rats (Groups V and VI). Moreover the levels of haemoglobin observed in tapioca fed groups of kids (Groups II and III) were almost similar (Table 42 and 44) to those not fed with tapioca (Group I). The levels of haemoglobin in all the groups of kids studied were almost in normal range given by Benjamin (1978) for goats and by Reddy (1982) for crossbred kids.

SUMMARY

A study was carried out in different varieties of tapioca to assess the level of their cyanogenic glycoside (CNG) content, the effect of various processing methods on the CNG content and the effect of CNG on metabolism in rats and kids. The role of dietary protein and iodine in modifying the effects of cyanide was investigated. Histopathological studies on the liver, pancreas and heart were also carried out in rats to assess the effect of tapioca.

The first phase of the study revealed that CNG content in terms of cyanide varied from 40.86 $\pm$ 2.79 to 186.31 $\pm$ 5.30 ug cyanide/g of fresh tuber in the seven varieties of tapioca analysed namely, 'M-4', 'Sree Visakh', 'Thottakolly', 'Sree Prakash', 'Sree Sahya', 'H-165' and 'Karkidakkan'. It was lowest in 'M-4' while higher concentration was recorded in 'H-165' and 'Karkidakkan'. Processings like boiling and sun drying lowered the CNG content by about 50 per cent of the original.

In the second phase of the study 60 albino Wistar rats (30-40 d old) were divided into six groups, of ten each and given a diet with or without tapioca and iodine alongwith different levels of protein. The first group was fed a diet

without tapioca but with 15 per cent protein. Group II was given a diet containing 15% protein and 30% tapioca. The level of protein was reduced to 7.5 per cent in the diet of Group III containing 30% tapioca. Similar diet was fed to Group IV but with tapioca made free of cyanide. A diet containing 15% protein and 30% tapioca with iodine @ 0.17 mg/kg was fed to Group V. Similar diet with higher level of protein (22.5%) was given to Group VI. Tapioca incorporated in the diets contained 182.6 mg cyanide/kg.

Response of rats to different diets was evaluated by recording growth rate, feed consumption and feed efficiency for a period of 12 weeks. Dry matter digestibility for a period of 24 h was also determined. At the end of 10th, 11th and 12th week of the study rats were sacrificed for endocrinological, biochemical and histopathological evaluation of blood/serum and tissues.

Performance of rats was poor on tapioca-based diet which were not supplemented with iodine (Groups II and III). Rats fed on protein deficient diet (Group III) with cyanide containing tapioca were the most affected. Group III showed stunted growth while Group II (15% protein + 30% tapioca without supplementation of iodine) did not gain as much weight as the animals in Group I. However, there was no significant variation in the performance in Groups V and VI compared to

Group I when tapioca-based diet were supplemented with iodine. The digestibility of dry matter in six groups of rats was almost inversely related to the quantity of feed consumed.

There was significant increase in DNA and decrease in protein content leading to an increase in the DNA:protein ratio in the thyroid gland of Group III. This was due to deficiency of protein which was aggravated further by the liberation of cyanide from tapioca. Increase in DNA content of thyroid in Group III was indicative of compensatory hyperplasia resulting from an increased TSH secretion under the influence of extremely low levels of thyroxine culminating in significant enlargement of the thyroid. A marginal increase in DNA content of thyroid in Group IV was due to lower levels of dietary protein. A significant compensatory hypertrophy of the thyroid in Group II was primarily due to depletion of iodine resulting in significant decrease in the circulating levels of thyroxine.

Dietary protein and iodine was found to play a major role in counteracting the inhibitory effects of cyanide and thiocyanate of tapioca origin on thyroid functions.

The observed differences in DNA and protein content of pancreas between the groups of rats were mainly due to differences in the relative availability of protein for tissue

protein synthesis. Consequently, the DNA:protein ratio per unit weight of tissue was increased in groups fed with low levels of dietary protein (Groups III and IV).

Insulin levels in different groups of rats were found to be related primarily to the blood glucose levels. There was no significant difference in insulin-to-glucose ratio between the various groups. The synthesis and release of insulin from the pancreas was not found influenced by cyanide containing tapioca present in the diet.

Rats showed no definite pattern of response in their blood glucose levels due to cyanide present in the tapioca-based diet. The differences, if any, found between the groups were attributed to individual variations.

There was elevation in the serum total protein level in tapioca fed groups not supplemented with iodine, despite the fact that bioavailability of protein was reduced in the presence of cyanide of tapioca origin. The elevation in serum total protein was more pronounced in Group III even though there was deficiency of dietary protein in this group. This indicated the development of hypothyroidism leading to an accumulation of amino acids which were not being utilized for protein synthesis and growth.

There was elevation in the levels of serum total cholesterol and lipid in rats fed tapioca without supplementation of iodine (Groups II and III) probably due to deficiency of thyroxine resulting in retarded metabolic processes. However, the increase in serum total lipid level in Group II was not significant.

The results of the study indicated that the estimations of serum total protein, cholesterol and lipid could be used as reliable indicators of thyroid functions.

A significant reduction in haemoglobin concentration in Groups II and III reflected the deleterious effects of cyanogens on thyroid functions as well as making dietary protein not available for haemoglobin synthesis.

Compared to Group I, the hepatic cells in Group II (15% protein + 30% tapioca) appeared slightly swollen with occasional cells showing vacuolar changes. Pancreas showed slight oedema and occasional mild degenerative changes of exocrine cells. Also there was slight interstitial oedema in the myocardium. Intensity of pathological changes in liver, pancreas and heart was more severe in rats fed on protein deficient, tapioca diet (Group III) compared to that of Group IV, the tapioca in the diet of which was made cyanide free. The hepatic cells in Group III showed marked and diffused

vacuolar changes with condensed or vesicular nuclei and occasional cell necrosis. Many pancreatic exocrine cells showed vacuolar and degenerative changes. Interstitial oedema was prominent. It was important to note that in spite of all the above histological changes in the pancreatic cells, all the endocrine component were intact in all the groups studied which may be the reason why the insulin level in almost all the experimental groups remained unaffected. Myocardium showed interstitial oedema with swollen sarcoplasm and fragmented sarcolemma. At the same time only moderate vacuolar changes in the liver, distention of interlobular space and interstitial oedema in myocardium were observed in Group IV. Since the diets of Groups V and VI were supplemented with iodine, no significant cytological alterations could be noticed in their organs.

The effect of different levels of dietary protein along with iodine on tapioca based diet was studied in three groups of 2½ to 3 months old, Alpine-Malabari kids of both sexes. The study included recording of performance of kids and determination of certain endocrinological and biochemical parameters in blood at fortnightly intervals for three months. Group I was given a diet containing 15% protein without tapioca while the other two groups were fed 30% tapioca either with 15% protein (Group II) or 25% protein (Group III),

supplemented with iodine in both cases @ 2 mg/kg. The tapioca incorporated contained 186.3 mg cyanide/kg.

There was no significant effect on the performance (body weight gain, dry matter intake and feed efficiency) of kids fed on tapioca-based diet supplemented with iodine, compared to Group I. The significant difference in body weight gain between Groups II and III was probably due to the difference in the relative availability of protein in their diet.

The results of the study in kids indicated that there was no significant influence of cyanogenic principles in tapioca on the thyroxine synthesis when iodine was supplemented in the diet.

The effect of feeding tapioca-based diets on secretion and release of insulin in kids was also not significant.

There was no significant effect produced by cyanide present in tapioca on the blood glucose levels in kids. However, there was almost a steady decrease in blood glucose concentration in all the groups which could be explained on the basis of the production of more and more VFA as rumen development progressed with age in kids.

There were no significant differences in the serum total protein, cholesterol, lipid and haemoglobin concentrations between the groups of kids studied. The elevation in serum total lipid concentration recorded in all the groups in the later half of the experiment might be an age dependent phenomenon.

The results of the present investigation suggested that cyanide is liberated from ingested tapioca. Metabolic detoxification of cyanide requires the supply of dietary source of sulfur containing amino acids. As a result, a portion of dietary protein is utilized for detoxification of cyanide leading to a reduction in the bioavailability of dietary protein calling-forth its additional supplementation. The resultant metabolic product - thiocyanate - exerts an inhibitory effect on thyroid function resulting in the development of hypothyroidism. This virtually affects almost all aspects of metabolism resulting in a retarded growth rate which could be corrected by supplementation of iodine in the diet. The protein deficiency brings about structural changes in the cells and potentiate the organs to the toxic effects of cyanide. Therefore, to ensure normal growth on tapioca-based diet sufficient quantity of good quality protein along with iodine should be incorporated in the diet.

In the present investigation the nutritional basis of goitrogenic effect of tapioca has been elucidated.

The effect of dietary cyanide through the ingestion of tapioca superimposed by protein deficiency was evident on the exocrine part of the pancreas of rats. However, any direct correlation between heavy and prolonged tapioca consumption and the genesis of pancreatic diabetes could not be established since the endocrine functions of pancreas remained intact, during the period of the present investigation.

The effect of dietary cyanide toxicity and protein deficiency on liver and heart has also been illustrated.

The results of the present study indicated that fresh tapioca could be incorporated in the rations of kids only if it is fortified with protein and iodine.

From the above data, it may be inferred that the lurking fears of the common man on the toxic effects of heavy and prolonged consumption of tapioca may be dispelled, to some extent, by consuming tapioca supplemented with sufficient amount of proteins and iodine from the easily available and comparatively cheaper source, the salted dry sea fish. However, further studies are required to confirm this hypothesis.

REFERENCES

REFERENCES

Allison, M.J. (1978). The role of ruminal microbes in the metabolism of toxic constituents from plants. In Effects of Poisnous Plants on Livestock. Keeler, R.F., Vankampen, K.V. and James, L.F., Eds., Academic Press, New York. p. 105.

Annamma Mathew (1979). Tapioca in the genesis of pancreatic diabetes. MD. thesis submitted to Kerala University, Trivandrum, India.

Aregheore, E.M. (1992). Effects of protein source on cassava peel utilization by growing sheep. Tropical Science, 32 : 313-317.

Armed Forces Institute of Pathology (1968). Manual of Histologic Staining Methods. McGraw-Hill Book Company, New York. 3rd Ed. pp. 12-184.

Barry, T.N. and Manley, T.R. (1985). Endocrine regulation of metabolism in sheep given kale (Brassica oleracea) and ryegrass (Lolium perenne) - clover (Trifolium repens) fresh-forage diets. Brit. J. Nutr. 54: 165-173.

Bassett, J.M. (1975). In Digestion and Metabolism in the Ruminant. McDonald, I.W. and Warner, A.C.I., Eds. Armidale: University of New England Publishing Unit. pp. 383-398.

Basu, S.K., George, S., Maharda, N.S., Shukla, R.K. and Sharma, S.K. (1984). Effect of goitrogens on the thyroidal handling of iodine and its modification by iodide supplementation. Indian J. Physiol. Pharmac. 28 (5): 23.

Benjamin, M.M. (1978). Outline of Veterinary Clinical Pathology. The Iowa State University Press, Ames, Iowa, U.S.A. 3rd Ed. pp. 60-63 and 250.

Bierman, E.L. and Glomset, J.A. (1981). Disorders of lipid metabolism. In Text Book of Endocrinology. Williams, R.H. Ed., IGAKU-SHOIN/Saunders, TOKYO. 6th Ed. p. 888-893.

Blood, D.C., Henderson, J.A. and Radostits, O.M. (1979). Veterinary Medicine. The English Language Book Society and Bailliere and Tindall, London. 5th Ed. p. 967.

Bourdox, P., Seghers, P., Mafuta, M., Vanderpas, J., Vanderpas-Rivera, M., Delange, F. and Ermans, A.M. (1982a). Cassava products : HCN content and detoxification processes. In Nutritional Factors Involved in the Goitrogenic Action of Cassava. Delange, F., Iteke, F.B., Ermans, A.M. Eds., International Development Research Centre, Ottawa, Canada, IDRC-184e, pp. 51-58.

Bourdox, P., Seghers, P., Mafuta, M., Vanderpas, J., Vanderpas-Rivera, M., Delange, F. and Ermans, A.M. (1982b). Traditional cassava detoxification processes and nutritional education in zaire. In Cassava Toxicity and Thyroid : Research and Public Health Issues. Delange, F. and Ahluwalia, R., Eds., International Development Research Centre, Ottawa, Canada, IDRC-207e, pp. 134-137.

Bovin, A., Vendrely, R. and Vendrely, C. (1948). Compt. rend. 226: 1061 (cited by Chargaff and Davidson, 1955).

Burstein, P.J., Draznin, B., Jhonson, J. and Schalch, D.S. (1979). The effect of hypothyroidism on growth, serum growth hormone, the growth hormone-dependent somatomedin, insulin like growth factor and its carrier protein in rats. Endocrinology. 104: 1107-1111.

Burton, K. (1956). A study of the condition and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxypentose ribonucleic acid. Biochem. J. 62: 315-322.

Chargaff, E. and Davidson, J.N. (1955). In The Nucleic Acids - Chemistry and Biology. Chargaff, E. and Davidson, J.N. Eds., Academic Press Inc., New York. Vol.II. pp. 1-12.

Chopra, I.J. (1981). Thyroid function tests : thyroxine and reverse triiodothyronine measurements. In Radioassay Systems in Clinical Endocrinology. Abraham, G.E. Ed., Marcel Dekker, Inc. New York and Basel. p. 110.

Clarke, E.G.C. and Clarke, M.L. (1975). Veterinary Toxicology. Bailliere Tindall, London. p. 251.

Cohen, A.M. (1957). Interrelation of insulin activity and thyroid function. Am. J. Physiol. 188: 287-294.

Cohn, C., Berger, S. and Norton, M. (1968). Relationship between meal size and frequency and plasma insulin response in man. Diabetes 17: 72.

Coiro, V., Braverman, L.E., Dana christianson, Shih-liehFang, Goodman, M. (1979). Effect of hypothyroidism and thyroxine replacement on growth hormone in the rat. Endocrinology 105: 641-646.

Conn, E.E. (1978). Cyanogenesis, the production of hydrogen cyanide, by plants. In Effects of Poisnous Plants on Livestock. Keeler, R.F., Vankampen, K.V. and James, L.F., Eds., Academic Press, New York. pp. 301-309.

Cooke, R.D. and Maduagwu, E.N. (1978). The effect of simple processing on the cyanide content of casava chips. J. Food Technol. 13: 299-306.

Cooke, R.D., Blake, G.G. and Blattershill, J.M. (1978). Phytochem. 17: 381-383. (cited by Nambisan and Sundaresan, 1984).

Coursey, D.G. (1973). Cassava as food : toxicity and technology. In Chronic Cassava Toxicity. Nestel, B. and McIntyre, R. Eds., International Development Research Centre, Ottawa, Canada IDRC-010e, pp. 27-36.

Crampton, E.W. and Lioyd, L.E. (1959). Fundamentals of Nutrition. W.H. Freeman and Company, San Francisco.

Crispel, K.R. and Wilson, E.C. (1964). Pathology of myxoedema. In The Thyroid. Hazard, J.B. and Smith, D.E. Ed., Williams and Wilkins Co., Baltimore. pp. 236-238.

DeBruijn, G.H. (1973). The cyanogenic character of cassava (Manihot esculenta). In Chronic Cassava Toxicity. Nestel, B. and McIntyre, R., Eds., International Development Research Centre, Ottawa, Canada, IDRC-010e, pp. 43-48.

Delangé, F., Bourdox, P. and Ermans, A.M. (1982). Summary and general conclusions. In Nutritional Factors Involved in the Goitrogenic Action of Cassava. Delange, F., Iteke, F.B., Ermans, A.M., Eds., International Development Research Centre, Ottawa, Canada, IDRC-184e, pp. 84-86.

Delange, F., Van der Velden, M. and Ermans, A.M. (1973). Evidence of an antithyroid action of cassava in man and animals. In Chronic Cassava Toxicity. Nestel, B. and McIntyre, R. Eds., International Development Research Centre, Ottawa, Canada, IDRC-010e, pp. 141-157.

Disbery, B.D. and Rack, J.H. (1970). Histological Laboratory Methods. E&S Livingstone, Edinburgh. pp. 99-101.

Donati, R.M., Warnecke, M.A. and Gallagher, N.I. (1965). Ferrokinetics in hyperthyroidism. Ann. Intern. Med. 63: 945.

Dorozynski, A. (1978). Cassava may lead to mental retardation. Nature 272: 121.

Douglas, R.C. and Jacobson, S.D. (1957). Pathologic changes in adult myxoedema : survey of 10 autopsies. J. Clin. Endocr. 17: 1354.

Ekpechi, O.L., Dimitriadou, A. and Fraser, R. (1966). Goitrogenic activity of cassava (a staple Nigerian food). Nature 210: 1137-1138.

Elgee, N.J. and Williams, R.H. (1955). Effects of thyroid function on insulin I^{131} degradation. Am. J. Physiol. 180: 13.

Ensinck, J.W. and Williams, R.H. (1981). Disorders causing hypoglycemia. In Text Book of Endocrinology. Williams, R.H. Ed., IGAKU-SHOIN/Saunders International, Tokyo and W.B. Saunders Company, London. 6th Ed. p. 864.

Ericsson, J.L.E., Orrenius, S. and Holm, I. (1966). Alterations in canine liver cells induced by protein deficiency - ultrastructure and biochemical observations. Exptl. Mol. Pathol. 5 (4): 329-349.

Ermans, A.M. (1980). General conclusions. In Role of Cassava in the Etiology of Endemic Goitre and Cretinism. Ermans, A.M., Mbulamoko, N.M., Delange, F. and Ahluwalia, R., Eds., International Development Research Centre, Ottawa, Canada, IDRC-136e, pp. 147-152.

Ermans, A.M., Kinthaert, J., Van der Velden, M., and Bourdox, P. (1980). Studies of the antithyroid effects of cassava and of thiocyanate in rats. In Role of Cassava in the Etiology of Endemic Goitre and Cretinism. Ermans, A.M., Mbulamoko, N.M., Delange, F. and Ahluwalia, R., Eds., International Development Research Centre, Ottawa, Canada, IDRC-136e, pp. 93-110.

F.A.O. (1980). Production yearbook, Vol.33, Rome, Italy.

Fletcher, K. and Myant, M.B. (1958). Influence of thyroid on the synthesis of cholesterol by liver and skin in vitro. J. Physiol. **144**: 361-372.

Flux, D.S., Butler, G.W., Jhonson, J.M. and Glenday, A.C. (1956). N. Z. J. Sci. Technol. **36**: 88-102 (cited by Annamma Mathew 1979).

Geevarghese, P.J. (1968). Pancreatic Diabetes. Popular Prakashan, Bombay, India. p. 93.

Geevarghese, P.J. (1982). Tropical calcifying pancreatitis and pancreatic diabetes. In Cassava Toxicity and Thyroid : Research and Public Health Issues. Delange, F. and Ahluwalia, R. Eds., International Development Research Centre, Ottawa Canada, IDRC-207e, pp. 77-78.

Geevarghese, P.J. (1986). Chronic Pancreatitis in the Tropics. Popular Prakashan, Bombay, India. pp. 58-65.

Gilbert, C. (1984). Endemic goitre : the cassava factor. World Health Forum 5: 170-174.

Gomez, G. (1982). Cassava, cyanide and animal nutrition. In Cassava Toxicity and Thyroid : Research and Public Health Issues. Delange, F. and Ahluwalia, R. Eds., International Development Research Centre, Ottawa, Canada, IDRC-207e, pp. 109-113.

Gomez, G. and Valdivieso, M. (1983a). Changes in cyanide content of cassava tissues as affected by plant age and variety. Proc. 6th Symp. Int. Soc. Tropical Root Crops. 21-26 February 1983, Lima, Peru.

Gomez, G., Santos, J. and Valdivieso, M. (1983). Least-cost rations containing cassava meal for broilers and growing pigs. Proc. 6th Symp. Int. Soc. Tropical Root Crops, 21-26 February 1983, Lima, Peru.

Gomez, G., Valdivieso, M., De la Cuesta, D. and Salcedo, T.S. (1984). Effect of variety and plant age on the cyanide content of whole-root cassava chips and its reduction by sun-drying. Anim. Feed. Sci. Technol. 11: 57-65.

Green, W.L. (1971). Mechanisms of action of antithyroid compounds. In The Thyroid. Werner, S.C. and Ingbar, S.H. Eds., Harper and Row Publishers, New York. 3rd Ed. pp. 41-51.

Harkness, J.E. and Wagner, J.E. (1989). The Biology and Medicine of Rabbits and Rodents. Lea & Febiger, Philadelphia, London. 3rd Ed. p. 49.

Hellman, L., Bradlow, H.L., Zumoff, B., Fukushima, D.K. and Gallagher, T.F. (1959). Thyroid-androgen inter-relations and the hypocholesteremic effect of androsterone. J. Clin. Endocr. **19**: 936.

Himwich, W.A. and Saunders, J.P. (1948). Am. J. Physiol. 153: 348-354 (cited by Annamma Mathew 1979).

ICAR (1985). Nutrient Requirements of Livestock and Poultry. pp. 7-8.

Ingbar, S.H. (1971). Clinical considerations. In The Thyroid. Werner, S.C. and Ingbar, S.H. Ed., Harper and Row Publishers, New York, pp. 243-253.

Ingbar, S.H. and Woeber, K.A. (1981). The thyroid gland. In Text Book of Endocrinology. Williams, R.H. Ed., IGAKU-SHOIN/Saunders International Tokyo and W.B. Saunders Company, London. 6th Ed. pp. 172-173.

Joachim, A.W.R. and Panditteseker, D.G. (1944). Investigations of the hydrocyanic acid content of manioc (Maninot utilissima). Tropical Agriculturist **100**: 150-163.

Jones, R.J., Megarrity, R.G. (1983). Comparative toxicity responses of goats fed on Leucaena leucocephala in Australien and Hawaii. Aust. J. Agric. Res. **34**: 781-790.

Kamalu, B.P. (1991). Digestibility of a nutritionally-balanced cassava (Manihot esculenta Crantz) diet and its effect on growth in young male dogs. Brit. J. Nutr. **66**: 199-208.

Kawano, K. (1978). Genetic improvement of cassava (Manihot esculenta Crantz) for productivity. Trop. Agric. Res. Ser. No. 11, pp. 9-21.

Keele, C.A. and Neil, E. (1971). Samson Wright's Applied Physiology. Oxford University Press, New York. 12th Ed. p. 433.

King, E.J. and Wootton, I.D.P. (1959). Microanalysis in Medical Biochemistry. J&A Churchill Ltd., London. 3rd Ed. pp. 25-27.

Kingsbury, J.M. (1975). Phytotoxicology. In Toxicology - The Basic Science of Poison. Casarett, L.J. and Doull, J. Eds., McMillan Publishing Company, Inc., New York. p. 598.

Kochupillai, N., Deo, M.G., Karmarkar, M.G., McKendrick, M., Weightman, D., Evered, D.C., Hall, R. and Ramalingaswamy, V. (1973). Pituitary thyroid axis in Himalayan endemic goitre. Lancet. May 12. pp. 1021-1024.

Koppers, L.E. and Palumbo, P.J. (1972). Lipid disturbances in endocrine disorders. Med. Clin. North Am. 56: 1013.

Kourides, I.A. (1981). Clinical application of the radio-immunoassay for human TSH. In Radioassay Systems in Clinical Endocrinology. Abraham, G.E. Ed., Marcel Dekker, Inc., New York and Basel. pp. 62-64.

Lamberg, B.A. and Grasbeck, R. (1955). The Serum Protein pattern in disorders of thyroid function. Acta Endocr. 19: 91-100.

Larsen, P.R. (1981). The use of serum T_3 measurements by radioimmunoassay in the diagnosis of thyroid disease. In Radioassay Systems in Clinical Endocrinology. Abraham, G.E. Ed., Marcel Dekker, Inc., New York and Basel. p. 127.

Lenzen, S., Joost, H.G. and Blatt, H.A. (1976). The thyroid function and insulin secretion from the perfused pancreas in the rat. Endocrinology 99: 125-129.

Loireau, A., Dumas, P., Autissier, N. and Michel, R. (1987). Influence of thyroid status on body weight gain, food intake and serum lipid levels in genetically obese zucker rats. J. Nutr. 117: 159-163.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Maduagwu, E.N. and Adewale, A.F. (1980). Loss of hydrocyanic acid and its derivatives during sun drying of cassava. In Tropical Root Crops : Proceedings of 1st triennial Symposium of International Society of Root Crops, Africa Branch, IDRC-163e, pp. 149-151.

Mason, R. and Wilkinson, J.S. (1973). Thyroid gland - A review. Aust Vet. J. 49: 44-49.

McDonald, P., Edwards, R.A. and Greehalgh, J.F.D. (1982). Animal Nutrition. The English Language Book Society, Longman Group Ltd., Essex, U.K. 3rd Ed. pp. 196-198.

McMillan, D.E. and Geevarghese, P.J. (1979). Dietary cyanide and tropical malnutritiion diabetes. Diabetes Care 2: 202-208.

Means, J.H., Degroot, L.J. and Stanbury, J.B. (1963). The Thyroid and its Diseases. McGraw-Hill Book Company, Inc., New York. 3rd Ed. pp. 18-19 and 386.

Meittinen, T.A. (1968). Mechanism of serum cholesterol reduction by thyroid hormones in hypothyroidism. J. Lab. Clin. Med. 71: 537.

Mercy, A.D., Sivraman, E., Kunjikutty, N. and Annamma Kurian (1981a). Studies on the growth rate, feed efficiency and digestibility coefficients of nutrients in Alpine-Malabari crossbred kids. Kerala J. Vet. Sci. 12 (1): 164-170.

Metzger, B.E. and Freinkel, N. (1971). Metabolic changes. In The Thyroid. Werner, S.C. and Ingbar, S.H. Ed., Harper and Row Publisher, New York. 3rd Ed. pp. 744-747.

Miale, J.B. (1967). Laboratory Medicine Haematology. The c.v. Morby Company, St. Louis. 3rd Ed. pp. 1143-1144.

Montgomery, R.D. (1969). Cyanogens. In Toxic Constituents of Plant Food Stuffs. I.E. Liener (Ed.), Academic Press, New York. pp. 143-157.

Muldowney, F.P., Crooks, J. and Wayne, E.J. (1957). The total red cell mass in thyrotoxicosis and myxoedema. Clin. Sci. 16: 309.

Nambisan, B. and Sundaresan, S. (1984). Spectrophotometric determination of cyanoglucosides in cassava. J. Assoc. Off. Anal. Chem. 67: 641-643.

Nambisan, B. and Sundaresan, S. (1985). Effect of processing on the cyanoglucoside content of cassava. J. Sci. Food Agric. 36: 1197-1203.

Nambisan, B. and Sundaresan, S. (1991). Cyanoglucosides in cassava. Central Tuber Crop Research Institute, Kerala, India. CTCRI, Tech. Bull. Ser. No.12.

Nangia, O.P., Dixit, U.P. and Agarwal, V.K. (1975). Studies on some blood constituents in chicken with modified thyroid activity. Haryana Agric. Univ. J. Res. 5: 254-259.

Nartey, F. (1968). Studies on cassava, Manihot utilissima Pohl. 1. Cyanogenesis : The Biosynthesis of Linamarin and Lotaustralin in Etiolated Seedings. Phytochem. 7: 1307-1312.

Nartey, F. (1973). Biosynthesis of cyanogenic glucosides in cassava (Manihot spp.). In Chronic Cassava Toxicity. Nestel, B. and McIntyre, R., Eds., Interntional Development Research Centre, Ottawa, Canada, IDRC-010e, pp. 73-87.

Nartey, F. (1978). Manihot esculenta (Cassava). Cyanogenesis, Ultrastructure and Seed Germination. Muksgaard, Copenhagen, Denmark. p. 262.

Nestel, B. (1973). Summary of the general discussion. In chronic cassava toxicity. Nestel, B. and McIntyre, R. Eds. International Development Research Centre, Ottawa, Canada. p. 161.

Oke, O.L. (1982). Processing and detoxification of cassava. In Cassava Toxicity and Thyroid: Research and Public Health Issues. Delange, F. and Ahluwalia, R., Eds., International Development Research Centre, Ottawa, Canada, IDRC-207e, pp. 129-133.

Okeke, G.C. and Oji, U.I. (1987). The nutritive value of grass ensiled with cassava peel and poultry excreta for goats. In Goat Production in the Humid Tropics: Proceedings of a Workshop at the University of Ife, Ile-Ife, Nigeria, 20-24 July 1987, pp. 101-106.

Padmaja, G. and Panikkar, K.R. (1989b). Intermediary metabolic changes in rabbits administered linamarin or potassium cyanide. Indian J. Exp. Biol. 27: 635-639.

Peters, J.P. and Man, E.B. (1950). The significance of serum cholesterol in thyroid diseases. J. Clin. Invest. 29: 1-7.

Phillips, T.P. (1982). An overview of cassava consumption and production. In. Cassava Toxicity and Thyroid: Research and Public Health Issues. Delange, F. and Ahluwalia, R., Eds., International Development Research Centre, Ottawa, Canada, IDRC-207e, pp. 83-88.

Pitchumoni, C.S. (1973). Pancreas in primary malnutrition disorders. Am. J. Clin. Nutr. 26: 374-379.

Porterfield, S.P. and Hendrich, C.E. (1976). The effects of growth hormone treatment of thyroid deficient pregnant rats on maternal and fetal carbohydrate metabolism. Endocrinology 99: 786-792.

Paulose, K.P., Soman, T.N. and Mathew, K.J. (1984). Incidence of goitre in Kerala. Kerala Med. J. 25: 180-183.

Pushpa, M. (1980). Chronic cassava toxicity. An experimental study. MD thesis submitted to Kerala University, Trivandrum, India.

Radeleff, R.D. (1970). Veterinary Toxicology. Lea & Febiger, Philadelphnia, 2nd Ed. p. 52.

Rajan, A. (1989). Incidence and Nature of Hypothyroidism in Domestic Animals: project report. Centre of Excellence in Pathology, KAU, Mannuthy (Trichur), India.

Rao, V.S. (1964). J. Nutr. Dietetics 1 (1): 8-13. (Cited by Annamma Mathew, 1979).

Ratnakumar, J.N. (1989). Prevalence and pathology of hypothyroidism in cattle. M.V.Sc. thesis submitted to Kerala Agricultural University, Mannuthy (Trichur), India.

Reddy, N.M. (1982). Pathology of the reproductive organs in experimental hypothyroidism in goats. Ph.D. thesis submitted to Kerala Agricultural University, Mannuthy (Trichur), India.

Rogers, D.J. (1963). Studies of Manihot esculenta Crantz (Cassava) and related species. Bull. Torrey bot. Cl. 90: 43-54.

Sasaki, H., Takahashi, H., Hikosaka, A.K., Hagino, A. and Oda, S. (1984). Insulin response to glucose and glucose tolerance following feeding in sheep. Brit. J. Nutr. 52: 351-358.

Sasaki, Y., Weekes, T.E.C. and Bruce, J.B. (1977). Effect of glucose and butryrate on insulin release from perfused fragments of sheep. J. Endocr. 72: 415-416.

Schneider, W.C. (1957). Determination of nucleic acids in tissues by pentose analysis. In ' Methods in Enzymology. Colowick, S.P. and Kaplan, N.O. Eds., Academic Press Inc., Publishers, New York. Vol.III. pp. 680-684.

Schooley, R.A., Friedkin, S. and Evans, E.S. (1966). Re-examination of the discrepancy between acidophil numbers and growth hormone concentration in the anterior pituitary gland following thyroidectomy. Endocrinology 79: 1053.

Seo, H., Refeloff, S. and Fang, V.S. (1977). Induction of hypothyroidism and hypoprolactinemia by growth hormone producing rat pituitary tumors. Endocrinology 100: 216-226.

Shenoy, K.T., Leena, K.B., Nair, R.B. and Praseeda, K.I. (1993). Cardiovascular changes in an experimental model fed on cassava-based diet. Proc. 4th World Congress on Clinical Nutrition, 2-5 October 1993, Cochin, Kerala, India.

Shyama, K. (1994). Effect of dried spleen as growth stimulator in kid rations. M.V.Sc. thesis submitted to Kerala Agricultural University, Mannuthy (Trichur), India.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. The Iowa State College Press, Ames, Iowa. 6th Ed.

Sobel, B.E. and Braunwald, E. (1971). Cardiovascular system. In. The Thyroid. Werner, S.C. and Ingbar, S.H. Eds., Harper and Row Publishers, New York. pp. 727-734.

Soto, R.J., DeNicola, A. and Blaquier, J. (1981). Physiopathology of Endocrine Diseases and Mechanisms of Hormone Action. Alan, R. Liss, Inc., New York. pp. 71-73.

Spiegel, C., Bestetti, G.E., Rossi, G.L. and Blum, J.W. (1993a). Normal circulating triiodothyronine concentrations are maintained despite severe hypothyroidism in growing pigs fed rapeseed presscake meal. J. Nutr. 123: 1554-1561.

Spiegel, C., Bestetti, G.E., Rossi, G.L. and Blum, J.W. (1993b). Lower food intake is a primary cause of reduced growth rate in growing pigs fed rapeseed presscake meal. J. Nutr. 123: 1562-1566.

Swai, A.B.M., McLarty, D.G., Mtinangi, B.L., Tatala, S., Kitange, H.M., Mlingi, N., Rosling, H., Howlett, W.P., Brubaker, G.R. and Alberti, K.G.M.M. (1992). Diabetes is not caused by cassava toxicity. Diabetes Care 15: 1378-1385.

Swan, H. and Lewis, D. (1976). Feed energy sources for livestock. ButterWorths, London. pp. 21-22.

Takama, S. and Kishino, Y. (1985). Dietary effects on pancretic lesions induced by excess arginine in rats. Brit. J. Nutr. 54: 37-42.

Tasker, P.K. (1962). Food Science 11(7): 205-210. (Cited by Annamma Mathew, 1979).

Teuscher, T., Baillod, P., Rosman, J.B. and Teuscher, A. (1987). Absence of diabetes in rural West African population with a high carbohydrate/cassava diet. Lancet. April 4. pp. 765-768.

Tewe, O.O. (1982). Thyroid cassava toxicity in animals. In Cassava Toxicity and Thyroid: Research and Public Health Issues. Delange, F. and Ahluwalia, R., Eds., International Development Research Centre, Ottawa, Canada, IDRC-207e, pp. 114-118.

Tewe, O.O. (1984). Effect of cassava-based diets varying in cyanide content on the performance and physiopathology of the African giant rat (Cricetomys Gambinus Waterhouse). Anim. Feed Sci. Technol. 11: 1-9.

Tewe, O.O. and Maner, J.H. (1978). Influence of the cyanogenic glucoside fraction of cassava on performance, thiocyanate concentration and rhodanase activity of rats during growth and reproduction. Nigerian J. Anim. Prod. 5(2): 207-212.

Tewe, O.O. and Maner, J.H. (1980). Cyanide, protein and iodine interactions in the performance, metabolism and pathology of pigs. Res. Vet. Sci. 29: 271-276.

Tewe, O.O. and Maner, J.H. (1981). Performance and patho-physiological changes in pregnant pigs fed cassava diets containing different levels of cyanide. Res. Vet. Sci. 30: 147-151.

Thomas, C.T. (1966). Studies on the biological utilisation of carbohydrates. M.Sc. thesis submitted to the University of Kerala, India.

Turnock, B.J.W. (1937). An investigation of the poisnous constituents of sweet cassava (Manihot utilissima) and the occurrence of hydrocyamic acid in foods prepared from cassava. J. Trop. Med. Hyg. 40: 65-66.

Udupa, K.N., Mishr, S.K. and Agarwal, J.K. (1983). Disorders of thyroid gland in tropics. Vikas Publishing House Pvt. Ltd., New Delhi. pp. 73-81 and 219-220.

Wyngaarden, J.B., Stanbury, J.B. and Rapp, B. (1953). The effects of iodide, perchlorate, thiocyanate, and nitrate administration upon the iodide concentrating mechanism of the rat thyroid. Endocrinology 52: 568.

Yoshida, M., Hishi, H., Kosaka, K. and Moromoto, H. (1966). Japanese Poult. Sci. 3: 39-44. (Cited by Annamma Mathew, 1979).

Zak, B. (1957). A simple rapid micro-technique for serum total cholesterol. Am. J. Clin. Path. 27: 583-588.

INFLUENCE OF DIETARY SUPPLEMENTATION OF PROTEIN AND IODINE ON TAPIOCA TOXICITY

By

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ABSTRACT OF A THESIS

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ABSTRACT

A three phase experiment was carried out to assess the level of cyanogenic glycoside (CNG) content in seven varieties of tapioca, its removal by various processing methods and its effect on metabolism in rats and kids. The role of dietary protein and iodine in modifying the deleterious effects of cyanide was investigated. Histopathological studies on the liver, pancreas and heart were also carried out in rats.

The first phase of the study revealed that CNG content in terms of cyanide varied from 40.86 ± 2.79 to 186.31 ± 5.30 µg/g of fresh tuber with the lowest concentration in 'M-4' and higher concentrations in 'H-165' and 'Karkidakkan'. Processings like boiling and sun drying lowered the CNG content by about 50 per cent of the original.

In the second phase 60 male albino Wistar rats, divided into six groups were given the following diet.

Group I - Protein = 15%; Tapioca = Nil; Iodine = Nil

Group II - Protein = 15%; Tapioca = 30%; Iodine = Nil

Group III - Protein = 7.5%; Tapioca = 30%; Iodine = Nil

Group IV - Protein = 7.5%; Tapioca without HCN = 30%; Iodine = Nil

Group V - Protein = 15%; Tapioca = 30%; Iodine = 0.17 mg/kg

Group VI - Protein = 22.5%; Tapioca = 30%; Iodine = 0.17 mg/kg

Performance of rats was evaluated by recording growth rate, feed consumption and feed efficiency for a period of 12 weeks. Dry matter digestibility was also determined over a period of 24 h. At the end of 10th, 11th and 12th week of the study the rats were sacrificed and endocrinological, biochemical and histopathological evaluation of blood/serum and tissues were made.

Performance of rats was poor on tapioca-based diet not supplemented with iodine (Groups II and III). Rats fed on protein deficient diet in the presence of cyanide containing tapioca were the most affected (Group III). However, there was no significant variation in the performance in Groups V and VI compared to Group I when tapioca-based diet were supplemented with iodine. The digestibility of dry matter in six groups of rats was almost inversely related to the quantity of feed consumed.

There was significant increase in DNA and decrease in protein content of thyroid thereby increasing the DNA: protein ratio, followed by extremely low levels of thyroxine and hyperplasia of the thyroid in Group III. In Group II also there occurred a significant reduction in the levels of thyroxine and a resultant hypertrophy of the thyroid.

Higher levels of dietary protein and iodine supplementation were found to play a decisive role in

counteracting the inhibitory effects of cyanide and thiocyanate of tapioca origin especially on thyroid functions.

The DNA: protein ratio per unit weight of pancreatic tissue was increased in groups fed on low levels of dietary protein (Groups III and IV).

Insulin levels in different groups of rats were found to be primarily related to the levels of blood glucose. There was no significant difference in insulin-to-glucose ratio between the groups. The synthesis and release of insulin from the pancreas was not affected by cyanide present in the diet of tapioca fed groups.

Rats showed no definite pattern of response in their blood glucose levels to cyanide contained in the diet of tapioca fed groups.

There was elevation in the levels of serum total protein, cholesterol and lipid concentrations in rats fed tapioca without supplementation of iodine (Groups II and III). This may be the result of the development of hypothyroidism. However, the increase in serum total protein and lipid in Group II was not significant. The results indicated that the above parameters could be used as reliable biochemical indicators of thyroid activity.

A significant reduction in haemoglobin concentration

in rats of Groups II and III compared to Groups I and IV reflected the deleterious effects of cyanogens on thyroid functions as well as the importance of availability of dietary protein for haemoglobin synthesis.

The hepatic cells in Group II appeared slightly swollen with occasional cells showing vacuolar changes. Pancreas showed slight oedema and occasional mild degenerative changes of exocrine cells. Also there was slight interstitial oedema in the myocardium. Intensity of pathological changes in the liver, pancreas and heart were more severe in rats fed on protein deficient, tapioca diet (Group III) compared to that of Group IV in the diet of which tapioca was made cyanide free. The hepatic cells in Group III showed marked diffused vacuolar changes with condensed or vesicular nuclei and occasional cell necrosis. Many pancreatic exocrine cells showed vacuolar and degenerative changes. Interstitial oedema was prominent. Inspite of these pancreatic exocrine changes, it is important to note that the islets remained almost intact in all the groups studied. Myocardium showed interstitial oedema with swollen sarcoplasm and fragmented sarcolemma. At the same time only moderate vacuolar changes in the liver, distention of interlobular space and interstitial oedema in myocardium were observed in Group IV. Since the diet of Groups V and VI were supplemented with iodine, no significant cytological alterations could be seen in their organs.

In the third phase of the study thirty 2½-3 months old Alpine-Malabari kids of both sexes were divided into 3 groups. They were given the following diet.

Groups I Protein = 15%; Tapioca = Nil; Iodine = Nil

Groups II Protein = 15%; Tapioca = 30%; Iodine = 2 mg/kg

Groups III Protein = 25%; Tapioca = 30%; Iodine = 2 mg/kg

The study included evaluation of the performance of kids and determination of certain endocrinological and biochemical parameters in their blood at fortnightly intervals for 3 months.

There was no significant effect on the performance (body weight gain, dry matter intake and feed efficiency) of kids fed on tapioca-based diet supplemented with iodine, compared to Group I. However, the significant difference in body weight gain between tapioca fed groups (Groups II and III) was probably due to the difference in the relative availability of protein in their diet.

The results of the study in kids indicated that there was no significant influence of cyanogenic principles present in tapioca on the thyroxin and insulin levels when iodine was supplemented in the diet. Similarly the blood glucose, serum total protein, cholesterol, lipid and haemoglobin levels were also not influenced throughout the study.