BIOLOGICAL UTILIZATION OF B-CAROTENE FROM AMARANTH AND LEAF PROTEIN IN PRESCHOOL CHILDREN

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TABLE OF CONTENTS

Chapter			Page No.
	LIST OF TABLES		
	LIST OF ANNEXURES		
	LIST OF FIGURES		
I.	INTRODUCTION	..	1
II.	REVIEW OF LITERATURE	..	6
	Global incidence of vitamin A deficiency	..	6
	Clinical and biochemical manifestations of vitamin A deficiency	..	7
	Dietary intake of vitamin A and B-carotene of Indian children	..	8
	Effect of cooking on provitamin content of foods	..	9
	Factors affecting utilization of vitamin A and B-carotene	..	10
	Influence of fat on the absorption of B-carotene and vitamin A	..	11
	Effect of protein nutritional status on absorption and utilization of B-carotene and vitamin A	..	12
	Influence of vitamin E on the absorption of vitamin A and B-carotene	..	15
	Effect of Emulsification on the absorption of vitamin A	..	16
	Effect of infection on the absorption of vitamin A and B-carotene	..	16
	Studies on Biological Availability of B-carotene	..	18
	Promising sources of B-carotene in poor dietaries	..	19

Chapter			Page No.
III.	EXPERIMENTAL PROCEDURE	..	22
	A. Selection of subjects	..	22
	B. Estimation of B-carotene in the foods	..	22
	C. Conducting food weighment survey	..	23
	D. Formulation of the Balwady Supplement diet		23
	E. Conducting the feeding trials	..	24
	F. Collection of faeces for analysis	..	26
	G. Estimating the availability of B-carotene	..	28
	H. Estimation of serum vitamin A	..	28
	I. Estimation of serum total protein and albumin	..	28
	J. Estimation of Haemoglobin	..	28
	K. Record of incidence of illness	..	29
IV.	RESULTS AND DISCUSSION	..	30
	General background of the participating children and their families	..	30
	The body heights and weights of the target children at the beginning of the study	..	31
	Diet and Nutrient intake of the target children	..	32
	Identification of different fractions obtained		37
	B-carotene content of amaranth and LP	..	37
	Effect of cooking on the B-carotene content of amaranth and LP	..	38
	B-carotene intake by the target children during the various phases of the study	..	40

Chapter		Page No.
	Excretion of B-carotene in the faeces of the children fed with balwady supplement along with the home diet ..	41
	Biological utilization of B-carotene from three different sources namely amaranth, LP and standard B-carotene ..	43
	Cost analysis of the supplement ..	49
	Changes in serum vitamin A after feeding various sources of B-carotene ..	50
	Serum total protein and albumin level of the target children ..	54
	Estimation of blood Haemoglobin ..	57
	Episodes of infection ..	58
	Deficiency symptoms among the target children ..	58
V.	SUMMARY AND CONCLUSION ..	59
VI.	BIBLIOGRAPHY ..	61
VII.	ANNEXURES ..	72

LIST OF TABLES

Table		Page No.
I.	SUPPLEMENT FED AT THE BALWADY PER CHILD PER DAY ..	23
II.	DIFFERENT SOURCES AND AMOUNTS OF B-CAROTENE FED TO THE TARGET CHILDREN DURING THE FOUR PHASES OF STUDY ..	24
III.	AGE AND SEX STATUS OF THE TARGET CHILDREN AND THEIR BODY HEIGHTS AND WEIGHTS AT THE BEGINNING OF THE STUDY ..	31
IV.	MEAN DAILY HOME FOOD CONSUMPTION OF THE TARGET CHILDREN ..	32
V.	MEAN CALORIE PROTEIN AND B-CAROTENE INTAKE OF THE TARGET CHILDREN ..	34
VI.	SPECTRA OF FRACTIONS OF CAROTENE AS SEPARATED BY AN ALUMINA COLUMN ..	37
VII.	B-CAROTENE CONTENT OF AMARANTH AND LP ..	38
VIII.	B-CAROTENE CONTENT OF RAW AND COOKED AMARANTH AND LP ALONG WITH THE COOKING LOSSES ..	39
IX.	PERCENTAGE OF B-CAROTENE AS A FRACTION OF TOTAL CAROTENE IN RAW AND COOKED AMARANTH ..	40
X.	QUANTITY AND SOURCES OF B-CAROTENE FED DURING VARIOUS PERIODS OF THE STUDY ..	41
XI.	B-CAROTENE EXCRETION THROUGH FAECES DURING THE BASAL PERIOD BY THE TARGET CHILDREN ..	42
XII.	PERCENTAGE UTILIZATION OF B-CAROTENE FROM AMARANTH BY THE 15 TARGET CHILDREN ..	44
XIII.	PERCENTAGE UTILIZATION OF B CAROTENE FROM LP ..	45
XIV.	PERCENTAGE UTILIZATION OF B CAROTENE FROM STANDARD B-CAROTENE ..	46

Table			Page No.
XV.	COMPARISON OF PERCENTAGE UTILIZATION OF B-CAROTENE FROM THREE DIFFERENT SOURCES IN FIFTEEN TARGET CHILDREN	..	47
XVI.	QUANTITY AND COST OF FOODS THAT PROVIDE THE DAILY B-CAROTENE OR RETINOL REQUIREMENT OF A PRE SCHOOL CHILD	..	50
XVII.	MEAN SERUM VITAMIN A LEVELS OF THE 15 TARGET CHILDREN BEFORE AND AFTER SUPPLEMENTATION	..	51
XVIII.	SERUM TOTAL PROTEINS, ALBUMINS AND GLOBULINS	..	53
XIX.	VITAMIN A AND PROTEIN CONTENT OF PLASMA IN 15 TARGET CHILDREN	..	54
XX.	CORRELATION COEFFICIENT BETWEEN VITAMIN A AND PROTEIN	..	55
XXI.	MEAN SERUM PROTEIN AND ALBUMIN LEVELS OF THE TARGET CHILDREN BEFORE AND AFTER THE STUDY PERIOD	..	55
XXII.	MEAN HAEMOGLOBIN CONTENT OF THE TARGET CHILDREN BEFORE AND AFTER THE STUDY	..	57

LIST OF ANNEXURES

Annexure			Page No.
I.	a. ESTIMATION OF B-CAROTENE FROM LEAF PROTEIN	..	72
	b. ESTIMATION OF CAROTENE FROM AMARANTH	..	73
II.	PREPARATION OF UPPUMA	..	74
	PREPARATION OF LADDU	..	75
III.	PREPARATION OF LEAF PROTEIN	..	76
IV.	DETERMINATION OF FAECAL CAROTENE	..	78
V.	ESTIMATION OF SERUM VITAMIN A (RETINOL)	..	79
VI.	BIURET METHOD FOR TOTAL PROTEIN AND ALBUMINS IN PLASMA OR SERUM	..	81
VII.	ESTIMATION OF HAEMOGLOBIN BY CYANMETHAEMOGLOBIN METHOD	..	83
VIII.	EXCRETION OF B-CAROTENE DURING THE THREE SUBSEQUENT TERMINAL DAYS BY THE TARGET CHILDREN WHEN AMARANTH WAS FED AS A SOURCE OF B-CAROTENE	..	85
IX.	EXCRETION OF B-CAROTENE DURING THE THREE SUBSEQUENT TERMINAL DAYS BY THE TARGET CHILDREN WHEN LEAF PROTEIN WAS FED AS A SOURCE	..	86
X.	EXCRETION OF B-CAROTENE DURING THE THREE SUBSEQUENT TERMINAL DAYS BY THE TARGET CHILDREN WHEN STANDARD B-CAROTENE WAS FED AS A SOURCE OF B-CAROTENE	..	87

LIST OF FIGURES

Figure			Page No.
1.	THE SCHEME OF THE STUDY	..	27
2.	NUTRIENT INTAKE OF THE TARGET CHILDREN	..	35
3.	PERCENTAGE ABSORPTION OF B-CAROTENE FROM THREE DIFFERENT SOURCES NAMELY AMARANTH LEAF PROTEIN AND STANDARD B-CAROTENE	..	48
4.	INITIAL AND FINAL SERUM VITAMIN A LEVELS OF THE TARGET CHILDREN	..	52
5.	REGRESSION LINE OF SERUM PROTEIN LEVELS ON SERUM VITAMIN A LEVELS	..	56

I. INTRODUCTION

It is one of those anomalies of nature and man that the countries with the highest rates of xerophthalmia are among the perennially greenest of the world, but the nutritious green leaves do not find their way into the mouth of the small child (oomen 1974). Days and nights become meaningless for lakhs of children in India (Alan Berg 1973). Light is shut for them for ever. Not that they were born blind but they only did not have proper diet and nourishment. If only they could have enough of vitamin A or its precursor B-carotene in their food, this tragedy could have been averted (Beck 1974).

Vitamin A deficiency is a major public health problem in India contributing as it does, to a sizable proportion of preventable blindness (Srikantia 1975). About one fifth of the world's blind are in India. The number of blind persons in India today is estimated to be 5 million (WHO, 1976). Vitamin A deficiency can be expected where ever poor living conditions exist for sometime, such as among impoverished and illiterate farm labourers, displaced households or in the septic fringes of tropical conurbations (Oomen 1973). Its prevalence is due to a combination of poor social and economic factors. (Ramalingaswami, 1975; Gopalan, 1975).

Vitamin A deficiency and the epidemiology of xerophthalmia have been studied by several groups of scientists (Mclaren (1962), Rodger et al (1964), Oomen (1969 and 1972), Patwardhan (1969)). In any country an ecological approach is needed to delinate the causes for the deficiency of vitamin A. (Van Veen et al 1974; Brown, 1975) One of the important cause of the widespread prevalence of this deficiency is the inadequate dietary intake of the vitamin.

The most rational method of prevention of vitamin A deficiency in children would be to improve the diets consumed by them, so as to ensure adequate intakes of the vitamin (Vinodini Reddy and Lala, 1970 and Devadas et al, 1975). By encouraging consumption of green leafy vegetables which are of low cost and rich in provitamin A compounds namely carotenoids, the incidence of vitamin A deficiency can be considerably reduced among poor communities (Rajalakshmi et al 1974; Jean Mayer, 1975).

Foods containing preformed vitamin A are expensive and beyond reach of the poor. Carotene derived from plant foods therefore need to be the chief source of vitamin A in the diet of poor population groups in India (Rao et al 1970). Amaranth is one of the perennial green leafy vegetable actually or potentially available and can be even grown with minimum effort in the kitchen garden (PAG, 1973). Hence such foods are worth using frequently as they are very good sources of B-carotene. Their consumption in adequate amounts especially by children should be encouraged.

Leaf protein with its relatively high protein content offer much promise (Olatunbosun, 1972) and is derived from green leaves and is one of the richest source of provitamin A obtainable (Bray, 1975). Its B-carotene content is 1.4 mg/g according to Pirie (1971) and its content overbeats any other plant food stuff which are considered to be very rich in B - carotene. Such a nutritious delight which in small amounts and at low cost provides large amounts of B-carotene should certainly be incorporated along with other diets for the public health improvement. From this view point the use of LP strictly as a source of vitamin A would seem justified, particularly in countries like Inida where the average diet provides far less than the recommended amount (Devadas and Kamalanathan 1975).

Plant carotenes are not fully absorbed by human subjects and the absorption has been found to vary over a wide range (FAO/WHO Expert Group 1967). There are also reports that suggest that both the absorption of B-carotene and its conversion to vitamin A are influenced by the protein nutritional status (NAS, 1974) Besides dietary protein, fat, minerals, acute and chronic infections, and growth rate also affect the absorption of B-carotene. (Sivakumar, 1972; Underwood, 1974 and Srikantia, 1975). The FAO/WHO group (1967) however considered that the available data on carotene absorption are few and variable and indicated the necessity for further research. In two recent studies, one conducted in adults (Nageswara Rao 1970) and the other in children (Lala et al 1970) absorption of B-carotene from green leafy vegetables was found to vary between 52 and 99 per cent.

However studies relating to the utilization of carotenes from different vegetable food stuffs by Indian children who are sub-clinically malnourished have so far been limited (Rao et al 1970). It becomes therefore important to determine the extent to which B-carotene from plant foods can replace vitamin A especially in the diets of poorly nourished preschool children. The present study was undertaken to find out the efficacy of utilisation or the availability of B-carotene from two vegetable foods namely amaranth and LPC.

The present study was designed to estimate the biological utilisation of B-carotene from amaranth and leaf protein as compared with standard B-carotene in preschool children at four consequtive periods.

Fifteen preschool children were selected for the study from Sri Avinashilingam Balwady who were fed with Balwady supplement. The study consisted of four phases. During the first phase Balwady supplement alone was fed to the target children. During the following months in the second, third and fourth phases along with the balwady diet amaranth, LP and standard B-carotene respectively were fed as a source of B-carotene each for a period of one month. Towards the termination of each period, faeces was collected and analysed to estimate the excretion of B-carotene. The determination of B-carotene intake and excretion facilitated the estimation of availability of B-carotene. The indicies of evaluation included the estimation of serum vitamin A levels, serum proteins and blood haemoglobin levels. Thus the study on biological utilization of B-carotene from amaranth and LP was carried out.

II. REVIEW OF LITERATURE

Global incidence of vitamin A deficiency

Vitamin A deficiency among preschool children is a widespread problem in many parts of the world occuring most frequently against a background of poverty. It has been reported to occur in Burma, Ceylon, East Pakistan, India, the Philippines, North and South Vietnam, South Korea and Thailand. (McLaren, 1958; Oomen et al 1964; Glick et al 1963; Vijayaraghavan et al, 1975).

Over 1000 preschool children were recorded as blind from vitamin A deficiency in a one year period in North West Brazil. (Simmons et al 1975) xerophthalimia is also frequently encountered in countries of the Middle East and African countries - Iran, Iraq, Jordan, Lebanon, Libya, Sudan, Tanganiyaka and UAR. Many cases have been reported from central and south American countries - Brazil, El Salvador, Guatamale, Mexico and Harti. (Srikantia, 1975)

Vitamin A deficiency, as evidenced by xerosis of the conjunctivae and Bitot spots has been recorded in 5 to 11 per cent of children in Southern and Eastern India (Swaminathan et al 1960; Someswara Rao et al, 1954; Rao et al, 1960; Gopalan, 1957; Lal, 1956).

Clinical and biochemical Manifestation of vitamin A Deficiency

To assess vitamin A nutritional status, the most commonly used clinical tests are predominantly ocular and they generally provide clear-cut evidence of the dificient state. The ocular manifestations include xerophthalmia, xerosis of the conjunctivae, Bitot spots as well as manifestations in the cornea. The most severe sign of vitamin A deficiency, keratomalacia, is not commonly recorded as it progresses rapidly to blindness, and an examination of the shrunken or staphylomatous globe does not give any definite indication of its aetiology. (Pereira, 1968).

It has been reported that impaired dark adaption was frequently seen in subjects whose blood vitamin A levels were below 18 µg/100 ml. (Hassan et al, 1947) In children with corneal involvement, however it has been consistantly found that vitamin A either cannot be detected at all or , if found, the levels are very low (Pereira et al, 1966 and Vinodini et al 1966). While in individual cases serum vitamin A levels do not always correlate well with clinical status, in groups of individuals there is a rough parallelism between serum vitamin A, on the one hand, and clinical manifestations on the other. A gradient from 25 µg/100 ml in apparently normal children without ocular signs to below 3 µg/100 ml in children with out severe manifestations such as corneal xerosis and keratomalacia, has been found with intermediate values being seen in children with conjuctival xerosis and Bitot spots. In children suffering from severe forms of Protein Calorie Malnutrition, however, even in the absence of ocular manifestations of vitamin A deficiency, levels of serum vitamin A have been found to be generally lower than in apparently normal children. (Din et al, 1966; Pereira et al, 1966; Smith et al, 1970; and Konno et al, 1968).

In Indonesia, preschool children whose habitual diets contained adequate amount of B-carotene but low amounts of fat had low levels of vitamin A in serum (Roels et al 1963).

Thus night blindness, conjunctival and corneal lesions along with low serum vitamin A levels are manifestations of vitamin A deficiency among the preschool children.

Dietary intake of vitamin A and B-carotene of Indian Children

The diet of an average Indian was low in foods of animal origin and was therefore, practically devoid of preformed vitamin A. Milk and other animal foods, because of their relatively high cost were not eaten by the majority in India who depended almost entirely on carotene source for their daily requirement of vitamin A. However, vegetables which are inexpensive and easily available are good sources of provitamin A.

Though readily available, green leafy vegetables were not eaten in adequate amounts. In a typical of the area, the children's food provided an average of just 180 µg of B-carotene a day. (Sundararaj et al 1969). In Tamil Nadu consumption of green leafy vegetables is negligible and only 8 g per day per person is the consumption as against the recommended allowances of 110 g. (Devadas 1974) Aykroyd and Doughty (1964) have repeatedly warned the low intakes of the vitamin. Aykroyd (1944) suggested that Indians should increase the consumption of leafy vegetables to balance their diets. In all states, of India intakes of vitamin A were far below the recommended value of 750 µg especially in the lower income groups. The highest values were found in west Bengal (533.4 µg) and the lowest in Kerala (106 µg). In most states, intakes tended to increase with income (NIN 1975).

Sundararaj and Pereira (1975) studied the diets of lactating women in a Harijan Community of a South Indian Village and found that only 4-8 per cent of the recommended allowance was covered.

<u>Effect of cooking on provitamin A content of foods:</u>

It has been found by Nageswara Rao et al (1967) that as much as 40 per cent of the carotene originally present in the food was lost because of cooking. When green leafy vegetables were cooked, the losses varied from 27 to 40 per cent (Rajalakshmi <u>et al</u> 1974). Deep frying or frying followed by heating with small amounts of water caused marked destruction of vitamin A, but boiling leafy vegetables and potatoes in in water resulted in little or no loss of carotene. High temperatures of deep fat frying may result in considerable losses of B-carotene from plant foods (Spencer 1973).

However the possibility that the cooking process might denature some of the carotenoids originally present in the new food stuffs has not been explored. Cooking resulted in losses of carotene in the leafy vegetable, the losses due to frying being double the losses due to boiling. When 100 grams of carrots were cooked in a pressure cooker for five minutes it provided about 1890 µgs of carotene.

Factors affecting utilization of vitamin A and B-carotene

There are several physiological factors which are seldom considered but which can influence absorption of vitamin A and its precursors (Underwood 1974). These include availability of the previtamins, presence of other dietary components like fat, vitamin E, protein, emulsified vitamin A and pathological conditions such as acute infections and fever (Lewis _et al_, 1941; Kimble _et al_, 1946), tuberculosis, diabetes (Pepper and Steigmann 1943) heart conditions or thyroid abnormalities. Any disorder which affects fat utilization such as celiac disease, sprue, intestinal obstruction, bile duct abnormalities or pancreatic disorders also reduces the utilization of vitamin A compounds. (May _et al_,1941, Kahan, 1970).

Influence of fat on the utilisation of β-carotene and vitamin A:

Dietary lipids do not appear to be as important for utilising preformed vitamin A as they are for the provitamins (Wilson, 1937). Oey Khoen Lian *et al* (1967) and Figueira et al (1969) concluded that dietary fat is not essential for vitamin A absorption since young children on low fat diet, supplemented with vitamin A and dried skimmed milk, had normal plasma values.

In premature infants the amount of vitamin A absorbed appeared to paralled the amount of fat absorbed (Henley, 1944). Very low fat diets may significantly decrease absorption, especially of carotenoids, since dietary fat from which monoglycerides and phospholpids are produced by normal digestive processes facilitate absorption. On the other hand, if digestion is incomplete, the presence of unhydrolysed fat could contribute to malabsorption of vitamin A active substances leading to an increased dietary requirement.

Many workers have suggested that the presence or absence of small amounts of fat as the vehicle for vitamin A could significantly influence the absorption of vitamin A in Kwashierkor the absorption being poor in the absence of fat. (Srikantia,1975)

Study conducted by Chandrasekharan (1975) say that higher levels of dietary proteins and lipids generally favoured the utilisation of vitamin A and the carotenoids. Senger *et al* (1975) said that there was no relation between essential fatty acid uptake and vitamin A and E plasma levels.

In a study conducted by Roels et al (1958) small amounts of fat given to young boys without any other change in the diet increased absorption of B-carotene from 5 per cent to 50 per cent and at the same time raised their serum vitamin A levels. In Indonesia, preschool children whose habitual diets contained adequate amounts of B-carotene, but low amounts of fat, had low levels of vitamin A in serum and this was raised by mere supplements of coconut oil (Roels et al 1963).

These data indicate that the fat content of the diet may be an important factor in the absorption and utilisation of B-carotene and vitamin A.

Effect of Protein Nutritional Status on Absorption and Utilisation of B-carotene and vitamin A:

An inter relationship between proteins and B-carotene both at the nutritional and metabolic levels has been suggested by several workers (Trowell, 1954; Scrimshaw, 1958; Gopalan, 1960) in recent years. (Kothari et al 1971). Thus McLaren in 1958 had observed that a decrease in plasma proteins might impair the blood transport of vitamin A. This has been reaffinned by Arroyave and his co-workers (1963) in the case of children suffering from Kwashiorkor. More recently, Arroyave (1969) has reviewed much of clinical and experimental evidence pertaining to the influence of protein deficiency on the metabolism of vitamin A.

Studies regarding the influence of protein nutritional status on absorption and utilisation of dietary carotene in man are also limited. It rats, both the quality of dietary protein and content of protein have been shown to influence absorption of B-carotene. In animals maintained on diets providing 4 per cent protein, the absorption and conversion of B-carotene has been found to be impaired (Mathews et al 1963). Dietary carotene has been found to be more efficiently utilised by rats when casein was the source of protein than when zein or gluten was the source (Berger et al 1962; James et al 1953). On the other hand activity of the intestinal enzyme, carotene dioxygense, which converts B-carotene to vitamin A has been found to be higher in animals maintained on diets providing 10 per cent protein as compared to those maintained on 20 and 40 per cent protein diets (Gronowska-senger and Wolf 1970). Also the activity of the enzyme has been found unaltered in human subjects, after an 8 day period of fasting.

In man, protein nutritional status does not appear to be critical in the metabolism of B-carotene. In some parts of Africa, habitual diets contain red palm oil, which is a rich source of B-carotene. In such population through protein calorie malnutrition is widespread among children, ocular signs of vitamin A deficiency are extremely rare even among the most serious cases of Kwashierkor (Scragg et al 1960). In Java, among urban school children of the poor income group who are predominantly rice-eaters, the protein intake was found to be around 38 g daily but the vitamin A intake was low, being only 350 μg, nearly 6 per cent of these children had Bitot spots. In sharp contrast, Javanese rural children who ate cassava, had only protein intakes (10 g. daily) but a good amount of vitamin A (1000 μg daily). In this area Kwashierkor was frequently seen, but xerophthalmia was very rare (Klerks, 1969).

These observations show that, even in severe states of protein calorie malnutrition, dietary vitamin A and its precursons are satisfactorily utilised.

Recent studies in undernourished children suffering from mild and moderate grades of malnutrition drawn from communities among whom ocular signs of vitamin A deficiency are frequently seen, have shown that such children absorb and utilise B-carotene satisfactorily (Lala et al 1970 and Pereira et al 1968) Children receiving supplements of about 1000 μg of B-carotene through green leafy vegetables for as short a period as two weeks showed considerable increase of vitamin A levels in serum from 20 to 30 μg/100 ml and in some of the children who had Bitot spots this amount of B-carotene brought about a clinical cure.

Influence of vitamin E on the Absorption of Vitamin A and β-carotene:

Vitamin E is a factor which affects the biological utilisation of vitamin A. (Redregues and Irvin, 1972) low vitamin E status may exist in children suffering from hypovitaminosis A and PCM, and because vitamin E is needed for efficient vitamin A utilisation, liver storage and may also alleviate hypervitaminosis A it is recommended that vitamin E be included in the intermittent massive dose formulation. (Bauernfeind et al 1974)

Vitamin A absorption is markedly impaired in vitamin E deficiency. Oral supplementation with d-alpha-tocopherol increased the utilisation of orally administred vitamin A about sixfold. Even when vitamin A was administered intramuscularly in emulsified from form, utilisation in the vitamin E deficient animal was low. With simultaneous injection of vitamin E, vitamin A utilisation was markedly increased (Ames 1969).

The effect of vitamin E supplements on the absorption of a massive dose of vitamin A was studied by Kusin et al (1974) in 17 normal children. The addition of 40 and 100 mg of vitamin E to the massive dose of vitamin A had no effect on the absorption or retention of vitamin A, where as when 500 mg of vitamin E was given, there was a significant increase in the intestinal absorption of vitamin A and also increase in urinary excretion.

Thus the influence of vitamin E in improving high vitamin A utilisation is not limited to improving intestinal absorption. Vitamin E is essential for the normal in vivo utilization of vitamin A.

Effect of Emulsification on the absorption of vitamin A:

Following oral administration, plasma vitamin A levels rise more rapidly with emulified vitamin A than vitamin A in oil. Emulsification increases the speed of absorption of an oral dose of vitamin A but does not increase its biological utilization. (Ames 1969). However, emulisification greatly enhances the biological response of intramuscularly administesed vitamin A. In general, the greater the proportion of emulsifier, the greater the biological response.

Effect of infection on the absorption of vitamin A and B-carotene:

Recent studies with the use of 11-12 $^3H_2$ retinyl acetate in physiological amounts have shown that during acute infective episodes, the intestinal absorption of vitamin A is considerably impaired (Sivakumar et al 1972). In communities where vitamin A deficiency is widespread, children are exposed to recurrent infections, and if the dietary intake of vitamin A is marginal or inadequate these episodes of infection may be expected to lead to depletion of hepatic stores.

Infective episodes are associated with a fall in circulating levels of vitamin A. In subjects suffering from diseases such as lobar pneumonia, pleural effusions, acute tonsillitis, rheumatic fever, erythema nodosa, jaundice, malarial fever induced for therapy of syphylis and infective hepatitis, the concentration of serum vitamin A is known to fall substantially but returns to normal, however, following recovery without any dietary supplements (Harris et al 1947, Jacobs et al, 1954; May et al 1940; Moore 1937, Popper et al 1943, Shank et al 1944).

In many parts of the world ocular signs of vitamin A deficiency in children and heavy infestation with Ascaris lumbricoides co-exist. Recently, Singh et al (1968) have found that mature round worms contained as much as 1.33 Iu/g vitamin A in contrast to earthworms which had no vitamin A in their bodies. They have also found that in children with round worms the mean serum vitamin A level was 14 μg/100 ml as against a mean value of 21 μg/100 ml.

In subjects suffering from Parasitic diseases in Thailand, Migasena (1969) has observed low levels of vitamin A in blood, and has attributed this to reduced absorption of vitamin A, as judged by vitamin A tolerance test.

Sheehy et al (1962) have reported that absorption of vitamin A is impaired in severe hook worm infestation while Leonard and Sanewell (1964) have found in their subjects with severe hook worm infestations, in Africa, that vitamin A absorption tests were normal and that the low levels of serum vitamin A seen in them could not be explained on the basis of impaired absorption.

Heymann (1936) has shown that infection decreases the absorption of carotene and Lawrie et al (1941) have reported that in some diseases, like pneumonia and influenza, there was considerable excretion of vitamin A in the urine.

Repeated attacks of respiratory infections and gastero-enteritis may enhance the vitamin A requirements by interfering with intestinal absorption of the vitamin (Reddy et al 1972). In children, whose dietary intakes are low and whose body stores of the vitamin are marginal this may precipitate clinical manifestations of vitamin A deficiency. High incidence of infections may thus contribute significantly to the widespread prevalence of vitamin A deficiency in children of poor communities.

Studies on Biological Availability of B-Carotene

Studies on human absorption of food carotene are limited and the data available are variable. It is difficult to predict with confidence, the availability of B-carotene, as in many studies, the method of estimation of B-carotene has included both α and B-carotene and in some studies other carotenes as well (Srikantia 1975). Absorption of B-carotene has, however, been found to be much higher than that of total carotenes. Absorption of B-carotene from different foods varied widely.

Balanced studies conducted by Vinodini Reddy (1970) in six undernourished preschool children to determine the efficiency of absorption of B-carotene from amaranth showed that the absorption of B-carotene was about 70 per cent similar to that of normal adults. There was also a significant increase in serum vitamin A after the feeding trial.

Murthy _et al_ (1972) conducted a study on 10 women college students, who were given a daily supplement of 100 g of carrots providing 1890 µg of B carotene for 20 days and estimated the serum vitamin A levels initially and on 21st day which showed a significant increase of 2.8 µg/ml of the serum.

A study was conducted by Rao et al (1970) on the absorption of carotenes from a leafy vegetable a root vegetable, a fruit, crystalline B carotene and a mixed vegetable source in four adult volunteers on diets low fat was studied. While crystalline B-carotene was completely absorbed, the mean percentage absorption of carotene from amaranth, carrot, papaya and the mixed vegetable source were 58, 36 46 and 33 respectively.

<u>Promising sources of B carotene in poor dietaries</u>

Preformed vitamin A is present only in foods of animal origin, which are expensive. On the other hand rich sources of B-carotene such as green leafy vegetables are in expensive and can totally meet vitamin A requirements of children. But in the diets of children belonging to the poorcommunities among whom vitamin A deficiency is a major public problem, green leafy vegetables are not included for reasons other than cost and availability.

The use of leafy green vegetables as a source of vitamins in young child feeding has been widely endorsed (Protein Advisory group, 1973) but, of course, green plants have the drawbacks of high moisture content, 85 per cent or higher, dietary fibre. Because of these factors it is often difficult, particularly for children to consume sufficient green plant material to provide significant amounts of many of the vitamins.

Leaf protein concentrate since it derives from leaves, would be expected to contain substantial amounts of most vitamins found in plants. (Devadas and Kamalanathan, 1975) The actual quantity will, of course, depend on a number of factors among them the following.

1. The plant source: both the species and the variety published date on green vegetables clearly shows the difference (Walt et al 1963).

2. The conditions under which the plant is grown the amount of water and fertiliser the plant receives as well as

3. The protein processing conditions. These would include such items as the method used for coagulation, the amount and type of washing, and the drying conditions.

In the preparation of leaf protein concentrate just as in the cooking of green vegetables there is a considerable loss of the various water soluble vitamins. It is estimated that 25 to 40 per cent of the B complex vitamins are lost. The most extreme loss however, is that of ascorbic acid.

The losses of fat-soluble vitamins are less-work done by the U.S department of Agriculture group involved with leaf protein has shown that the loss of B-carotene is only 15 per cent (Knuckles *et al*, 1970) when the process is carried out in recommended manner.

As can be seen the Leaf Protein concentrate is an extremely rich source of B carotene, provitamin A. In fact just a few grams of Leaf Protein concentrate a day will provide the entire daily vitamin A requirement, leaf protein is the richest natural source of Vitamin A obtainable. It has almost twice the vitamin A activity of leaf lives and ten times that of fresh green vegetables. (Bray, 1975)

It may be concluded that leaf protein concentrate is a particularly rich source of most vitamins needed in the human nutrition. Unfortunately, it is a completely overlooked source at present; a situation which it is hoped will be changed in the near future.

III. EXPERIMENTAL PROCEDURE

The present study was designed to estimate the biological utilization of B-carotene from amaranth and Leaf Protein foods as compared with standard B-carotene in fifteen preschool children at four different periods.

A. Selection of subjects:

The study was undertaken on fifteen children of 3-5 years of age selected from Sri Avinashilingam Balwady. The sample included seven boys and eight girls. They were studied four times during the calendar year 1975, in the month of September, October, November and December. None of the children possessed any inherant disorders which might interfere with the study. Their eyes were also tested to check the presence of any vitamin A deficiency symptom. Their age, height and weight were also noted down.

B. Estimation of B-carotene in the foods:

The amount of total carotene and that of B-carotene present in Leaf Protein and amaranth were estimated using the procedures of Thompson et al (1946) and that of Nageswara Rao (1967) using alumina column. The detailed procedure is given in Annexure I. The B-carotene values determined by the above procedures were made use of while formulating the diets for the feeding programmes.

C. Conducting Food Weighment Survey:

A five day food weighment survey was done in the families of the subjects. The children lived at home and ate their usual family fare without any dietary modifications for the study. During the study period the children were not supplied with any vitamin or vitamin mineral supplement. The average B-carotene intake of every children per day from their home diets was calculated. This facilitated the estimation of the amount of B-carotene rich supplement that had to be added to the diet at the Balwady.

D. Formulation of the Balwady supplement diet:

Based on the food weighment survey a supplement diet was formulated. This balwadi supplement was same but the source of B-carotene fed was different for the various phases of the study. The balwady supplement is given in Table I.

TABLE I

SUPPLEMENT FED AT THE BALWADY PER CHILD PER DAY

No.	Food stuff	Mean daily intake	B-carotene µg.
1.	Maize	70	63
2.	Pulse	30	25
3.	Jaggery	20	34
	Total	110	122

The source of B-carotene added to the balwadi supplement during the different periods included amaranth, LP and the standard B-carotene. The B-carotene source was fed to the subjects along with uppuma and laddu since balwadi supplement was given in that form. The preparation of uppuma and laddu are given in Annexure II. The different sources of B-carotene given are shown in Table II.

TABLE II

DIFFERENT SOURCES AND AMOUNTS OF B-CAROTENE FED TO THE TARGET CHILDREN DURING THE FOUR PERIODS OF THE STUDY

Period	Source of B-carotene	Amount given	B-carotene content
1.	(Basal period)	NIL	Negligible
2.	Amaranth	40 g.	1200
3.	Leaf protein	8 g.	1200
4.	Standard B-carotene	1 ml of solution	1200

E. Conducting the feeding trials:

The feeding schedule was divided into four different periods.

Period 1:

During the first period, for one month the children were fed the balwadi supplement along with home diet without any extra source of B-carotene.

Period 2:

During the next month the same children were fed with amaranth as a source of B-carotene along with the balvady supplement. Amaranth was obtained from the nearby local market and the samples of the vegetables were analysed periodically for B-carotene content before and after cooking. The cooking losses were determined and accounted for in the finalisation of the quantity of the supplement. About 40 g. of amaranth was added along with uppuma. The average amount of B-carotene provided by amaranth was 1200 µg. This level was chosen as it represents one day's requirement of B-carotene for preschool children as recommended by ICMR.

Period 3:

During the following month children were fed with LP as a source of B-carotene along with the balvady supplement. Leaf Protein that was required for the feeding was obtained from the leaf protein plant* located at the college. This LP was incorporated along with the laddu and was given to the children. The cooking loss was only 10 per cent since the process of preparation of laddu included just five minutes of steaming. The average amount of B-carotene provided by it as estimated was 1200 mg. The preparation of LP is given in Annexure III.

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* IBP pulper with belt press

Period 4:

During the following month the same subjects were fed with 1200 µg of standard B-carotene a commercial BDH preparation. The tablet was carefully diluted in coconut oil to obtain a required dilution. 1 ml of this solution was equivalent to 1200 µg. of B-carotene which formed the source of B-carotene.

F. Collection of faeces for analysis:

The faeces was analysed for three days prior to the termination of treatment. During this period, fecal markers were used, plastic containers with covers were supplied after noting the weight ofthe containers, before giving them to the mothers of the individual children. Another set of containers was kept in the Balwady to collect defacation in the Balwady. After 24 hour collection, the collection of the individual children were pooled, the total weight of the faeces recorded correct to one gram after substracting the weights of the containers. A portion of the same weighing about 10 g. was taken up immediately for analysis. Total amount of B-carotene present in the faeces were estimated using the procedure of Jagannathan and Patwardhan (1960) Annexture IV.

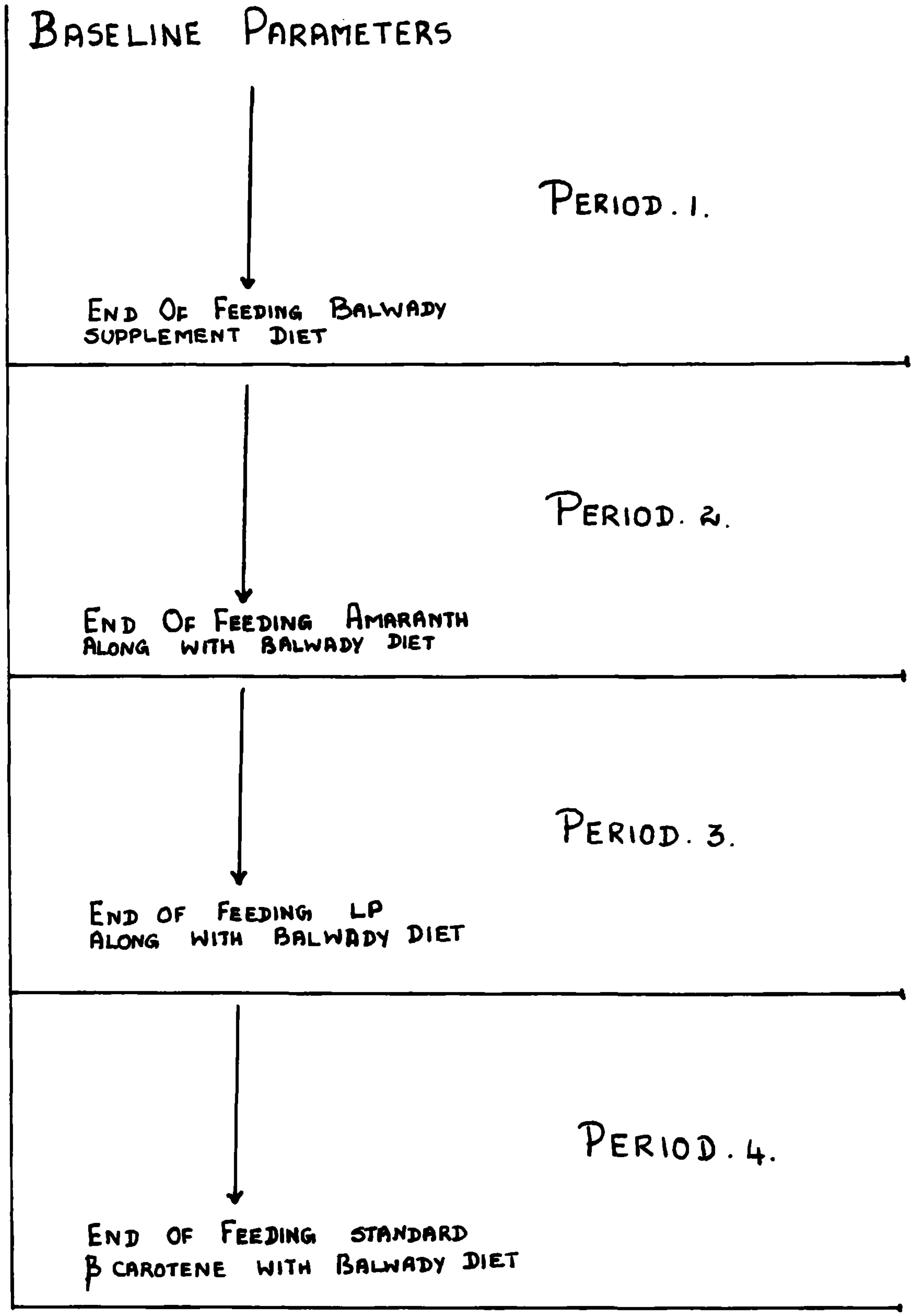

FIG. 1

SCHEME OF THE STUDY

G. Estimating the availability of B-carotene:

The amount of B-carotene available to the body was obtained by calculating the B-carotene intake through foods and B-carotene excretion through faeces. The difference between these two values gave the B-carotene utilization or availability.

H. Estimation of serum vitamin A:

Nearly 5 ml of blood was drawn from the antecubital vein, with heparin as an anticoagulant, and the plasma was separated. Serum vitamin A was estimated following the method of Neeld and Pearson (1973), and the solution was read on a Beckman Du Spectophotometer at 348 mμ following the procedure of NIN, 1971 (Annexture V).

I. Estimation of serum total protein and albumin:

Because of some of the interrelationships between proteins and vitamin A (Fry et al 1975) it was important to obtain an estimate of protein nutriture of the target children. Accordingly total proteins and albumin levels in serum were determined by the Biuret method. The method used is given in Annexture VI.

J. Estimation of Haemoglobin:

About 20 cubic millilitre of blood was drawn through finger prick and the sample was analysed for haemoglobin using cyanmethemoglobin method as given in NIN, 1971. The method used is given in Annexture VII. This was done at the beginning and at the end of the study period.

K. <u>Record of incidence of illness:</u>

As the children were participating in these feeding programme for a period of four months the incidence of sickness in these children was recorded all through the period to see whether or not supplement had any ill effects on the children. A monthly record was kept on the type of sickness and its duration.

The obtained dates were statistically analysed. The mean, standard deviation and coefficient of correlation were found out for different groups of values and were compared. 't' test was also used to find out the level of significance and for the comparison of groups.

IV. RESULTS AND DISCUSSION

The present study was aimed to assess the biological utilization of B-carotene from amaranth and Leaf Protein (L.P) as compared with standard B-carotene in fifteen pre school children with 38 to 55 months of age and belonging to poor socio economic status. The target children were participating in a Balvady feeding programme and their diets were further supplemented with three different sources of B-carotene namely amaranth, LP and standard B-carotene and the excretion of B-carotene in the faeces were analysed during each phase of the study period. The difference between both gave the biological availability of B-carotene. Serum vitamin A levels, serum total proteins and albumins and blood haemoglobin levels were also useful indices of evaluation for this study.

<u>General background of the participating children and their families:</u>

The families of the target beneficiaries belonged to a poor socio economic group. The monthly income of all the families of the target children was between rupees 200 and 300. Casual labour in a textile mill and cooli (day labour) were the main occupations of the parents of these fifteen target children. The educational attainment of the parents was poor. Fifty per cent of them were illiterates and the rest had had some school education. All the children were from nuclear families.

The body heights and weights of the target children at the beginning of the study:

The fifteen target children were drawn from a specified age group between 38 and 55 months, free from any known diseases and they were not participating in any other feeding programme. Table III shows the age and sex status of the target children along with their heights and weights at the beginning of the study.

TABLE III

AGE AND SEX STATUS OF THE TARGET CHILDREN AND THEIR BODY HEIGHTS AND WEIGHTS AT THE BEGINNING OF THE STUDY

S.No.	Age (months)	Sex	Weight ~~Cms.~~ Kilos	Height Cms.
1.	43	Boy	12.6	88.8
2.	42	Boy	12.5	91.9
3.	45	Boy	13.6	94.8
4.	44	Boy	11.9	84.9
5.	55	Boy	12.9	99.6
6.	49	Boy	11.7	88.5
7.	48	Boy	14.7	98.6
8.	45	Girl	12.4	91.3
9.	40	Girl	9.9	81.5
10.	42	Girl	11.3	85.6
11.	48	Girl	11.4	92.3
12.	40	Girl	12.0	91.5
13.	38	Girl	12.1	93.4
14.	55	Girl	12.5	99.4
15.	49	Girl	11.7	88.5
Mean	45.7		12.15±1.07	90.6±5.116

Among fifteen target children seven of them were boys and eight were girls. The target children did not have any obvious signs of malnutrition but their heights and weights were poor. When compared with the percentile values for height and weight of well-to-do preschool Indian children (NIN, 1975), the target children were below the 25th percentile. Thus the children who participated in the present study were subclinically malnourished; they were poor in their attainment of body heights and weights, showing growth retardation, when compared with their well nourished Indian counterparts, but did not have the obvious signs of PCM or vitamin A deficiency syndromes.

<u>Diet and Nutrient intake of the target children</u>

Food weighment survey was carried out for five consecutive days among the target children and their families. The mean food intake of the children are represented in Table ~~III~~. IV

TABLE ~~III~~ IV

MEAN DAILY HOME FOOD CONSUMPTION OF THE TARGET CHILDREN

Foods	R.D.A. I.C.M.R.(1971) g.	Quantity consumed by target children (g)
Cereals (Rice, Cholam, ragi or jowar	200	220
Pulses	60	12
Green leafy vegetables	75	10
Other vegetables and roots and tubers	50	20
Fruits	50	8
Milk and its products	200	25
Fats and oils	25	5
Sugar and jaggery	40	6

The consumption of cereals was even higher than the ICMR (1971) recommendations. But the consumption of pulses, vegetables, milk and fruits were below the recommended allowances of the ICMR, (1971). Consumption of green leafy vegetables was very low, and even if the families cooked the green leafy vegetables the pre school children were not fed this item of the menu.

The common breakfast items in the target families were left over rice, uppuma, iddli or chola kali. For lunch, rice or cholam was used with sambar. The same rice or chola kali and sambar was kept for dinner. Thus the family menu had no variation or variety. Target children who participated in the study were given rice, jowar or ragi. Food was cooked usually once a day or in a few cases twice a day. Children were given the sambar only if it was not spicy and hot (Pepper). A vegetable piece from the sambar was occasionally served to the children.

The nutrient intake of the target children was calculated from the food consumption weighment survey. The mean nutrient intake of the target children are given in the Table V.

TABLE IV.

*MEAN CALORIE PROTEIN AND B-CAROTENE INTAKE OF THE TARGET CHILDREN

No.	Nutrient	Diet		Total	R.D.A. ICMR (1971)
		Home	Balwady		
1.	Calories	860	420	1280	1500
2.	Proteins (g)	20	15	35	22
3.	B-Carotene (μg)	150	122	272	1200

* Data collected through the five day weighment survey, on 15 target children.

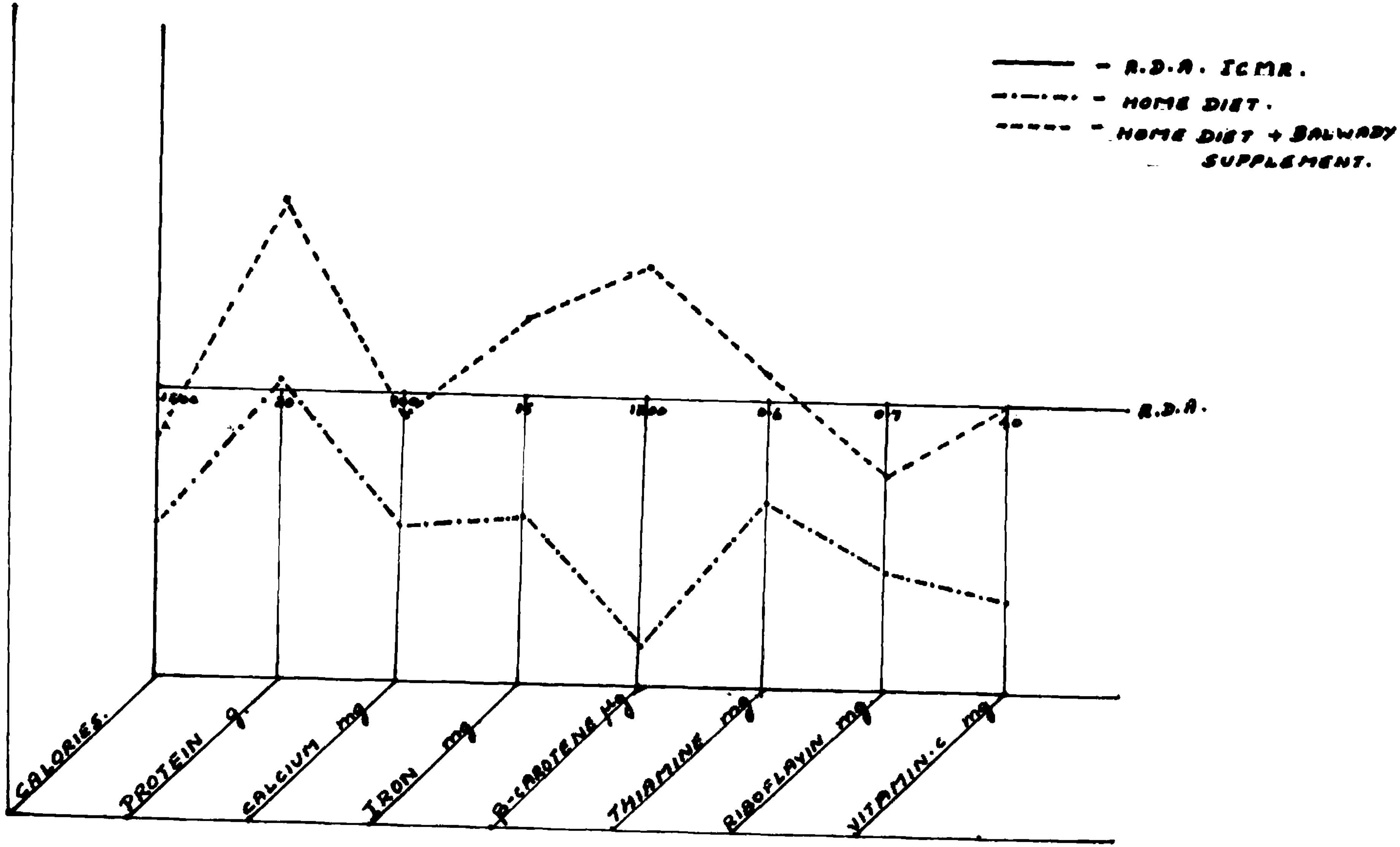

FIG. 2.

NUTRIENT INTAKE OF THE TARGET CHILDREN.

As shown in the table IV, there was not protein gap in the diet of the target children but there was a calorie gap. The main lacuna in the diets of the pre school children was a food gap and not a protein gap as reported by Gopalan 1973. Thus in a cereal based diet if the calorie gap is closed by an extra cereal pulse supplement, the calorie gap is overcome with an increased protein intake. But the deficiency of vitamins and minerals can not be overcome by a cereal pulse supplement alone.

The dietary intake of B-carotene of all the subjects was as low as 150 µg from their home diets. The home diet along with the balwady supplement provided 272 µg of B-carotene. The low intake of preformed vitamin A or B-carotene was because of the inadequate consumption of green leafy vegetables, other vegetables, milk and milk products. Their B-carotene intake from the home diet met only 10-13 per cent of the ICMR recommended allowances (1971).

The home diets were surveyed in the target children before supplementation was initiated, and the survey was repeated six months later. No significant differences in the intakes of home diets between the two periods were noticed.

In order to formulate the dietary supplements, the B-carotene content of amaranth and LP were analysed using alumina column.

Identification of different fractions obtained:

In the case of amaranth and LP, about three bands were separated on column chromatography. Spectra of all the fractions are shown in Table VI. The first band was B-carotene eluted with 1 per cent acetone. Other bands were not identified. The other bands required high polarity solution for elution. The fractionation was also done on calcium hydroxide column but comparing the result with alumina there was no difference. No isomers of B-carotene were found with either of these methods.

TABLE VI

SPECTRA OF FRACTIONS OF CAROTENE AS SEPARATED BY AN ~~ARE~~ ALUMINA COLUMN

No.	Band	Spectrum (mμ)	Identity
1.	1st band	420,450,478	B-carotene
2.	2nd band	425,445,470	Unidentified
3.	3rd band	425,445,475	Unidentified

B-carotene content of amaranth and LPC

B-carotene content of amaranth and LP which were fed to the target children as sources of B-carotene were analysed and the values obtained are presented in the table VII. These two foods were analysed at four different days taking duplicate samples each time to determine the mean B-carotene content.

TABLE VII

B CAROTENE CONTENT OF AMARANTH AND LP

No.	Food stuff	B carotene content: Range µg/100g.	B carotene content: Mean µg/100 g.	Standard Deviation
1.	Amaranth	2800-3100	3000	±200
2.	LP	13950-14100	14000	±100

Effect of cooking on the B-carotene content of amaranth and LP:

The B-carotene content of amaranth and LP before and after cooking and the cooking losses are presented in Table VIII. While amaranth was supplemented to the children through a uppuma preparation leaf protein (LP) was fed in the farm of a laddu.

Cooking green leafy vegetables by incorporating in uppuma resulted in losses of B-carotene. About 20 to 25 per cent of B-carotene was lost in cooked amaranth and about 10 per cent of B-carotene was lost in LP in laddu preparation. The cooking losses were minimum since frying which causes greater distraction of B-carotene was completely avoided in the present study.

TABLE VIII

B-CAROTENE CONTENT OF RAW AND COOKED AMARANTH AND LP ALONG WITH THE COOKING LOSSES

No.	Food stuff	B-carotene content of raw food ug	B-carotene content of cooked food ug	Cooking losses of B-carotene ug	Percentage of B-carotene lost in cooking
1.	Amaranth	3000	2250	750	25%
2.	LP	14000	12600	1400	10%

The percentage of B-carotene present as against total carotene in raw and cooked amaranth as observed in this study along with the values in the other published reports are given in table IX.

In the present study the cooking loss that occured when amaranth was cooked by boiling in small amount of water and incorporating the same in uppuma receipe was 25 per cent. According to Rajalakshmi et al (1974) when greens were cooked, the losses varied from 27 to 40 per cent.

Previous reports (Nageswara Rao (1970 and 1967), Vinodini Reddy(1970) have shown that B-carotene is the major carotene in raw amaranth and in other green leafy vegetables and the values reported ranged between 76 to 96 per cent. Nageswara Rao (1970) in his study found that in cooked green leafy vegetables the percentage of B-carotene was lowered compared to in the raw food.

Similar findings were shown by Vinodini Reddy (1970). In both the above reports the percentage of B-carotene present in amaranth after cooking were about 56 per cent and 42 per cent.

TABLE IX

PERCENTAGE OF B-CAROTENE AS A FRACTION OF TOTAL CAROTENE IN RAW AND COOKED AMARANTH

No.	Report	Percentage of B-carotene present in	
		Raw amaranth	Cooked amaranth
1.	Nageswara Rao (1970)	96%	56.8%
2.	Vinodini Reddy (1970)	-	42%
3.	Present study	86%	70%

<u>B-carotene intake by the target children during the various phases of the study:</u>

The target children were fed with three different sources of B-carotene namely amaranth, LPC and standard B-carotene at three subsequent periods. Prior to all these a basal balwady supplement diet was fed during the first period which provided very little quantity of B-carotene. The different sources of B-carotene and their amounts fed to the target children are given in Table X.

TABLE X

QUANTITY AND SOURCES OF B-CAROTENE FED DURING VARIOUS PERIODS OF THE STUDY

Period	Supplement diet with sources of B-carotene	Amount fed per child per day	B-carotene content µg	Recommended allowances ICMR (1971) µg
Period I	Balwady diet alone	110 g.*	122	1200
Period 2	Balwady diet + amaranth	110g + 40g	122 + 1200	1200
Period 3	Balwady diet + LP	110g + 8 g	122 + 1200	1200
Period 4	Balwady diet + standard B-carotene	110g + 1 ml of the solution	122+ 1200	1200

Excretion of B-carotene in the faces of children fed with balwady supplement along with the home diet:

Analysis of the Balwady supplement for its B-carotene content proved negligible (122 mg per day per child) when compared with the recommended allowances. Hence this was taken as the basal period of the study. The subjects were fed with balwady supplement for a period of one month. The mean faecal weight and the mean B-carotene excretion analysed during the last three terminal days of the first period were given in Table XI.

* 110 g of Balwady diet consisted of 70 g maize, 30 g pulse and 20 g. jaggery.

TABLE XI

B-CAROTENE EXCRETION THROUGH FAECES DURING THE BASAL PERIOD BY THE TARGET CHILDREN

(MEAN VALUE OF THREE DAY ANALYSIS)

No.	Weight of the faeces in g.	Excretion of B-carotene µg.
1.	85	8.1
2.	110	10.8
3.	130	14.2
4.	94	9.5
5.	120	38
6.	135	42
7.	97	25.2
8.	89	20.1
9.	129	42.2
10.	147	40.1
11.	79	14.4
12.	134	44.8
13.	98	10.5
14.	105	15
15.	101	12.4
Mean	110	23.2
Standard Deviation ±	20.22	±13.6

Biological utilisation of B-carotene from three different sources namely amaranth LP and standard B-carotene:

Duplicate samples of the cooked feeds fed with amaranth as a source of B-carotene was analysed for the B-carotene content. From this the intake of B-carotene by the target children were determined.

The average value of B-carotene excreted on a balwady supplement and home diet were substracted from the amount of B-carotene excreted in faeces after feeding the diet with amaranth. The difference was taken as excretion of B-carotene from amaranth. This calculation was done following the procedure of Vinodini Reddy (1970). The difference between the intake and excretion gave the amount of B-carotene utilized.

TABLE XII

PERCENTAGE UTILIZATION OF B CAROTENE FROM AMARANTH BY THE 15 TARGET CHILDREN

No.	Amount of B-carotene in cooked amaranth µg	Fecal excretion of B-carotene from amaranth µg	percentage utilization of B-carotene from amaranth
1.	900	351.9	60.9
2.	900	529.2	41.2
3.	900	441.8	50.9
4.	900	326.5	63.7
5.	900	538	60.3
6.	900	318	64.7
7.	900	394.8	56.2
8.	900	195.9	78.3
9.	900	281.8	68.7
10.	900	595.9	60.5
11.	900	285.6	68.3
12.	900	339.2	62.3
13.	900	241.5	73.2
14.	900	477	47
15.	900	323.6	64.1
	Mean	360	61.4
	Standard Deviation	±96.32	±9.35

After supplementation of amaranth, the excretion of B-carotene in faeces was markedly increased in all the subjects.

It is apparent that there was considerable individual variation for the fecal excretion of carotenes. Average percentage of utilization of B-carotene was ~~76.7~~ 61.4 per cent of this study and this is similar to that of Vinodini Reddy's (1971) where the utilization is 63.8 per cent.

TABLE XIII

PERCENTAGE UTILIZATION OF B-CAROTENE FROM LP

No.	B-carotene consumed from cooked LP µg	Fecal excretion of B-carotene from LP µg	Percentage utilization of B-carotene from LP
1.	1080	111.9	90
2.	1080	233.2	78.4
3.	1080	177.8	83.6
4.	1080	246.5	77.2
5.	1080	190	82.4
6.	1080	330	69.4
7.	1080	286.8	73.4
8.	1080	315.9	70.7
9.	1080	449.8	58.3
10.	1080	307.9	71.5
11.	1080	129.6	88
12.	1080	363.2	66.4
13.	1080	253.5	76.5
14.	1080	237	78.1
15.	1080	207.6	86.3
	Mean	256	76.7
	Standard deviation	±86.54	±7.483

The table XIII gives the mean B-carotene excretion during LP supplementation phase. Average percentage utilization of B-carotene from LP was 76.7 per cent, and the absorption ranged widely between 58.3 per cent and 90 per cent.

TABLE XIV

PERCENTAGE UTILIZATION OF B CAROTENE FROM STANDARD B-CAROTENE

No.	Standard B-carotene consumed µg	Fecal excretion of B-carotene from std. B-carotene µg	Percentage utilization of B-carotene
1.	1200	113.9	90.5
2.	1200	137.2	88.6
3.	1200	181.8	84.8
4.	1200	191.5	84
5.	1200	149	87.6
6.	1200	183	84.7
7.	1200	214.8	82.1
8.	1200	189.9	84.2
9.	1200	199.8	83.3
10.	1200	194.9	83.8
11.	1200	175.6	85.3
12.	1200	190.2	84.2
13.	1200	171.5	85.9
14.	1200	163	86.4
15.	1200	177.6	85.2
	Mean	175.6	85.4
		±25.86	±6.143

The mean values of three day faeces analysis for B-carotene excretion during the standard B carotene feeding regimen is given in table XIV. The mean utilization of B-carotene was 85.4 per cent, which is the highest utilisation compared to the earlier food sources.

TABLE XV

COMPARISON OF PERCENTAGE UTILIZATION OF B CAROTENE FROM THREE DIFFERENT SOURCES IN FIFTEEN TARGET CHILDREN

No.	Sources of B-carotene	Percentage of B-carotene utilized: Mean	Range	Standard Deviation
1.	Amaranth	61.4	41.2-78.3	±9.35
2.	LP	76.7	58.3-90	±7.483
3.	Standard B-carotene	85.4	82.1-90.5	±6.145

The percentage utilization of B-carotene from the three food sources are presented in table XV.

When the balwady diet was supplemented with 40 g. of amaranth per day per child supplying 1200 µg. of B-carotene, the absorption of B-carotene was 61.4 per cent. When amaranth was substituted by 8 g. of LP supplying an equivalent amount of 1200 µg. of B-carotene per day per person there was further increase of B-carotene absorption, the value being 76.7 per cent. This increase in absorption of B-carotene from LP ~~and~~ was significantly higher than from amaranth, among the target children.

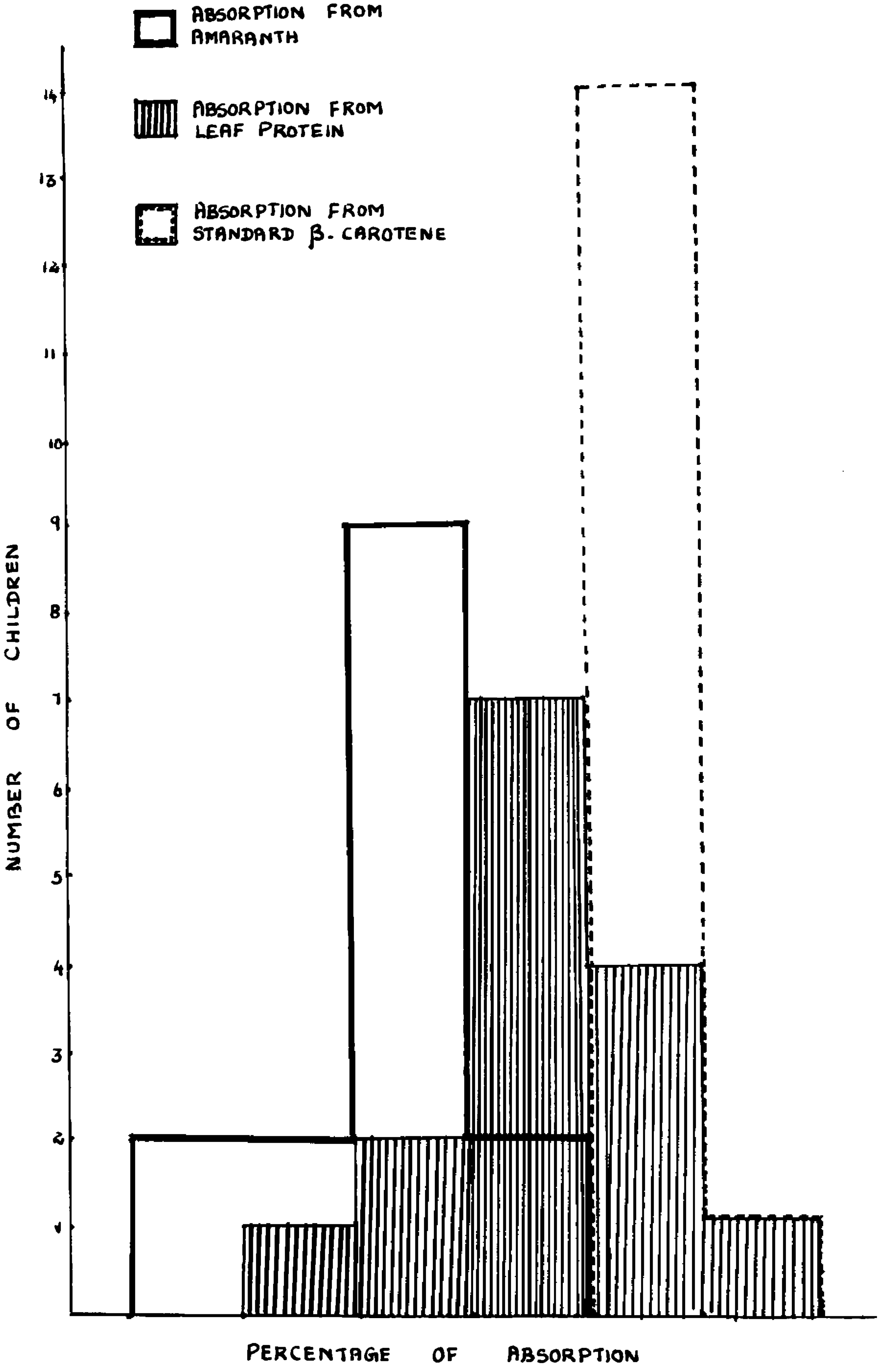

FIG. 3.

PERCENTAGE ABSORPTION OF β-CAROTENE FROM THREE DIFFERENT SOURCES NAMELY AMARANTH LEAF PROTEIN AND STANDARD β- CAROTENE.

When LPC was substituted by 1 ml standard B-carotene solution providing 1200 µg. of B-carotene per day per person the absorption further increased to 83.4 per cent. The absorption from standard B-carotene solution was significantly higherthan absorption from amaranth or LP.

The results of this study suggest that though the intake of B-carotene was same, the percentage absorption of B-carotene was different for different food sources. The absorption being maximum for the standard B-carotene, next for LP and then for amaranth. The study further confirms that vitamin A requirements can be successfully met by supplementing the pre-school child's diet with green leafy vegetables like amaranth or LP; Both amaranth and leaf protein contain several other nutrients besides B-carotene.

Cost analysis of the supplement:

Table XVI gives the quantity and cost of different foods that can provide 1200 µg of B-carotene or 300 µg of retinol to meet the daily requirements of a pre school child.

TABLE XVI

QUANTITY AND COST OF FOODS THAT PROVIDE THE DAILY B-CAROTENE OR RETINOL REQUIREMENT OF A PRE SCHOOL CHILD

No.	Food stuff	Quantity of food containing 1200 µg. of B-carotene g.	Cost*
1.	LP	8	1.6 paise
2.	Amaranth	40	4 paise
3.	Milk	137	50 paise
4.	Butter	50	125 paise
5.	Egg	50	45 paise

* as per prevailing prices during Feb. 1976 at Chinthameni Supermarket, Coimbatore.

As per table XVI, the best B-carotene food sources which are of low cost are amaranth and LP. Such economic and low cost source of B-carotene which in small amounts successfully provide the required amount daily should certainly be incorporated in the diets of the children to overcome vitamin A deficiency.

<u>Changes in serum vitamin A after feeding various sources of B-carotene:</u>

The mean serum, vitamin A values of the children who were fed different sources of B-carotene like amaranth LP and standard B-carotene along with balwady supploment diet are given in the table XVII. Initial and final serum vitamin A levels before and after 4 months of supplementation were analysed for their significance.

TABLE XVII

MEAN SERUM VITAMIN A LEVELS OF THE 15 TARGET CHILDREN BEFORE AND AFTER SUPPLEMENTATION

No.	Dietary intake of B-carotene	Serum vitamin A level ug/100 ml.
1.	272 µg	20.5±4.39
2.	1200 µg	29.2±2.09

The result showed that there was a significant increase in serum vitamin A levels after feeding, the different sources of B-carotene for 4 months among the target children. This indicates that in these children, B-carotene was not only absorbed adequately but also converted to vitamin A and utilized well. A similar observation has been reported by Pereira and Begum (1968) who found an increase in the serum vitamin A levels in children after feeding green leafy vegetables for three months. Vinodini Reddy (1970) obtained this effect in as short a period as fifteen days.

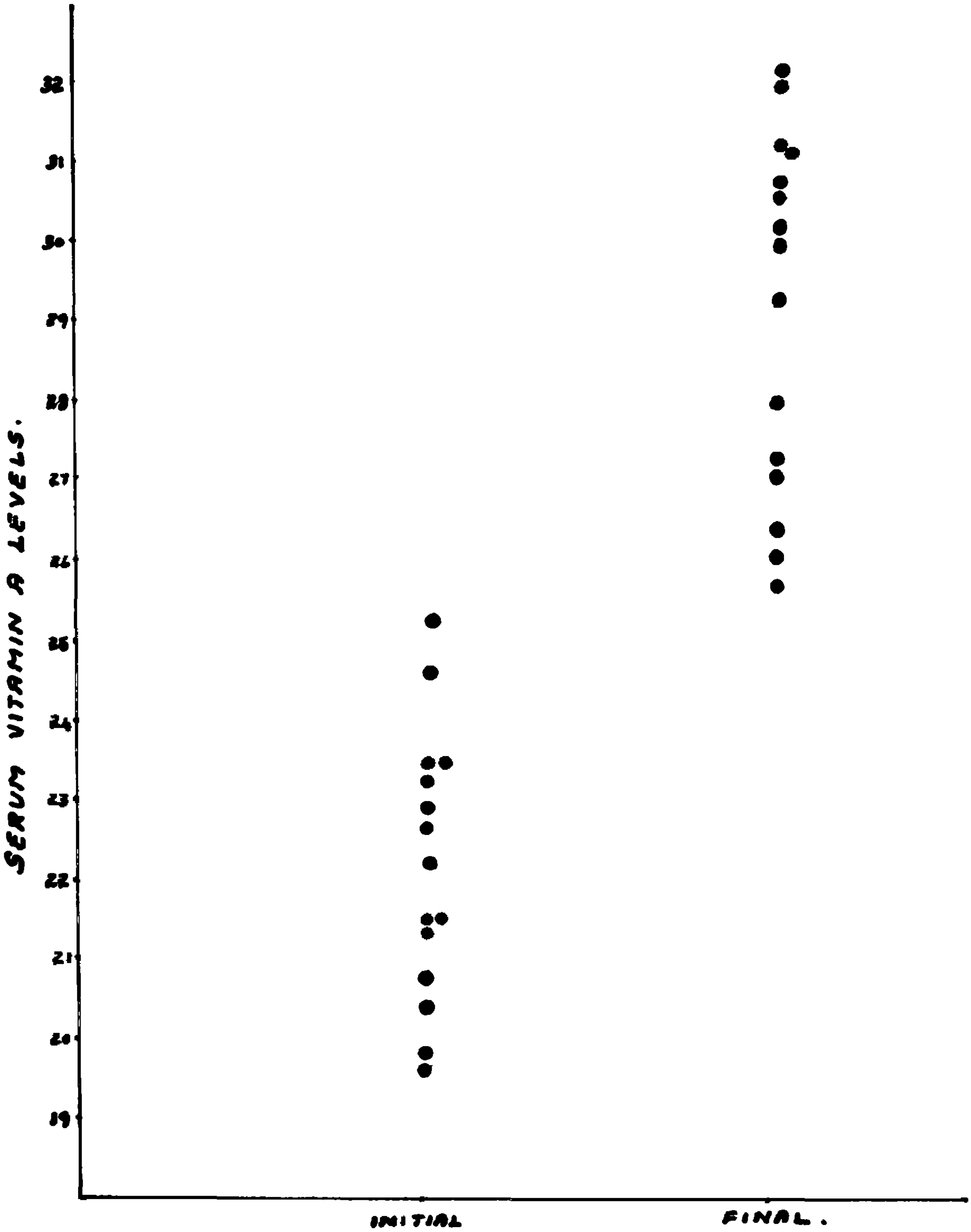

FIG. 4.

INITIAL AND FINAL SERUM VITAMIN A LEVELS OF THE TARGET CHILDREN.

<u>Serum total proteins and albumin levels of the target children:</u>

Table XVIII depicts the total proteins and albumin levels in the serum of the fifteen target children. During the fourth month of the supplementation period the serum proteins were analysed. The mean values of the serum total proteins were 6.7±0.68 g/100 ml, and of albumins were 3.5±0.35 g/100 ml and of globulins were 3.2±0.68. These values were similar to those reported by Pereira *et al*, (1969) and Vinodini Reddy, (1974).

TABLE XVIII

SERUM TOTAL PROTEINS, ALBUMINS AND GLOBINS

No.	Total proteins g/100 ml	Albumins g/100 ml	Globulins g/199 ml
1.	7	3.8	3.2
2.	6.2	3.4	2.8
3.	7.4	3.9	3.5
4.	5.9	3	2.9
5.	6	3.1	2.9
6.	7.7	4.1	3.6
7.	6.8	3.7	3.1
8.	5.7	2.9	2.8
9.	7.2	3.9	3.3
10.	7	3.7	3.3
11.	6.5	3.5	3.0
12.	7.9	4	3.9
13.	7.2	3.7	3.5
14.	6.6	3.3	3.3
15.	5.[illegible]	3.5	[illegible].7
Mean	6.7	3.5	3.2
Standard deviation	±0.68	±0.35	±0.680

<u>Correlation between vitamin A and protein in serum of the target children:</u>

Statistical analysis was done for estimating the correlation between plasma levels of vitamin A and proteins among the target children. The correlation co-efficients were câlculated between vitamin A and various protein fractions and also between vitamin A and total proteins.

TABLE XIX

VITAMIN A AND PROTEIN CONTENT OF PLASMA IN 15 TARGET CHILDREN

No.		Range	Mean	Standard Deviation
1.	Vitamin A µg/100 ml	26.1-32.2	29.2	± 2.09
2.	Total protein g/100 ml	5.7-7.9	6.7	±0.68
3.	Albumin g/100 ml	2.9-4.1	3.5	±0.35
4.	Globulin g/100 ml	2.7-3.9	3.2	±0.68

Table XIX summarises the vitamin A and protein values in the fifteen target children, all of which are within the normally acceptable limits for Indian children. The values obtained are similar to that of Prasad <u>et al</u> (1969) and Kothari <u>et al</u> (1971).

The correlation co-efficients between vitamin A and different protein fractions are given in table XX.

TABLE XX

CORRELATION COEFFICIENT BETWEEN VITAMIN A AND PROTEIN

	Correlation co-efficient r	Significance p.
Vitamin A and total proteins	0.55	0.001
Vitamin A and albumin	0.36	0.001
Vitamin A and globulin	0.008	0.005

Vitamin A and albumin showed a significant positive correlation even at the 0.1 per cent level of significance. On the other hand there was no significant correlation between vitamin A and globulin.

A significant positive correlation between plasma albumin and vitamin A has already been reported in some experimental animals by Friend <u>et al</u> (1961) and Ascarelli (1969) as well as in children by Kothari et al (1971) and in children suffering from kwashiorkor by Arroyave et al (1963).

TABLE XXI

MEAN SERUM PROTEIN AND ALBUMIN LEVELS OF THE TARGET CHILDREN BEFORE AND AFTER THE STUDY PERIOD (Study period-4 months. Number of children-15)

Dietary intake of protein g.	Total serum protein (g) per100 ml.	Albumin level g per 100 ml
20	5.9± 1.4	3.2 ± 1.7
35	6.7± 0.68	3.5 ± 0.55

REGRESSION EQUATION

$y = 1.683x + 14.92.$

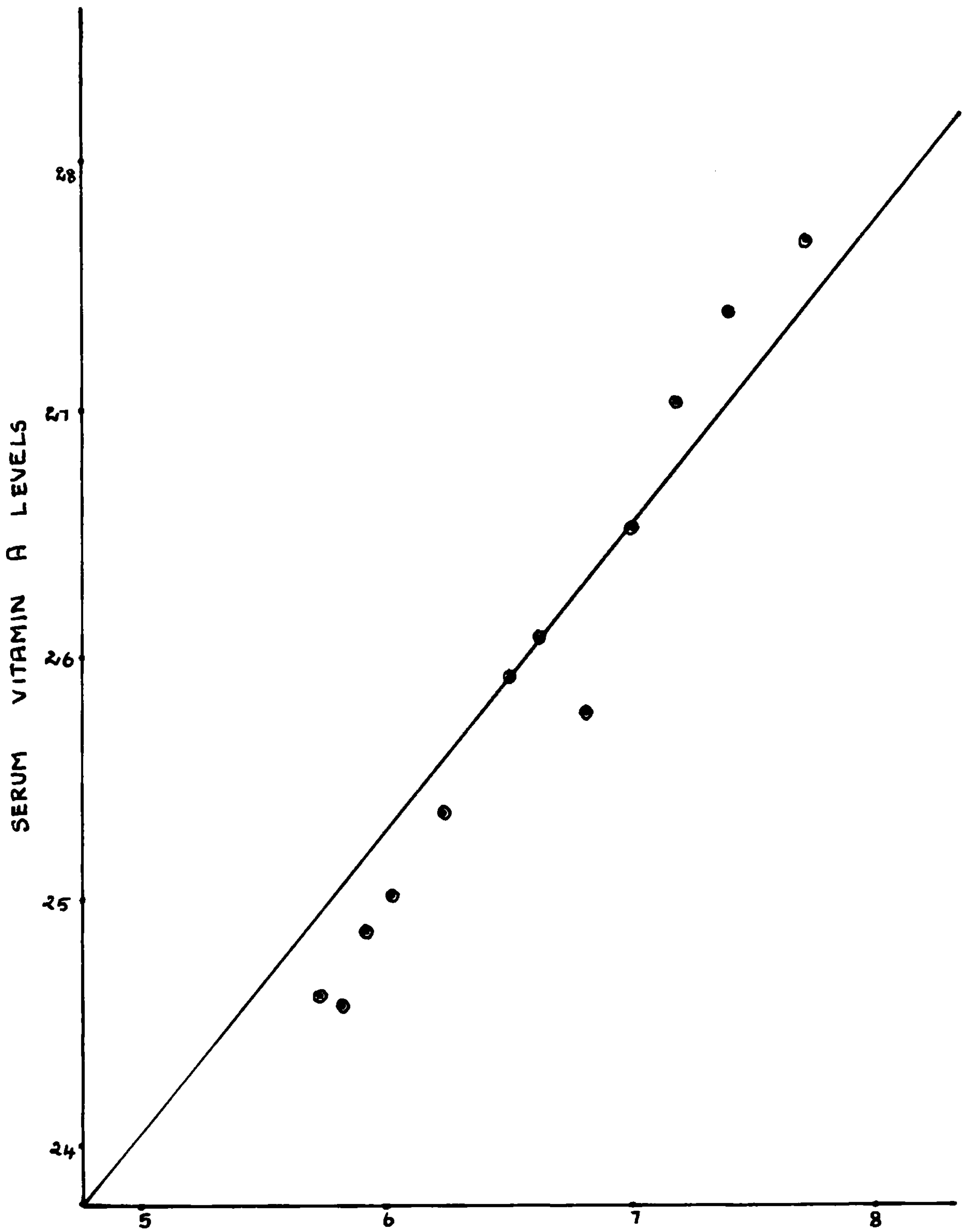

FIG. 5.

REGRESSION LINE OF SERUM PROTEIN LEVELS ON SERUM VITAMIN A LEVELS.

There was no significant increase in total serum protein levels before and after the study period as well as in serum albumin levels before and after the study, in the participating children.

Estimation of blood Haemoglobin:

The haemoglobin content of the finger prick samples of blood of the children estimated at the beginning and at the end of the study are presented in Table XXII.

TABLE XXII

MEAN HAEMOGLOBIN CONTENT OF THE TARGET CHILDREN BEFORE AND AFTER THE STUDY

Number of children = 15
Period of supplementation = : 4 months

	Dietary intake of iron mg.	Mean haemoglobin value of g/100 ml
Home diets alone	9	8.4 ± 1.4
Home diets + Balwady supplements + LP/ amaranth	19.2	12.0 ± 0.91

Judged by the WHO standards at the beginning of the study all the target children were anaemic but towards the end of the study period there was an increment in the blood haemoglobin level. An additional amount of iron provided by the supplements fed during the study may be considered to be the reason for increment of blood haemoglobin. Folic acid, vitamin C etc. present in the diet might have also contributed towards it.

Episodes of infection:

During the course of the experiment, occurence of any infections disease among the participating children were noted down. During the second month two of the children were affected by common cold and during the third month two of the children had fever. However that did not cause significant alteration in the absorption of B-carotene.

Deficiency symptoms among the target children:

No deficiency symptoms of vitamin A malnutrition such as Bitot spots or xerophthalmia were seen in the fifteen target children neigher at the beginning of the study nor towards the end of the study period.

V. SUMMARY AND CONCLUSION

A study was conducted in fifteen preschoolers belonging to low income families to determine the efficiency of absorption of B-carotene from amaranth and leaf protein (LP). The children belonged to Sri Avinashilingam Balvady and were fed with a Balvady supplement. The study consisted to four phases. During the first phase the target children were fed with a basal balvady supplement diet alone. During the following months in the second, third and fourth phases along with the balvady supplement diet amaranth, leaf protein and standard B-carotene respectively were fed as a source of B-carotene for a period of a month. During the termination of each period faeces was collected for subsequent three days and analysed for the excretion of B-carotene. The determination of B-carotene intake and excretion facilitated the estimation of utilization of B-carotene from different sources like amaranth, LP and standard B-carotene. The indices of evaluation included the estimation of serum vitamin A levels, serum proteins and blood haemoglobin.

The percentage of B-carotene absorbed from different sources of B-carotene namely amaranth, leaf protein and standard B-carotene were 61.4 per cent $\pm$9.35, 76.7 per cent $\pm$ 7.48 and 83.4 per cent $\pm$ 6.15 respectively by the preschool children.

There was a significant increase in the serum vitamin A levels from 20.5±4.39 ug/100 ml to 29.2±2.36 ug/100 ml in the target children after a supplementation of 1200 ug of B-carotene from 40 g. of amaranth, 8 g. of leaf protein and 1 ml of standard B-catorene solution containing 1200 ug of B-carotene.

The total serum protein levels were 5.9±1.4 g/100 ml and 6.7±0.68 g/100 ml before and after the study. The initial and final values of serum albumin were 3.2±1.7 g/100 ml and 3.5±0.35 g/100 ml respectively. There was a positive correlation between serum vitamin A levels and serum total proteins and albumins.

The two food sources namely 40 grams of amaranth and 8 grams of leaf protein which are obtainable at a very low cost of 4 paise and 2 paise provided the daily requirement of 1200 ug of B-carotene when compared with other costly and scarce animal foods such as egg, milk, butter etc.

Such economic and low cost sources âf B-carotene namely amaranth and Leaf Protein which successfully provide the required amount of vitamin A is highly recommended in the balwady diets. Consumption of such foods by the preschool children along with a cereal pulse mixture would certainly releive them from the hazards of vitamin A deficiency.

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ANNEXURES

ANNEXURE I

A. **Estimation of B-carotene from Leaf Protein:**

(Overnight extraction procedure of Thompson et al, 1946).

One to two grams of the sample was allowed to soak overnight in the dark room at room temperature in 100 ml stoppered amber coloured volumetric flask with 30 ml of a mixture of 3 parts of acetone and seven parts of petroleum ether. The extract was diluted to 100 ml using petroleum ether after 16 hours.

The absorption columns were prepared as for the estimation of carotene content from fresh leaves. The column was washed with 2 ml of petroleum ether. The suction pump was turned off when 5 ml of petroleum ether was still above the sodium sulphate. 5 ml of the extract to be chromatographed was poured on to the column and suction was applied. The flask was rinsed with 5 to 10 ml portions of eluent (1 part of acetone and 9 parts of petroleum ether), pouring each rinising on the column before the previous one had been completely absorbed on to the $Na_2 So_4$. The washing was continued until the desired pigments moved off the column and the filtrate was colourless. The content of the filter flask were transferred into a 25 ml flask and the volume was made up with the eluent. The transmission of the solution was read at 450 mµ. The instrument was set at 100 per cent transmission with petroleum ether. B-carotene content of LPC was determined in the same way using calcium oxide as absorbent also.

B. Estimation of carotene from amaranath:

Preparation of the extract:

The edible portion of the food stuff was cut into small pieces and mixed well. About 5 to 25 g. of the cut sample was ground well with successive 50 ml portions of 95 per cent alcohol till the alcohol layer was no longer coloured yellow. The pooled alcohol extract was saponified with sufficient sodium hydroxide. Enough water was added to bring the concentration of the alcohol down to 80 per cent.

The unsaponifiable matter was extracted with successive 50 ml portions of petrolium ether (40-60°C) till the petroleum ether layer was colourless. The pooled petroleum ether extract was washed with distilled water till it was free of alkali. About 10 to 15 g. of anhydrous sodium sulphate was added and kept aside for 60 minutes. It was then filtered through a cotton pling with a small amount of anhydrous sodium sulphate placed on it. The filtrate was then concentrated in vaccum between 40-50°C to volume between 10 to 25 ml.

Chromatographic separation of pigments:

30 cms x 1 cm columns were packed with 10g of alumina or 6g. of calcium hydroxide. About 1 to 25g of anhydrous sodium sulphate was placed on top of the packed column. 2 ml of the concentrated extracted was charged on the column. The separated bands were individually eluted and their spectra were studied in a Beckman Du spectrophotometer to identify the different carotene fractions.

ANNEXURE XX

Preparation of uppuma:

Ingredients:

Cereal pulse mixture	-	50 g.
Amaranth	-	40 g.
Onion	-	5 g.
Oil (Groundnut)	-	6 g.
Seasonings		

Procedure:

1. Roasted the cereal pulse mixture
2. Heated the oil
3. Seasoned with mustard, green chillies and cut onion.
4. Poured water and brought it to boiling
5. Sprinkled the roasted mixture, little at a time.
6. Stirred constantly to avoid lumping
7. Sprinkled salt and mixed
8. Cooked for twenty minutes
9. Added cleaned and washed greens and stirred
10. Cooked for 10 more minutes
11. Removed from fire.

Preparation of laddu:

Ingredients:

Cereal pulse mixture	- 30 g.
LP	- 8 g.
Jaggery	- 20 g.
Oil	- 2 g.

1. Roasted the cereal pulse mixture and kept aside
2. Added jaggery to water and prepared a syrup
3. Added the roasted mixture along with LPC and stirred constantly and cooked for 10 minutes
4. Heated the oil and added to the mixture
5. Removed from fire and made into balls.

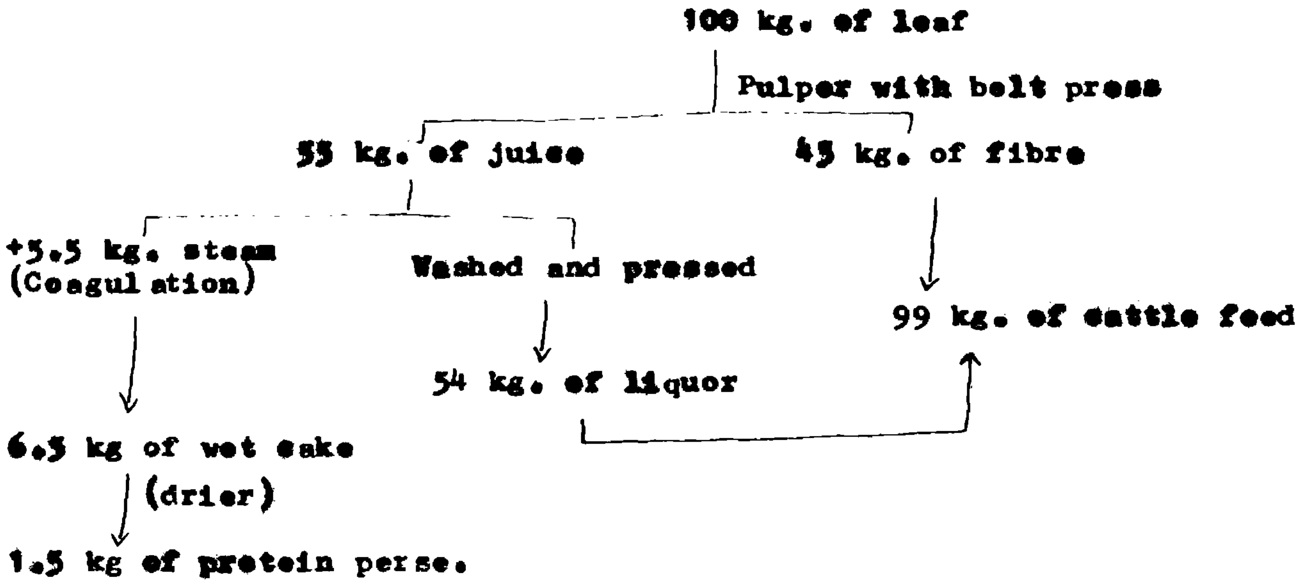
ANNEXURE III

Preparation of Leaf Protein:

Harvested lucerne plants were sequentially washed chopped, pulped using an IBP pulper with belt press, and filtered to separate the protein rich juice. The filtered juice was pumped to a 'U' tube where it was curdled by injecting steam from a boiler to heat it to 90°C. This coagulum was filtered out using a standard cloth stockings and pressed by a beam press. The pressed cake was then suspended smoothly in water, acidified to PH 4 with 2 N.Hcl filtered and pressed again. The product was air dried at 40°C to 50°C using a drying oven and ground to a fine powder.

On dry weight basis the leaf protein powder had 50 per cent protein, which was rich in lysine and limiting in methionine. It was a supplement to a diet based on cereals.

Flow diagram of the leaf protein extraction:



The crop lucerne was cut very 20 days from cultivation. The two biproducts namely the fibre and filtered liquor were used as cattle feed. The cost of leaf protein was 2 Rs./kg.

APPENDIX IV

Determination of faecal carotene:

The faecal carotene was estimated using the procedure of Jagannathan and Patwardhan (1960).

Procedure:

Reagents:

1. Alcohol - Distilled pure alcohol
2. Petrolium ether
3. Anhydrous sodium sulphate.

Method:

The faecal samples were soaked in alcohol overnight and they were transferred to a mortar and ground with alcohol repeatedly until the extract had no more yellow colour. The alcohol extracts were then transferred to a separating funnel. An equal volume of water and petroleum either were added, swirled well and the lower layer discarded. The process was repeated until the lower layer showed no trace of yellow. The petroleum ether extracts were then transferred to a separating funnel to which an equal volume of water was added, swirled and the lower layer was discarded. The extract was freed from moisture by filtering through a bed of anhydrous sodium sulphate. This was washed with small quantities of petroleum ether until the filtrate was free from traces of carotene. The petroleum ether extract was made upto volume and the absorption of yellow colour measured in a coleman Junion Spectrophotometer at 450 m.

APPENDIX V

Estimation of serum vitamin A (retinol):

The extraction procedure was done, following the method of Neeld and Pearson (1963) and the solution was read at 348 m. (in ultra violet region), following the procedure given by NIN (1971).

Procedure:

Reagents:

1. Alcoholic potassium hydroxide 0.5%:- 0.5 of potassium hydroxide was dissolved in 100 ml of purified alcohol.
2. N hexane (MPC) B.P. 65°-75°C.
3. Chloroform
4. Stock vitamin A solution: 344 mg. of vitamin A acetate. (300 mg of vitamin A) was dissolved in chloroform and made upto 100 ml. 1 ml of stock contains 3000 mg of retinol.

Intermediate standard:

1. 0.25 ml of stock diluted to 25 ml = 30 mg/ml
2. 0.50 ml of stock diluted to 25 ml = 60 mg/ml
3. 0.75 ml of stock diluted to 25 ml = 90 mg/ml
4. 1.00 ml of stock diluted to 25 ml = 120 mg/ml

Working standard:

Each intermediate was again diluted in the ratio 1:10 and from each standard finally 1ml was taken and 2.0 ml of chloroform was added and read at 438 mμ in Beckman Du. spectrophotometer.

<u>Method:</u>

1 ml of serum was placed in a test tube. An equal volume of alcoholic potassium hydroxide was added. 1,5 ml of n hexane was added to the serum alcoholic potassium hydroxide in the test tube. The test tube was stoppered and the contents were shaken well for 45 seconds. It was centrifudged for 10 minutes at a speed of 3000 revolutions/minute. The supernatant was evaporated to dryness in a 40°C water bath. The residue was immediately taken up in one ml of chloroform, added 2 ml of chloroform again and the OD of the solution was measured at 348 mμ in the spectrophotometer.

The solution is now transferred to a soft glass tube, with a stopper irradiated with U.V.light (the lamp should be turned on 10 minutes before use). The tube should be kept at a distance of 20 cm from the lamp. The OD at 348 mμ is again read and the difference in OD is taken as a measure of vitamin A in the solution.

ANNEXURE VI

Biuret Method for total proteins and albumins in plasma or serum:

Principle:

The (-CONH) groups in the protein molecule react with copper sulphate in alkaline medium to give a purplish violet or pinkist violet colour which is then read at 540 m.

Reagents:

1. **Biuret Reagent:**

1.5 g. cupric sulphate and 6.0 g sodium potassium tartrate were dissolved in 500 ml of distilled water. To this was added, with swirling, 300 ml of 10 per cent (W/V) sodium hydroxide (CO_2 free) and diluted to 1 litre in a plastic bottle. 0.1 per cent potassium iodide was then added and stored at room temperature. This reagent was stable for upto six months.

2. **28% sodium sulphate solution:**

3. **Protein standard:**

The standard protein solution might be either a pooled normal human serum, the protein content of which had been accurately determined by a kjeldahl method or solutions of pure albumin. Use of albumin solutions permited concentrations above the normal range. The serum or albumin solutions were diluted with 0.9 per cent saline to given exact standards of ~~0.0g.~~ 1.0g. to 8.0g. per 100 ml.

Procedure:

Total proteins:

0.1 ml serum was diluted with 1.9 ml of water. 8 ml of biuret reagent was added and mixed well by shaking, let stand for 30 minutes and read in a spectrophotometer at 540 m.

Albumin:

To 0.2 ml serum in a tube added 4.8 ml of 28 per cent sodium sulphite from a burette (Pipette should not be used as the salt crystallises out easily) mixed and let stand for 10 minutes, filtered with whatman 40 or 44 filter paper. Added 8 ml of biuret reagent to 2.0 ml of the filtrate and proceeded as for total proteins. Ordinary centrifugation would not yield a clear globulin free supernatant and use of other reagents at room temperature was not suitable.

Reagent blanks were prepared with 2.0 ml of distilled water and 8.0 ml biuret reagent for total proteins and with 2.0 ml 28 per cent dodium sulphite and 8.0 ml biuret reagent for albumin.

ANNEXURE VII

Estimation of haemoglobin by cyanmethaemoglobin method:

Reagents:

1. Drabkin's Diluent solution

Sodium bicarbonate	-	1 g.
Potassium cyanide	-	0.05 g.
Potassium ferricyanide	-	0.20 g.
Distilled water	-	1 litre

This solution should not be used after it forms a precipitate on the botton of the storage bottle. The solution is preserved in dark brown bottle and preferably under cold storage. Its preparation and handling should be done with great care.

Procedure:

1. Exactly 5 ml of Drabkin's diluent solution is measured into a dry test tube from a burette or a pipette with suction bulb.
2. Exactly 0.02 ml of blood is transferred from a standard haemoglobin pipette into a diluent solution. Usual care is filling and cleaning of loaded haemoglobin pipette must be observed.
3. The pipette is rinsed three times with the diluent solution, without allowing the formation of air bubbles in the solution.

4. The blood and the diluent are thoroughly mixed by rotating the tube.

5. 10 minutes time is allowed for the formation of the cyanmethaemoglobin.

6. 5 ml of the diluent solution is used as blank

7. With green filter No.540 the readings are taken in a photo electric calorimeter.

Calibration Procedure:

1. Total blood iron is determined by Wong's method. This determination would give absolute amount of haemoglobin.

2. Exactly 0.02 ml of this known blood sample measured as above into 5.0, 7.5, 10.0, 12.5 and 15.0 ml respectively of diluted solutions are now equivalent to blood samples containing respectively 100,67,50, 40 and 30 per cent that of the original solution.

3. The intensity of the colour is read using green filter 540 against a blank set at zero. O.D.

4. On a graph paper a standard graph is drawn using these haemoglobin concentration and corresponding density values.

ANNEXURE VIII

Excretion of B-carotene during the three subsequent terminal days by the target children when amaranth was fed as a source of B-carotene

No.	B-carotene excretion µg			
	First day	Second day	Third day	Mean
1.	280	420	380	360
2.	590	480	550	540
3.	410	523	435	456
4.	339	219	450	336
5.	612	546	570	576
6.	292	378	410	360
7.	408	440	412	420
8.	232	224	192	216
9.	342	350	280	324
10.	335	410	437	396
11.	290	330	280	300
12.	414	392	346	384
13.	218	267	271	252
14.	566	485	425	492
15.	298	322	388	336

ANNEXURE IX

Excretion of B-carotene during the three subsequent terminal days by the target children when Leaf Protein was fed as a source of B-carotene

No.	B-carotene excretion µg			
	First day	Second day	Third day	Mean
1.	90	140	130	120
2.	300	248	244	264
3.	148	202	226	192
4.	289	268	211	256
5.	300	224	160	228
6.	312	380	424	372
7.	288	296	352	312
8.	309	330	369	336
9.	468	484	524	492
10.	360	342	342	348
11.	162	110	260	144
12.	392	422	410	408
13.	270	274	248	264
14.	264	248	244	252
15.	294	202	164	220

ANNEXURE X

Excretion of B-carotene during the three subsequent terminal days by the target children when standard B-carotene was fed as a source of B-carotene

No.	B-carotene excreted µg.			
	First day	Second day	Third day	Mean
1.	142	100	124	122
2.	138	160	146	148
3.	184	214	190	196
4.	210	199	194	201
5.	186	186	189	187
6.	230	195	250	225
7.	280	240	200	240
8.	220	215	195	210
9.	246	268	212	242
10.	230	230	245	235
11.	175	210	185	190
12.	270	220	215	235
13.	190	210	146	182
14.	198	126	210	178
15.	160	230	180	190