BIOLOGICAL AVAILABILITY OF β CAROTENE FROM FRESH AND DRIED GREEN LEAFY VEGETABLES ON PRESCHOOL CHILDREN

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I INTRODUCTION

It is one of the anomalies of nature and man that the countries with the highest rate of xerophthalmia are among the perennially greenest of the world, but the nutritious green leaves do not find their way into the mouth of small children (Oomen, 1974).

Im most, if not at all developing countries, vegetables rather than animal products are the main available source of dietary vitamin A, that is, carotene. Increased consumption of carotene rich foods can ensure also increased consumption of other vitamins and minerals (Gopalan 1976). The dark green leafy vegetables eaten traditionally in tropical countries have a much higher carotene content than do pale green leafy vegetables. Hence the World Health Organisation (WHO, 1976) has recommended that deficiency of vitamin A can be overcome by encouraging cultivation of green leafy vegetables in kitchen gardens, scho l gardens and com unity gardens.

In India people are governed to a large extent by superstitious beliefs. This is the reason why food stuffs like green leafy vegetables are avoided. Green which are within the easy reach of the low income groups is strictly avoided during childhood for they are termed as 'hot' foods or believed to cause diarrhoea, dysentery and other illness (NIN, 1973 and Apte, 1977).

The National Institute of Nutrition (NIN, 1975) has reported the intake of vitamin A among the lower income groups of the country to be far below the recommended value of 750 μg. A nation wide dietary survey revealed the highest intake of βcarotene in West Bengal (533.4μg) and the lowest in Kerala (106 μg). According to Devadas (1974) the daily consumption of green leafy vegetables in Tamil Nadu is as low as eight grams per person as against the recommended allowance of 100 g. Leafy vegetables which are the only practicable source of carotene in the poor diet are hardly ever consumed by more than 30 per cent of poor rural families. The consumption of green leafy vegetables in the rural areas among the low income group is estimated to be five to ten grams by Rajalakshmi (1976).

Owing to the low intake, the deficiency of vitamin A has become a major public health problem in India contributing to five million cases of preventable blindness

(Oomen, 1969; Srikantia, 1975 and WHO, 1976). For these blind children days and nights remain meaningless. Light is shut for them for ever. Not that they were born blind but they only did not have proper diet and nourishment. Clinical signs of vitamin A deficiency have been found in school children in sev ral states with as high a prevalence as 10 per cent. Occular manifestations of vitamin A deficiency are frequently seen among preschoolers — bitot spots 3.1 per cent; conjunctival xerosis 4.3 per cent and corneal xerosis 0.1 per cent (Alan Berg, 1973 and Wilson, 1977).

The inadequate dietary intake of the vitamin is mainly due to the poor socio economic background of the population. In India 23 per cent of the rural and 48 per cent of the urban population lie below the poverty line. So much so that even if they spend 70 to 80 per cent of their income on food they cannot afford even the least cost balanced diet (Srikantia, 1975 and Gopalan, 1977). Its prevalence is also due to parasitic infestation, ignorance and illiteracy (Oomen 1973; Gopalan, 1975; Ramalingaswamy, 1975; Cravioto, 1976; IPPF, 1976 and Ng and Chong, 1977).

Lala and Reddy (1970) and Beck (1974) opine that the most rational method of preventing this tragedy of vitamin A deficiency in children would be to improve their diets. Foods rich in vitamin A such as meat, fish, milk, egg yolk and liver are expensive and beyond the reach of the poor. Hence, carotenes derived from inexpensive plant sources should be encouraged (Rao and Rao, 1970; Rajalakshmi, 1974; Devadas and Murthy, 1975 and Jean Mayer, 1975).

Plant food carotenes are not fully absorbed by human subjects and the absorption has been found to vary over a wide range. Besides, greens being used fresh, they are also dried and used in the powdered form. Sundrying of vitamin A rich foods is a technique followed in Africa to overcome scarcity of such foods. Mc Dowell (1976) states that carotene content of foods is destroyed under direct exposure of leafy vegetables to sunlight. However studies relating to the utilization of carotenes from different leafy vegetables by Indian children who are subclinically malnourished have so far been limited (FAO/WHO, 1967 and Rao _et al_, 1970) and also the effect of drying and preserving the leafy vegetables on the utilisation of β carotene is not completely explored.

Hence it is imperative to determine the extent to which β carotene from plant foods can replace vitamin A especially in the diets of poorly nourished children. Though a few studies have been reported in the literature not all leaves have been examined fully along this line. Two of such unidentified sources of green leafy vegetables are fenugreek and drumstick leaves with the β carotene content of 2340 μg and 6780 μg per 100g respectively (NIN, 1976).

Both fenugreek and drumstick leaves are grown all over India, throughout the year. Both these greens have good medicinal value, and have been used by homemakers in both rural and urban areas (Aman, 1969).

In the present investigation an attempt has been made to study the biological availability of B carotene from fenugreek and drumstick leaves in the fresh and dried forms on a group of rural preschool children. For comparison one group of children were fed standard β carotene. The feeding was carried out for a period of two and a half months and the effect of supplementation was evaluated using serum vitamin A levels, blood haemoglobin levels, clinical picture and faecal excretion levels as the criteria.

II REVIEW OF LITERATURE

The literature pertaining to the study on "Biological Availability of β Carotene from Fresh and Dried Green Leafy Vegetables on Pre School Children" is reviewed under the following headings:

A. Factors influencing the biological utilization of β carotene and vitamin A

B. Assessment of vitamin A nutritional status

C. Prevalence of vitamin A deficiency and the preventive measures taken

and D. Green leafy vegetables - A source of inexpensive vitamin A

A. Factors influencing the biological utilization of β carotene and vitamin A:

1. Utilization of β 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 β carotene, as in many studies, the method of estimation of β carotene has included both α and β carotene and in some studies other carotenes as well (Srikantia, 1975). Absorption of β carotene has, however, been found to be much higher than that of total carotenes. Absorption of β carotene from different foods varies widely.

There are several physiological factors which are seldom considered but which can influence the absorption and utilization of vitamin A and its precursors. These include the availability of the provitamin, presence of other dietary components like fats, vitamin E and pathological conditions such as acute infection or thyroid abnormalities as has been put forth by Underwood (1974).

A study conducted by Chandras kharan (1975) reports that higher levels of dietary protein and lipids generally favoured the utilization of vitamin A and the carotenoids. Sengar (1975) suggests that there is no relation between essential fatty acid intake and vitamin A and E plasma levels.

The absorption and utilisation of carotene from a leafy vegetable, a root vegetable, a fruit, a crystalline β carotene and a mixed vegetable source in four adult male volunteers was investigated by Rao and Rao (1970). While crystalline β carotene was completely absorbed, the mean percentage absorption of carotene from amaranthus, carrot, papaya, and mixed vegetable source were 58, 36, 46 and 33 respectively.

A study by Murthy _et al_ (1972) on 10 college students, who were given a daily supplement of 100 g of carrots for 20 days registered a significant increase of vitamin A namely, 2.8 ug/100 ml of the serum.

Murthy _et al_ (1976) compared the efficiency of absorption of β carotene from amaranthus and leaf protein with that from standard B carotene on 15 preschool children and the results showed that 88.4 per cent of B carotene was absorbed from standard B carotene 61.4 per cent from amaranthus and 76.7 per cent from leaf protein. The serum vitamin A levels showed increment from 20.5 to 29.2 µg/100 ml. The serum total protein and albumin levels showed a positive correlation with serum vitamin A levels.

2. Utilization of vitamin A:

The factors incluencing the absorption and utilization of vitamin A has been discussed under the following heads, as postulated by Underwood (1974).

a. Effects of cooking

b. Dietary protein levels

c. Vitamin E intake

d. Presence of emulsified fats

and e. Pathological conditions of the body

a. Effect of cooking:

According to Nageswara Rao et al (1967) as much as 40 per cent of the carotene originally present in the food is lost by cooking. Deep frying or frying followed by heat with small amounts of water caused marked destruction of vitamin A, but boiling leafy vegetables and potatoes in water resulted in little or no loss of carotene. High temperature of deep fat frying may result in considerable loss of β carotene from plant foods according to Spencer (1973). When green leafy vegetables were cooked, the losses varied from 27 to 40 per cent as studied by Rajalakshmi et al (1974).

However, the possibility that the cooking process might denature some of the carotenoids originally present in the new foodstuffs 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 100g of carrots were cooked in a pressure cooker for five minutes it provided about 1230 μg of carotene as against 1890 μg present in fresh samples (Spencer, 1973).

b. Dietary protein levels:

An interrelationship between protein and B carotene both at the nutritional and metabolic levels has been suggested by Kothari et al (1971).

Dietary carotene has been found to be more efficiently utilized by rat when casein was the source of protein than when zein or gluten was the source as referred to by Berger <u>et al</u> (1962). In rats both the quality and the quantity of dietary protein have been shown to influence the absorption of β carotene. In animals maintained on diets providing four per cent protein the absorption and conversion of β carotene has been found to be impaired as asserted by Mathews (1963).

On the other hand activity of the intestinal enzyme carotene dehydrogenase, which converts β carotene to vitamin A has been found to be higher on animals maintained on diets providing 10 per cent protein as compared to those maintained on 20 and 40 per cent protein diets as remarked by Gronowska-Senger and Wolf (1970) and Iimori (1975). Also the activity of the enzyme has been found unaltered in human subjects after an eight day period of fasting.

The effect of time on the utilisation of a fixed dose of carotene in young rats on diets with 10, 20 and 40 per cent protein was studied by Kamath (1972).

It was demonstrated that protein had no effect on effect on the utilization of the lowest level of the retinyl acetate supplementation. In contrast, utilization of large amounts of retinyl acetate was diminished in protein deficiency, but was not affected by excessive protein intake.

On protein starvation, a decrease in serum vitamin A was observed by Thapar et al (1973, 1975). No significant change was noticed after feeding on protein diets for three days but after seven days the highest liver vitamin A, and serum vitamin A values were seen in rats given casein followed by rats given gluten and lysine, and gluten alone.

Rats deprived of protein stored more vitamin A per gram hepatic tissue and kidney than normal rats, but plasma contained less vitamin A. Rao et al (1974) postulates that during repletion vitamin A in all three tissues became comparable with that in the normal. The activities of the enzymes of vitamin A metabolism were low compared with controls, but they returned to normal when rats were given the high protein diet.

Vitamin A utilization and weight gain are lineanly related as pointed out by Adhikari *et al* (1976). Impairment of vitamin A is reflected in lower excretion of polar metabolites of the vitamin. The storage of 3000 μg of vitamin given with H^3 retained by stomach tube was maximum after 24 hours but uptake was slower for rats given 3 per cent casein.

According to Scragg and Rubidge (1960) in man protein nutritional status does not appear to be critical in the metabolism of β carotene. In some parts of Africa even among the most serious cases of Kwashiorkor the occular signs of vitamin A deficiency are extremely rare. Their habitual contain red palm oil, a rich source of β carotene.

Lala *et al* (1970) indicate that the under nourished children with occular signs of vitamin A deficiency could absorb and utilize β carotene satisfactorily. Children receiving supplements of about 1000 μg of β carotene through green leafy vegetables for two weeks showed an increase in serum vitamin A levels from 20 to 30 μg and also an improved clinical picture. Lala and Reddy (1970) reported the efficiency of absorption of B carotene from amaranthus in a group of undernourished preschool children to be about 70 per cent, a value similar to that reported for normal adults.

Smith et al (1975) observed that the plasma vitamin A, retinol binding protein and prealbumin concentration were much more sensitive to caloric and protein therapy in protein-calorie malnutrition than were plasma albumin and total protein levels in children. A balance between dietary calories and protein may be an important variable in the response of plasma protein to treatment.

c. Vitamin E intake:

Vitamin E is a factor which effects the biological and utilization of vitamin A according to Rodriquez and Irwin (1972) and Bauernfeind et al (1975). Ames (1969) puts forward the hypothesis that vitamin A absorption is markedly impaired in vitamin E deficiency. Oral supplementation with d-alpha-tocopherols increased the utilization of orally administered vitamin about sixfold. Even when vitamin A was administered intramuscularly in emulsified form utilisation in the vitamin E deficient animal was low.

Kusin *et al* (1974) demonstrated that the addition of 40 and 100 mg of vitamin E to the massive dose of vitamin A in normal children had no effect on the absorption or retention of vitamin A, but on addition of 500 mg of vitamin A resulted in a significant increase im the intestinal absorption of vitamin A and also in urinary excretion.

d. Presence of Emulsified Fats:

Following oral administration, plasma vitamin A levels rise more rapidly with emulsified 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 emulsification greatly enhances the biological response to intramuscularly administered vitamin A. In general, the greater the proportion of emulsifier, the greater the biological response.

Dietary lipids do not appear to be as important for utilizing preformed vitamin A as they are for the provitamin (Oey Khoen Lien *et al*, 1967).

Figueira *et al* (1969) concluded that dietary fat is not essential for vitamin A, absorption since young children on low fat diets, supplemented with vitamin A and dried skimmed milk had normal plasma values.

However, Kahan (1970) explained that in premature infants the amounts of vitamin A absorbed was parallel to the amo nt of fat absorbed. Very low fat diets may significantly decrease absorption, especially of carotenoids, since dietary fats from which monoglycerides and phospholipids are produced by normal digestive process facilitates absorption.

e. Pathological condition of the body:

Repeated attacks of respiratory infection and gastroenteritis may enhance the vitamin A requirement by interfering with the intestinal absorption of the vitamins as reported by Reddy and Sivakumar (1972). In children whose stores of the vitamin are marginal this may precipitate clinical manifestations of vitamin A deficiency. In a study by Mukherjee and Barat (1972) all the children with keratomalacia had a history of diarrhoea, the number of loose motions being 6 to 12 daily. There was associated vitamin B-complex and protein deficiency as well.

Ascariasis in populations on marginal intakes of vitamin A and the precursors is an important contributing factor in producing vitamin A deficiency (Mahalonabis <u>et al</u>, 1976). According to them the oral administration of a large single dose of vitamin A, at long intervals to the population may be ineffective in the presence of ascariasis.

Thyroid activity and the conversion of carotene to vitamin A was implicated by Gambhir <u>et al</u> (1975). Thyroxine treated animals showed higher levels of vitaminA than control rats. Thiourea fed animals showed opposite effects on liver tissue but little effects on kidneys and serum. It has been suggested that thyroxine has three effects on the carotene metabolism;

a. increased absorption of carotene through the gut
b. enhanced activity of the enzyme responsible for conversion of carotene to vitamin A
c. regulated release of stored vitamin A from the liver into the blood stream.

B. <u>Assessment of vitamin A nutritional status</u>:

To assess the vitamin A nutritional status, the most predominantly used clinical tests are occular and they generally provide clear cut evidence of the deficient state.

The occular manifestations include xeróphthalmia, xerosis of the conjunctivae, bitot's spots as well as manifestations of the cornea. For circulating vitamin A the plasma may be regarded as an extension of the body pool. The ICNNE recommends the following interpretation of plasma levels.

high	-	over 50 μg/100 ml
normal	-	20-50 μg/100 ml
low	-	10-20 μg/100ml
deficient	-	below 10 μg/100ml

With respect to these categories, the deficiency state less th n 10 μg/100ml, tends to be universally associated with both low liver reserves of vitamin A and an increased prevalence of clinical signs of deficiency (WHO, 1976).

It has been reported by Reddy et al (1966) and Pereira et al (1968) that impaired dark adaptation was frequently seen in subjects whose blood vitamin A levels were below 18 μg/100ml. In children with corneal involvement, however, it has been consistently found that vitamin A either cannot be detected at all or, if found the levels are very low.

Rankov (1976) found that in 27.3 per cent of cases with impaired dark adaptation serum vitamin A was lower than in the normal subjects by 36 µg/100ml for men and 32 µg/100ml for women. In subjects with impaired adaptation the incidence of skin and mucosa membrane abnormalities was increased, gingivitis was about twice as common, and chronic conjunctivities five times as common as in persons withnormal dark adaptation.

The most severe signs 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 as suggsted by Perzira and Begum (1968).

A gradient from about 25 µg/100ml in apparently normal children without severe manifestations such as corneal xerosis and keratomalacia has been found with immediate values being seen in children with conjectional xerosis and bitot spots.

C. Prevalence of vitamin A deficiency and the preventive Measures taken:

Most of the present century physicians, public health officials and nutritionists have been concerned about vitamin deficiency diseases. Methods of control of beri-beri, scurvy, rickets and pellagra have to a considerable degree b en widely effective, but not for xerophthalmia of vitamin A deficiency.

Vitamin A deficiency and the epidemiology of xerophthalmia have been studied by several groups of scientists (Patwardhan, 1969; and Oomen, 1972). It has been reported to occur in Burma, Ceylon, Bangladesh, India, the Philippines, North and South Vietnam, South Korea, and Thailand as reported by Glick et al (1973), Kamel (1974) and Vijayaraghavan et al (1975). Xerophthalmia is also frequently encountered in countries of the Middle East and African countries. Many cases have been reported from Central and outh American countries (Srikantia, 1975). Over 1000 preschool children become blind due to vitamin A d ficiency in a period of one year in North West Brazil (Simmons et al 1975).

As put forth by Brown (1975) one of the important causes for the widespread prevalence of this deficiency is the inadequate dietary intake of the vitamin and poverty. The prevalence of vitamin A deficiency both in rural and urban populations of India is very high. A survey carried out in the city of Calcutta by the USAID (1972) reveals that 71 per cent of the preschool children and 91 per cent of the adults do not receive the minimum requirements of vitamin A. Seven states of India, four in the South and three in the east, where most cases of malnutrition are observed, some 13,000 young children are going blind for this reason (Bagchi, 1976). The overall peak prevalence of xerophthalmia is in the third and fourth years. The condition frequently is associated with PCM, but often overlooked by the physicians. The peak for PCM without accompanying xerophthalmia is between 30-36 months of age (Smith et al, 1973). The association of hypoalbuminemia and low serum vitamin levels in PCM is accompanied by low levels of retinol-binding protein (Arroyave et al, 1963 and Zaklama et al, 1972, 1973). Treatment with protein feeding without additional vitamin A is usually followed by an increased in the serum retinol concentration due apparently to utilization of previously unavailable liver stores (Smith et al, 1973). The study at Surabaya suggests that of the blind survivors about 25 per cent are in the fourth year of life. Blindness in this case means about 60 per cent abinocular blindness and 40 per cent monocular. The effection is not always symmetrical as proposed by Oomen (1976).

A study conducted on the serum vitamin A levels for a Malayan and an Indian community in rural Malaysia showed the following evidence-32 per cent of the preschool children of both communities had serum vitamin A levels that may be regarded 'at risk', namely levels below 20 μg/100ml while 16 per cent and 27 per cent of the Malaya and Indian school children respectively were f und to be in a similar state of hypovitaminosis. In contrast the rural adult population did not appear to suffer any measur able lack of this vitamin as studied by Ng and Chong (1977).

The main objective of the prevention of vitamin A deficiency is to reduce the prevalence of blindness. Prevention can be carried out through periodic massive dose programmes aimed at children at risk or fortification of suitable food that is consumed regularly in populations and education may contribute substantially to the prevention of xerophthalmia (WHO, 1976).

In a study conducted by Pereira and Begum (1969) in an orphanage in South India, when the children were administered 90,000 µg of vitamin A orally as a single dose, had levels of serum vitamin A which were above pretreatment levels for periods up to four months after dosing. The administration of small amounts of dietary intake of β carotene (120 µg daily) was found to be capable of elevating and maintaining blood levels of vitamin A at levels higher than those seen in a single group of similar children who had not received the massive dose for periods upto 16 weeks.

In the studies carried out at the National Institute of Nutrition, (Srikantia and Reddy, 1970) the administration of massive dose in malnourished children could maintain the serum vitamin A level at 27 µg/100 ml even at the end of six months and that by 9 to 14 months it had fallen to about

14 μg/100ml a value above the predosing level. As a preventive measure Indonesia many ophthalmologists and pediatricians also administer 90,000 μg of vitamin A to all preschool children Karyadi et al (1975).

With the objective of preventing vitamin A deficiency about 2000 preschool children were administered an annual massive, single dose of 300,000 ml of vitamin A. The r sults of the follow up study ov r a period of two years indicate a considerable redyction in the prevalence of signs of vitamin A deficiency. In order to attain better prophylaxis Swaminathan et al (1970) suggested that the frequency of administration of the massive dose be increased to once in every six months instead of once every year.

Since vitamin A deficiency mostly affects young children the UNICEF with the scientific and technical advice of WHO took upon itself to enrich the dried skim milk used in its programme with vitamin A. The level recomm ended for stabilised form of the vitamin A is 1,500 μg retinol (Eg. to 5,000IU of vitamin A) per 100 g of skim milk powder, assuming intakes in the range of 40 to 80g skim milk powder per day (PAG, 1976; WHO,1977)

A survey of food habits in the states of Gujarat and Maharashtra in Western India revealed that 87 per cent of the villagers in Gujarat including 84 per cent of those in the lowest income families, give tea to their children. Therefore the fortification of tea by dry mixing with fine powdered dry vitamin A palmitate is an ideal vehicle for conveying vitamin A to millions of Indians in all age groups whose diet is seriously deficient in this vitamin (WHO, 1976).

The effectiveness of nutrition education programmes aimed at improving the consumption of vitamin A rich foods has been adequately evaluated. The education must include aspects of production, storage, and utilization of food products and in some cases may also include methods of preservation (WHO, 1976).

D. Green leafy vegetables - A source of inexpensive vitamin A:

In most of not all areas of the world where xerophthalmia is prevalent vegetables, rather than animal products are the main available source of dietary vitamin A (i.e. carotene). Increased consumption of many of the carotene rich foods will also ensure the increased consumption of other essential nutrients. Hundred grams of amaranthus provides 5,520 μg of β carotene (ICMR, 1976). Many of the dark green leafy vegetables eaten traditionally in tropical countries have a much higher carotene content than do pale green leafy vegetables such as cabbage, raddish and turnips (WHO, 1976).

The absorption of carotene from a leafy vegetable, a root vegetable, a fruit and crystalline β carotene, and a mixed vegetable source in, four male volunteers was studied by Rao and Rao (1970). While crystalline carotene was completely absorbed, the mean absorption of carotene from amaranthus, carrot, papaya and mixed vegetable sources were 58, 36, 46, and 33 respectively. It appears from the study that availability of carotene from leafy vegetables may be 50 per cent or more.

In a study undertaken by Ramana and Singh (1971) rats from the progeny of mothers fed a vitamin A deficient diet during mating, pregnancy and lactation were given by mouth 32 IU of vitamin A daily, while one group of rats were on a vitamin A deficient diet. Then rehabilitated with a diet containing 680 µg carotene/100g of leaf protein. The rats maintained on a vitamin A free diet showed loss os body weight and had visible signs of vitamin A deficiency. The rats with continuous oral supply of vitamin A had heavier lives with greater vitamin A content than the deprived rats.

The efficiency of conversion of B carotene to vitamin A was in the region of 1/3 for pure B carotene and also for B carotene in leafy material such as fenugreek. No difference was found between rats on diets with five per cent or 10 per cent protein with respect to the apparent utilization of B carotene, but blood haemoglobin and serum protein and albumin were diminished in rats deprived of vitamin A suggesting that the vitamin A is involved in protein synthesis (Rajalakshmi et al, 1975).

In a study conducted by Murthy et al (1976) to determine the efficiency of absorption of B carotene from amaranthus and leaf protein as compared to standard B carotene showed that 85.4 per cent of B carotene was absorbed from the standard and 61.4 per cent from amaranthus and 76.7 per cent from leaf protein.

Studies were conducted by Rajalakshmi et al (1977) on adult men of the comparative response of serum carotene and vitamin A levels to supplementation with 1800 μg of carotene provided by leafy vegetables and 600 μg of vitamin A palmitate in oil. The increase in serum vitamin A (μg/100ml) in the groups fed leafy vegetables and vitamin A were 15 and 16 as against 2 and 1 in the fed and non fed controls. The corresponding increase for the serum carotene were 36, 2to1 and 5 respectively. Thus subjects given leafy vegetables showed at least as good a response as the group given vitamin A suggesting a high efficiency of utilization of carotene in subjects accustomed to diets based mainly on plant foods.

III EXPERIMENTAL PROCEDURE

The present investigation was designed to study the Biological Availability of β Carotene from Fresh and Dried Green Leafy Vegetables on Preschool Children. The procedure consisted of the following steps:

A. Selection and grouping of children

B. Supplementation of the existing diets with β carotene

and C. Evaluation of the feeding trial

A. Selection and grouping of children

A village Vadamadurai of the Perianaickenpalayam Block situated 11 km north of Coimbatore city was selected for carrying out the study. The village had a balwadi with 45 children of four to five years of age, participating in the CARE Feeding Programme. Another group of 30 children of the same age group, of attending the balwadi but participating in the CARE Feeding Programme were also selected for the study. All the children participating in the feeding programme received 80 g of Balahar daily in the form of Uppuma, but no green leafy vegetable of any kind was fed to them. A group of 15 children of the same age not participating in any feeding programme served as controls.

For all the 90 children selected initial heights were recorded using a measuring tape, fixed on the wall, to the nearest 0.1 cm. Weights were recorded using a spring balance to the nearest 0.1 kg. A socio economic cum dietary survey was carried out by the investigator to find out the economic background and dietary habits of all the children selected. The seventy-five experimental children were divided into five groups of 15 children each, comparable in their mean initial height, weight and their socio economic background and designated as A, B, C, D and E for receiving different supplements. The mean initial heights and weights of the selected groups of children are presented in Table I. The individual values are given in Appendix A.

TABLE I

THE MEAN INITIAL HEIGHTS AND WEIGHTS OF THE SELECTED CHILDREN

Group	Height (cm)	Weight (kg)
A	87.5	11.7
B	87.4	11.6
C	87.4	11.4
D	87.1	11.4
E	87.5	11.3
F	87.6	11.2

B. Supplementation of the existing diets with β carotene:

1. Selection and estimation of β carotene in the Supplements

The two β carotene sources selected for the investigation were fenugreek and drumstick leaves both in the fresh as well as dried forms. The drying was carried out in the out door but not under direct sunlight, to prevent the loss of β carotene. In this study a standard β carotene source, a commercial BDH preparation was chosen to compare the utilisation of the vitamin against natural food sources. The β carotene content of the fresh leaves were estimated using the overnight extraction procedure of Thompson et al (1946). Weighed quantities of the fresh leaves were dried as described above and these samples were estimated for β carotene content. The β carotene content of the commercial β carotene preparation was also estimated using the above procedure. The values obtained in comparison with the ICMR value are presented in Table II.

TABLE II

β CAROTENE CONTENT OF THE SUPPLEMENTS (ug/100g)

	Fresh values	ICMR values	Dried leaves	Percentage loss
Drumstick leaves	5,925	6,780	3,643	38.51
Fenugreek leaves	1,967	2,340	1,202	38.89

The contents estimated were found to be lower than the ICMR values. Drying of the leaves resulted in about 38 per cent loss of carotene in both the leaves.

2. Conducting food weighment survey:

Initially five children from each of the experimental and the control groups were selected randomly and a three day food weighment survey was carried out to determine the β carotene content of the normal home diets consumed by the children. The weight of the raw ingredients used for each meal, the total weight of the cooked food and the amount of food consumed by the individual preschooler were weighed for a period of three days and the raw ingredients consumed were computed. The mean β carotene, retinol, protein and iron content of the diets was calculated using the Food Composition Tables (ICMR, 1976). From the data it was found that the daily int ke of β carotene in the home diet was only about 137 μg as against the ICMR recom ended allowance of 1200 μg.

3. Feeding the children:

For the supplementation of the diets a quantity of 1200 μg of β carotene was decided as per the procedure followed by Lala and Reddy (1970). The details of the supplement given to different groups of children is presented in Table III.

TABLE III

SUPPLEMENTS GIVEN TO THE DIFFERENT GROUPS OF CHILDREN

Groups	Supplement given	Quality of the supplement	Carotene content of the supplement
A	Fresh Fenugreek leaves	50 g	1200 μg
B	Fresh Drumstick leaves	20 g	1200 μg
C	Dried Fenugreek leaves	75 g	1200 μg
D	Dried Drumstick leaves	45 g	1200 μg
E	Standard β carotene	--	1200 μg
F (control)	Nil	Nil	Nil

The daily supplement was given in the form of greens kootu. The preparation was first standardised and the most acceptable preparation was selected for feeding the children. The quantities required for feeding different groups were standardised in terms of cups and spoons.

Children in Group E were fed 1200 μg of standard β carotene. The table was diluted in coconut oil to obtain a required dilution. One ml of this solution was equivalent to 1200 μg of β carotene. The β carotene solution was given orally to the children to Group E every day after lunch.

The children in the balwadi were fed Balahar Uppuma every day. The Balahar was supplied by CARE at the rate of 80 g per child per day. The 30 children not attending the balwadi also received the same quantity of Up uma. The experimental groups were seated separately during the feeding time. The daily attendance records were maintained throughout the experimental period of two and a half months.

C. Evaluation of feeding trials:

The criteria used for the evaluation of the feeding were:

1. Serum vitamin A levels
2. Faecal excretion of B carotene
3. Clinical picture

and 4. Blood Haemoglobin levels

1. Serum vitamin A levels

The serum vitamin A level gives a picture of the vitamin A status of the individual, and it may be regarded as an extension of the body pool (WHO, 1976). In view of this the serum vitamin A levels of the children was estimated at the beginning and end of the study. The five children who were selected for the initial weighment survey were chosen for this also from all the six groups and three ml of venous blood from each of the selected child was drawn with the help of a trained technician. The serum vitamin A level was estimated using the tri-fluoroacetic acid method of Neeld and Pearson (1963) (Appendix B).

2. <u>Faecal excretion of B carotene:</u>

As 40 per cent of ingested B carotene is excreted in the faeces, stool examination will give the amount of β carotene utilized by the individual (Rao and Rao, 1970).

Stool samples of the selected five children from each group were collected for three consecutive days prior to the introduction of the supplements. Following the β carotene supplementation, Faecal samples were collected from the same children until the carotene content of faecal samples was the same in two consecutive days. It was observed by Rao and Rao (1970) and Lala and Reddy (1970) that most of the unabsorbed carotene from the ingested sources was excreted within 48 hours after consumption and on the third day the faecal excretion had returned to basal levels. Faecal samples were collected daily, weighed and blended. Aliquots were analysed for β carotene content.

The amount of carotene excreted on the unsupplemented diet was subtracted from the amount of carotene excreted after the supplement was given and the difference was taken as carotene unabsorbed from the dietary supplement. From this the per cent absorption of carotene was calculated. The estimation was done using the procedure of Jaganathan and Patwardhan (1960).

3. Clinical picture of the children:

The clinical assessment is based on the recognition of certain physical signs. This can be used to evaluate the change believed to be related to inadequate nutrition-superficial epithelial tissues, eye, hair, and buccal mucosa (Jelliffe, 1967).

Hence a clinical assessment was carried out for all the 90 children with the help of a physician both at the beginning and at the end of the study using the ICMR Clinical Survey forms.

4. Blood haemoglobin levels:

Results of some recent studies undertaken at the National Institution (1977) have suggested that vitamin A may play an important role in haemopoiesis. Daily supplements of vitamin A resulted in significant increase in levels of haemoglobin from 9.3±0.55 to 9.9 ± 0.49 g/100ml in children. In the light of this finding the blood haemoglobin level of the children selected for the weighment survey was estimated both at the commencement and at the termination of the feeding. The method followed was the cyanmethaemoglobin advocated by the ICNND (1963).

IV RESULTS AND DISCUSSION

The present investigation was aimed at assessing the biological availability of β carotene from fresh and dried green leafy vegetables on preschool children. Differnt sources of B carotene namely the fresh and dried forms of fenugreek and drumstick leaves and standard β carotene were supplemented in the diets of five groups of preschool children for a period of two and a half months in a rural area. The results obtained in this study are discussed under the following heads:

A. Socio-economic background of the children
B. Diet and nutrient intake of the children
C. Changes in serum vitamin A levels
D. Absorption of β carotene from different supplements
E. Changes in the clinical picture
and F. Changes in blood haemoglobin levels.

A. Socio economic background of the children:

The families of the target beneficiaries belonged to the poor as well as middle income groups with an income range of rupees 175 to 775 per mensum. Thirty per cent of the families spent 51 to 60 per cent of their income for food, 47.6 per cent spent 61 to 70 per cent for food and 22.4 per cent spent 71 to 80 per cent for food. Semi skilled labour in textile mills and couli (day labour) were the main occupation of the parents of these target children. All the children were from nuclear families. Among the parents 21.32 per cent were illiterate in the groups studied.

B. Diet and nutrient intake of the children:

A three day food weighment survey was carried out for the randomly selected five target children from each of the six groups. The mean food intake of the children as computed from the data are presented in Table IV with the details in Annexure C.

TABLE IV

MEAN DAILY FOOD CONSUMPTION OF THE TARGET CHILDREN*

Foods	RDA ICMR (g)	Quantity consumed by the target children (g)					
		A	B	C	D	E	F
Cereals	200	215	220	218	210	220	185
Pulses	60	25	22	27	20	25	19
Green leafy vegetables	75	15	15	10	8	12	10
Other vegetables Roots and Tubers	50	40	32	44	41	35	40
Fruits	50	15	10	8	11	10	--
Milk and milk products	250	40	30	25	35	40	20
Fats and oil	25	15	12	10	15	21	18
Sugar and jaggery	40	20	22	19	17	20	15

* The children in Group A, B, C, D and E received 80 g of Balahar and 7g of salad oil through the feeding programme.

RDA - Recommended Dietary Allowances (ICMR, 1976).

In all the groups of children studied the mean daily consumption of cereals was greater than the ICMR (1976) recommendations. This was mainly due to the supply of 80g of Balahar through the feeding programme. Children in the control group received a slightly lesser quantity through their home diets. The consumption of pulses, vegetables fruits, milk, fats and oil andsugar and jaggery were below recommended allowances of ICMR(1976). The mean consumption of green leafy vegetables was found to be very low, namely, 19g per day as against the recommended quantity of 75g per day.

The common breakfast items among the selected families were, left over rice, uppuma, iddli or cholam kali. For lunch rice or cholam was used with sambar, occasionally a separate vegetable preparation was also made. The same rice or cholam kali and sambar was kept for dinner also. In the lower income families the night meal usually consisted of either boiled tapioca or sweet potatoes. Thus the family menu did not have much variation or variety. The food was cooked only once a day either in the morning or at night, in a few cases it was twice a day.

The poor diet habits and deficient food intake were governed by several traditional, unscientific beliefs. The food beliefs prevailing in the society are as follows:

Leafy vegetables if consumed by a pregnant women will cause her baby to become dark skinned.

Papaya if eaten during pregnancy damages the foetus

No vegetables should be consumed after delivery as the wounds of the uterus take a long time to heal and may even turn septic.

Consumption of green leafy vegetables by young children causes diarrhoea, dysentry, itching and seizures.

The mean daily nutrient intake of the selected children was calculated from the food consumption data. The mean calorie protein, iron and carotene intake of the selected children are presented in the Table V with the individual values in Annexure D.

TABLE. V

MEAN TOTAL CALORIE, PROTEIN, IRON AND B CAROTENE INTAKE OF THE SELECTED CHILDREN

Nutrients	RDA	Groups studied					
		A	B	C	D	E	F
Calories (Cal)	1500	1154	1252	1127	1224	1250	1100
Protein (g)	22	21.94	24.55	22.06	21.10	23.54	18.38
Iron (mg)	15.20	12.57	13.29	12.50	12.35	11.83	10.91
β Carotene (μg)	1200	137	253	74	99	169	90

RDA - Recommended Dietary Allowances (ICMR, 1976)

Note: The balwadi diet supplied 305 calories, 11.84g protein, 3.2 mg iron and 16 μg β carotene to the children in Groups A, B, C, D and E.

The main lacuna in the diets of the preschool children was a food gap and not a protein gap as reported by Gopalan et al (1973) and Srikantia (1973). The mean calorie gap observed in these groups of children ranged from 16.5 to 26.7 per cent. The highest deficit was noticed in the control group children. Thus in a cereal based diet if the calorie gap is closed by an extra cereal pulse supplement, the caloric gap is shortened with an increased protein intake. But the deficiency of vitamins

The intake of protein was very disappointing as all the experimental children received more than 20g/day. The children in the control group received the lowest quantity of protein namely 18.38 g/day.

The daily intake of iron ranged from 10.91 mg in the control group to 12.57 mg in the Group A. It can be interpreted that all the children received a meagre quantity of green leafy vegetables resulting in a deficient intake of iron.

The mean dietary intake of B carotene of all the subjects was as low as 137 µg from their home diets. The balwadi supplements provided only 16 µg of B carotene. The low intake of preformed vitamin A or B carotene was also due to an inadequate consumption of green leafy vegetables, other vegetables and milk and milk products. The B carotene intake from the home diet met only 20 to 23 per cent of the ICMR recommended dietary allowance (1976).

C. Changes in serum vitamin A levels:

The mean initial and the final serum vitamin A levels of the control as well as the experimental groups of children who were fed different sources of B carotene, namely, fresh and dried fenugreek and drumstick leaves and standard β carotene along with the balwadi supplements are given in Table VI along with the statistical analysis and Figure I. The individual values are given in the Annexure E.

TABLE VI

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

Group	Source of supplement	Mean initial serum vitamin A level(μg/100ml)	Mean final serum vitamin A level (μg/100ml)	Mean difference (μg/100ml)	't' value for the mean difference	Groups compared	't' value
A	Fresh Fenugreek leaves	13.78±	21.34 ± 2.99	7.56± 1.05	1*	A VS B	0.83
						C	0.21
						D	0.66
						E	1.98
						F	10.80**
B	Fresh Drumstick leaves	11.90 ±	21.69 ± 3.07	8.14 ±0.92	1*		
						B VS C	1.03
C	Dried Fenugreek leaves	13.49 ±	20.89 ± 4.08	7.40 ±1.10	3*	D	0.04
						E	1.02
						F	13.26**
D	Dried Drumstick leaves	13.48 ±	21.66 ± 2.79	8.17 ±0.97	1*	C VS D	1.06
						E	2.16
						F	9.98**
E	Standard . B carotene	13.62 ±	22.25 ± 1.93	8.63 ±0.27	1*	D VS E	0.92
						F	12.57**
F	Control	10.53 ±	9.02 ± 2.87	-1.51 ±0.41	17	E VS F	

** Significant at one per cent level

* Significant at five per cent level

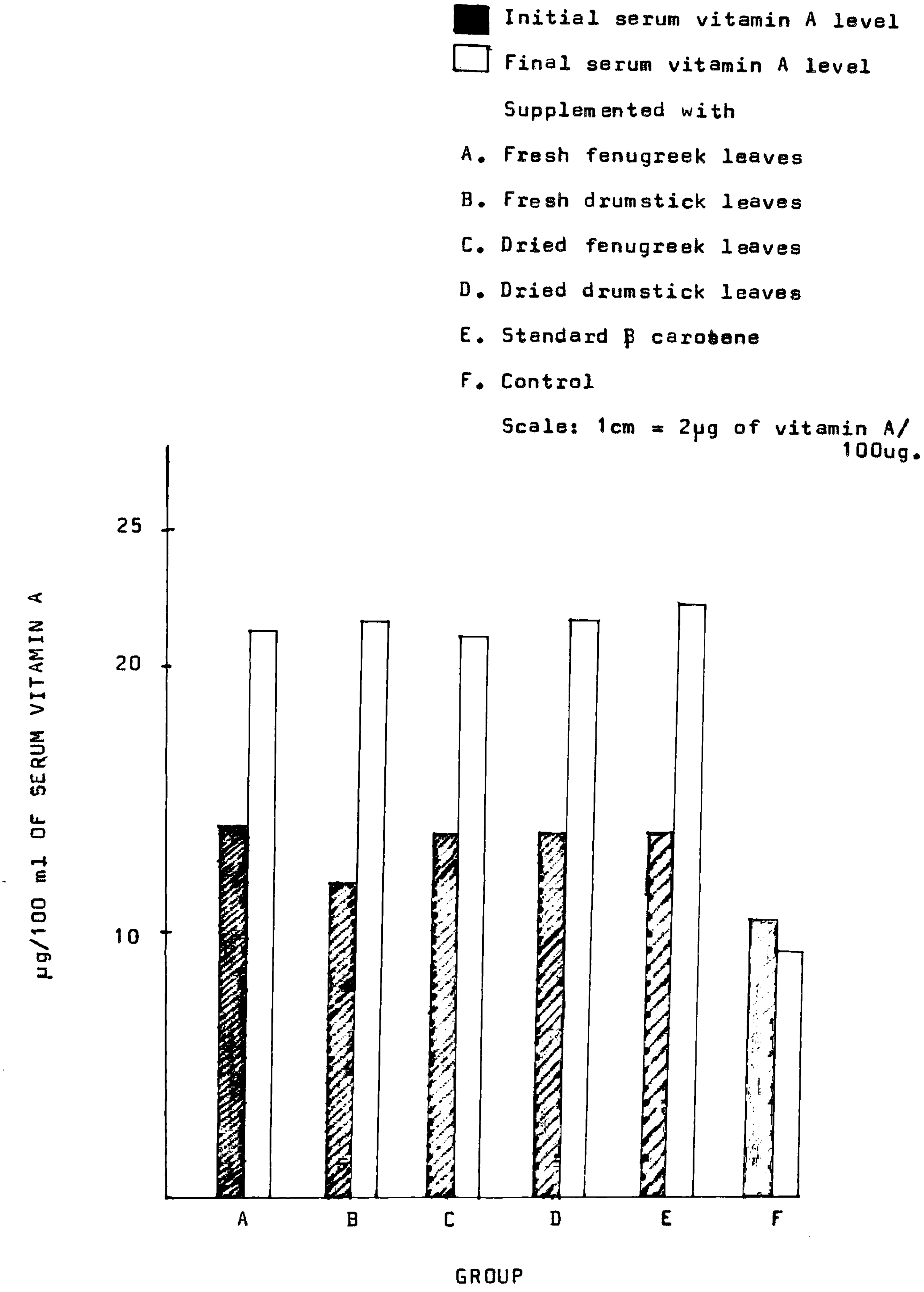

FIG I

MEAN SERUM VITAMIN A LEVELS OF THE SELECTED CHILDREN BEFORE AND AFTER SUPPLEMENTATION.

The main initial levels of serum vitamin A in all the selected children were below 14 µg/100 ml. When interpreted with the recommendations of WHO (1976) all the children selected for the investigation could be regarded as having the low body pool of vitamin A. It was also observed that the control children in group F had the lowest initial serum vitamin A levels. It could be related that these children did not participate in the balwadi feeding programme which supplemented 11.04 g protein and 305 calories per day. The low protein intake might be the cause for the lower initial serum vitamin A levels as Murth et al (1976) have observed a positive correlation existing between the protein intake and the serum vitamin A level.

Suplementing 1200 µg of β carotene for a period of two and a half months had increased the serum vitamin A levels in all the five experimental groups. The increments ranged from 7.4 µg in Group C supplemented with dried fenugreek leaves to 8.63 µg in Group E supplemented with standard β carotene. The increments observed in all the five supplemented groups were significantly higher than the initial values at five per cent level. This indicates that in these children, B carotene was not only obs rbed 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. Lala and Reddy (1969 and 1970) obtained this effect in as short a period as fifteendays.

The children in the control Group F, however registered a slightly low r value when compared with the initial value (-1.51 μg/100ml). But this decrease was not statistically significant.

When the mean difference in the serum vitamin A levels obtained for different groups were compared amongst themselwes it was observed that all the children in the experimental groups had regist red a statistically si nificant increase over the children of the control group who were not given any supplement. The Children in Group A and C who were fed with fresh and dried fenugreek leaves r gistered 7.56 and 7.40 μg of vitamin A/ 100 ml serum respectively. Whereas the children in Groups B and D who were fed with fresh and dried drumstick leaves registered a slightly higher value, namely 8.14 and 8.17 μg/100 ml respectively. The results of the study by Murthy et al (1976) also showed an increase of

8.7 μg/100ml in a period of three months after feeding 1200 μg of B carotene per day from different sources. The statistical comparison between the five experimental groups did not reveal any appreciable difference.

Correlation between the intake of β carotene and the serum vitamin A levels:

A positive correlation was observed between the intake of carotene and serum vitamin A levels. When the initial and the final data obtained for all the six groups of children were compared. The correlation for the total group was found to be + 0.83.

D. Absorption of β carotene from different supplements:

The mean intake of β carotene, the quantities excreted through faeces and the percentage retention of B carotene from the five different sources of supplements are presented in Table VII with the details of th individual values in the Annexure F.

TABLE VII

MEAN PERCENTAGE OF ABSORPTION OF β CAROTENE IN THE SIX GROUPS OF CHILDREN

Group	β Carotene Intake (ug)			β Carotene Excreted through faeces† (μg)	β Carotene absorbed (μg)	Percentage of β Carotene absorbed.	Groups compared		't' value
	Home diet	Supplement	Total						
A	137	1200	1337	523	764	59.10 ± 0.59	A VS	B	3.86*
								C	3.81*
B	253	1200	1453	575	878	60.99 ± 0.78		D	1.92
								E	37.47**
C	74	1200	1274	535	739	57.69 ± 0.96		F	3.62*
D	99	1200	1299	541	758	58.35 ± 0.52	B VS	C	7.33**
								D	5.61**
E	169	1200	1369	238	1131	84.58 ± 1.22		E	32.76**
								F	5.48**
F	90	--	90	42	48	55.59 ± 1.84	C VS	D	1.89
								E	41.37**
								F	2.21
							D VS	E	39.74**
								F	2.88†
							E VS	F	26.35†*

† Computed from the difference between the faecal excretion during the experimental period and the basal period.

** Significance at one per cent level.

* Significance at five per cent level.

All the children in the five experimental groups were consuming more than the recommended allowance of β carotene. The children in the control Group F were consuming the lowest quantity of β carotene namely 90 μg/1day and their mean daily excretion was also the lowest being 48 μg. The supplementation of 1200 μg of β carotene resulted in an increase in its faecal excretion in all the five groups. The β carotene excreted through faeces ranged from 238 μg to 541 μg in the supplemented groups.

In children in Group E fed with the standard β carotene received the highest rate of absorption being 84.58 per cent. Murthy et al (1976) have reported 85.4 per cent absorption of β carotene from standard β carotene supplementation.

The children in Groups A, B, C and D registered 59.10, 60.99, 57.69 and 58.35 per cent β carotene absorption respectively. Though the children in the control group had the very low intake and excretion the mean absorption was found to be 55.59 per cent.

Similar values have also been observed by Reddy (1970). It was noted that the mean absorption of B carotene observed in children of Group E was found to be significantly greater than that of all the other groups at one per cent level and at the same time the control group children revealed a significantly lower value when compared with that of groups A, B, D and E. The mean absorption obtained for Group A was significantly greater than that of Groups C ($P < 0.05$) and D ($P < 0.01$) and significantly lower than that of Group B ($P < 0.05$).

The children in Group B recorded a significantly greater increase in B carotene absorption than that of Groups C and D. and F at one per cent level.

E. **Improvement in the clinical picture:**

The initial and the final clinical picture of the experimental and control group children as evaluated by the physician is presented in Table VIII.

TABLE VIII

CHANGES IN THE CLINICAL PICTURE OF THE CHILDREN

Symptoms	Experimental Group				Control Group			
	Initial		Final		Initial		Final	
	No. of children	Percent age	No.of children	Percent age	No.of children	Percent age	No.of children	Per cent age
Anaemia	33	44	3297	38.7	13	86.7	13	86.7
Angular Stomatitis	21	28.0	3	24	11	73.3	11	73.3
Bleeding Gums	11	14.7	4	5.3	7	46.7	7	46.7
Discoloured Hair	7	9.3	7	9.3	9	60.0	9	60.0
Dry Skin	19	25.3	11	14.7	13	26.7	13	26.7
Night Blindness	3	4	0	0	2	13.3	2	13.3
Conjunctival xerosis	5	6.7	2	2.6	1	6.7	1	6.7
Bitot Spot	2	2.6	2	2.6	2	13.3	2	13.3

When the study was started many of the children were suffering from anaemia, B complex deficiency as well as mild forms of the vitamin A deficiency. The supplementation of B carotene reduced the symptoms in all the experimental children. The consumption of green leafy vegetables also helped them to be free from angular stomatitis and bleeding gums. However no change was observed in the case of the children in the control Group F.

F. <u>Changes in blood haemoglobin levels</u>:

The blood 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 IX with the individual values in Annexure G.

TABLE IX

MEAN INITIAL AND FINAL HAEMOGLOBIN LEVELS OF THE TARGET CHILDREN

Group	Initial haemoglobin level (g/100ml)	final haemoglobin level (g/100ml)	Difference (g/100ml)	Groups compared	't' value
A	10.84 ± 1.35	11.61 ± 1.24	0.77 ± 0.35	A vs B	0.35
				C	0.48
				D	0.06
B	10.93 ± 1.39	11.77 ± 1.54	0.84 ±0.072	E	0.05
				F	0.55
				B vs C	0.39
C	10.41 ± 1.43	11.26 ± 1.49	0.85 ±0.091	D	1.72
				E	0.48
D	11.17 ± 1.04	12.07 ± 1.16	0.90 ±0.08	F	0.73
				C vs D	1.23
				E	0.04
E	10.68 ± 1.50	11.46 ± 1.67	0.78 ±0.069	F	0.29
				D vs E	1.32
				F	0.97
				E vs F	0.29
F	9.65 ± 0.70	9.23 ± 0.98	-0.42 ±0.083		

Judged by the WHO standards (Davidson _et al_, 1973) all the selected children were found to be anaemic in the beginning of the study but towards the end there was an increment in the blood haemoglobin levels in all the children receiving the B carotene supplementation. The mean increments observed for the five experimental groups was 0.77, 0.84, 0.85, 0.90 and 0.78 g/100ml respectively. The results corroborate with the findings at the National Institute of Nutrition (1977) which has suggested an important role of vitamin A in haemopoiesis. It has been reported that the daily supplements of vitamin A resulted in a significant increase in the levels of haemoglobin from 9.3 to 9.9 g/100 ml in children.

The children in Group E, though registered the highest B carotene absorption did not record the highest increase in blood haemoglobin levels. This might be due to the fact that the green leafy vegetables not only supplied B carotene but also considerable quantities of iron and ascorbic acid, the two important haemopoietic agents.

When the mean changes in the blood haemoglobin levels was compared between the groups none of the differences were statistically significant.

Thus it could be concluded that the supplementation of β carotene through the fresh and dried forms of green leafy vegetables or standard β carotene preparation in the diets of preschool children would improve the vitamin A nutritional status of children within a short period of two and a half months. Hence the leafy vegetables could be used to reduce the menace of vitamin A deficiency in the developing countries.

V SUMMARY AND CONCLUSION

The present investigation was aimed at investigating the biological utilization of B carotene from fresh and dried genugreek and drumstick leaves on preschool children. Seventy five preschool children of the ge group four to five years were selected from a village Vadamadurai in the Perinaickanpalayam Block and divided into five groups of 15 each based on their mean initial heights, weights and socio economic levels. The target ch ldren were participating either in the balwadi fe ding programme or the CARE feeding programme and received 80g. Balahar per day in the form of uppuma. The four experimental groups, namely A, B, C and D received 1200 ug of B carotene through fresh fenugreek and drumstick leaves and dried fenugreek and drumstick leaves respectively, in the form of a kootu. The children in Group E were supplied with the same quantity of standard B carotene in the form of a pharmaceutical preparation. Another group of 15 children (Group F) not receiving any supplementation served as a control. The feeding was carried out for a period of two and a half months. Diet and nutrient intake of the children, changes in serum vitamin A levels, absorption of

B carotene, changes in the clinical picture of children and their blood haemoglobin levels served as the criteria for the evaluation of the study. The results of the study revealed the following:

1. The home diets of all the children supplied an adequate quantity of cereals but consumption of all the other foods were deficient. As a result the diets were found to be deficient in calories with a calorie gap of 26.1 per cent. The dietary intake of B carotene of all the children was found to be only 137 ug from their home diet, as against the recommended allowance of 750 ug per day.

2. The children with a lower dietary intake of B carotene and vitamin A had a lower body pool of vitamin A initially, reflected by less than 14 ug of vitamin A/100ml serum.

3. Supplementation of the diets with 1200 ug B carotene resulted in an increase in the concentration of serum vitamin A levels irrespective of the source of supplement.

The increments ranged from 7.4 ug/100 ml in the group fed with dried fenugreek leaves to 8.63 ug/100 ml in the group fed with the standard B carotene. In all the groups except the control group the final serum vitamin A levels were significantly greater than that of initial levels at five per cent level.

4. Though the children receiving the standard B carotene registered the highest increment in B carotene levels the differences between the groups were not statistically significant. However the children in the control group recorded a significantly lower serum vitamin A level than that of all the experimental groups at one per cent level.

5. The intake of B carotene was found to be positively correlated with the serum vitamin A levels. The correlation was + 0.83.

6. The children in Group E fed with the standard B carotene registered the highest rate of absorption being 84.58 per cent which was significantly greater than that of all the other groups at one per cent level. Whereas the control group children registered a significantly lower absorption of B carotene (55.59 per cent) than all the other test groups except C. The absorption of B carotene for the Groups A, B, C and D were found to be 55.10, 60.99, 57.69 and 58.35 per cent respectively.

7. Initially, the vitamin A deficiency symptoms such as night blindness, conjunctival xerosis and bitot spots were observed in all the children along with the symptoms of anaemia and B complex deficiency. After the supplementation of B carotene for a period of two and half months improvement in the clinical picture was noticed in all the children but no improvement or change was observed in the children of the control group.

8. There was an increment in the mean blood haemoglobin level in all the children receiving the B carotene

supplementation. The mean increments observed for the Groups A, B, C, D, E and F were 0.77, 0.84, 0.85, 0.90, 0.78 and -0.42 g/100 ml respectively. The difference observed between the groups were not statistically significant.

It could be concluded that the supplementation of the diet of preschool children with green leafy vegetables or standard B carotene of a pharmaceutical preparation for as short a period as two and a half months could greatly improve the vitamin A nutritional status of children. When the quantities of B carotene were kept constant the two sources of leafy vegetables did not show any significant difference in the utilization of B carotene and nor was any significant difference observed between fresh and dried forms of leafy vegetables. Though the B carotene absorption rate was the highest in the children fed standard B carotene, the children receiving green leafy vegetables could obtain additional quantities of minerals and other vitamins which was reflected in their blood picture. It should be noted that the consumption of leafy vegetables are less expensive when compared with the pharmaceutical preparations. Hence the addition of green leafy vegetables should be greatly encouraged in all the feeding programmes. People should be educated to include the leafy vegetables in their regular home diets. This is an easy workable solution to reduce the prevalence of nutritional blindness.

Further investigations should be carried out along this line to explore the biological utilization of all the locally available leafy vegetables. Studies on the effect of supplementing protein and vitamin E at different levels along with the B carotene through leafy vegetables would throw more light on the subject.

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ANNEXURES

ANNEXURE A

HEIGHT AND WEIGHT OF THE SELECTED CHILDREN

No.	Group A		Group B		Group C		Group D		Group E		Group F	
	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)
1.	105.0	13.5	102.0	13.5	101.5	13.0	99.0	14.5	98.0	13.0	97.5	13.5
2.	96.0	12.5	96.0	13.5	96.0	12.0	96.5	13.0	97.0	12.5	96.5	12.0
3.	95.0	13.0	94.0	12.0	94.0	12.5	94.0	13.5	94.0	12.0	93.5	12.5
4.	92.5	11.5	93.0	15.0	93.0	12.0	93.0	12.5	93.5	11.5	93.5	11.0
5.	92.0	11.0	92.0	11.5	92.0	13.5	92.0	12.0	91.5	12.5	92.0	12.0
6.	90.0	12.5	91.0	11.0	91.0	13.0	91.0	12.0	91.0	11.0	91.0	11.5
7.	90.0	12.0	89.5	13.0	89.0	11.0	89.0	11.5	89.0	11.5	89.5	11.5
8.	87.0	12.0	87.0	11.5	88.0	11.0	88.0	12.0	88.0	11.5	88.0	10.5
9.	87.0	11.5	87.0	11.0	87.0	11.5	86.0	10.5	86.0	10.0	87.5	10.0
10.	85.0	11.0	86.0	11.0	85.0	11.5	85.0	11.5	85.0	11.0	86.0	11.0
11.	84.5	11.5	84.0	10.0	84.0	11.0	83.5	10.0	82.5	12.5	84.0	11.5
12.	80.0	11.5	81.0	11.5	81.5	10.0	82.0	9.0	82.5	11.5	82.5	12.0
13.	79.5	10.0	79.0	10.5	78.5	10.5	78.0	9.5	78.0	10.5	81.5	10.5
14.	75.5	10.5	76.5	9.5	77.0	9.5	77.0	10.5	77.5	10.5	79.0	10.0
15.	74.0	11.0	73.5	10.0	73.0	10.0	72.5	10.0	72.0	9.5	80.0	11.5

ANNEXURE B

ESTIMATION OF SERUM VITAMIN A (RETINOL)

The extraction procedure was done, followed the method of Neeld and Pearson (1963).

Reagents

(i) Alcoholic KOH 0.5%: 5.6 g. of potassium hydroxide was was dissolved in 100 ml of purified alcohol.

(ii) N Hexane

(iii) Chloroform

(iv) Stock vitamin A solution: 344 mg of vitamin A acetate. was dissolved in chloroform and made upto 100ml. 1ml of stock contains 300mg of retinol.

Intermediate Standards

1. 0.1 ml of stock diluted to 100 ml : 3ug/ml
2. 0.1 ml of stock diluted to 50 ml : 6ug/ml
3. 0.15ml of stock diluted to 50 ml : 9ug/ml
4. 0.1 ml of stock diluted to 25 ml : 12ug/ml

Working Standard

Each intermediate was again diluted in the ratio 1:1 0 and from each standard finally 1.0ml was taken and 2.0ml of chloroform was added and read at 620 in Beckman Du Spectrophotometer.

Procedure

The serum (0.5 ml of less) is saponified with an equal volume of 1 N ethanolic potassium hydroxide in a water bath at 60°C for 20 minutes. The mixture was cooled and vigorously shaken in a glass-stoppered tube with an equal volume (1ml) of N hexane for 10 minutes. The tube is centrifuged for 1 minute at 100g to separate the layers. An aliquot (0.8 ml) of the N hexane layer is pipetted off for determination of retinol. The N hexane is evaporated from this aliquot in a water bath at 60°C in a stream of oxygen-free nitrogen. The last traces of N-hexame are removed by nitrogen blowing at room temperature. The residue is taken up in (0.5 ml) chloroform; 1 drop of acetic anhydride is added followed by (0.1 ml) trifluoacetic acid. The mixture was shaken vigorously and the optical density at 620 mu is determined exactly 30 seconds after addition of the trifluoroacetic acid.

ANNEXURE C

DAILY FOOD CONSUMPTION OF THE TARGET CHILDREN

Group	Cereals (g)	Proteins (g)	Green leafy vegetables (g)	Other vegetables(g)	Fruits (g)	Milk (g)	Fats and oil(g)	Sugar and jaggery (g)
A								
1.	235	23	12	42	16	41	13	23
2.	225	23	17	40	18	40	16	17
3.	205	27	16	39	13	39	17	20
4.	210	24	14	43	11	42	15	19
5.	200	22	18	36	17	38	14	22
B								
1.	238	19	13	33	10	31	13	19
2.	206	26	16	30	12	28	10	26
3.	212	17	17	35	9	33	14	18
4.	227	27	15	30	8	28	12	27
5.	217	21	14	32	11	30	11	20

Group	Cereals (g)	Proteins (g)	Green leafy vegetables (g)	Other vegetables (g)	Fruit (g)	Milk (g)	Fats and oil (g)	Sugar and jaggery (g)
C								
1.	235	26	48	46	7	28	9	21
2.	215	25	12	43	6	27	10	16
3.	205	29	11	44	9	23	7	20
4.	215	27	9	45	8	25	13	18
5.	220	26	10	42	10	22	11	20
D								
1.	235	23	6	43	11	35	43	22
2.	217	20	10	41	13	34	41	21
3.	192	19	7	39	10	37	38	18
4.	208	16	9	42	9	33	42	22
5.	210	22	8	40	12	36	36	17

Group	Cereals (g)	Proteins (g)	Green leafy vegetables (g)	Other vegetables (g)	Fruits (g)	Milk (g)	Fats and oil (g)	Sugar and jaggery (g)
E								
1.	238	28	13	36	7	43	24	21
2.	204	25	12	33	10	41	21	22
3.	214	23	10	38	11	38	20	19
4.	229	22	11	33	13	42	17	18
5.	215	27	14	35	9	36	23	20
F								
1.	195	22	12	41	--	22	21	12
2.	192	15	9	39	--	21	14	17
3.	172	21	8	42	--	18	20	16
4.	184	18	14	40	--	17	17	14
5.	192	19	7	38	--	22	18	18

ANNEXURE D

CALORIES, PROTEIN, IRON AND B CAROTENE INTAKE OF THE SELECTED CHILDREN

Group	Energy (cal)	Proteins (g)	Iron(mg)	B Carotene (ug)
A				
1.	1098	19.68	12.64	129
2.	1288	23.14	14.29	141
3.	997	23.00	10.64	146
4.	1254	22.80	12.63	122
5.	1133	21.08	12.65	149
B				
1.	1213	25.87	15.18	281
2.	1110	22.89	11.96	97
3.	1207	24.61	10.41	72
4.	1420	24.50	15.43	704
5.	1310	24.88	13.45	119
C				
1.	1100	20.03	11.46	107
2.	1212	23.78	13.64	59
3.	1020	27.69	12.50	67
4.	1103	22.64	13.40	52
5.	1200	21.86	11.50	84

Group	Energy (cal)	Proteins (g)	Iron(mg)	B Carotene (ug)
D				
1.	1242	22.80	11.21	74
2.	1133	25.00	13.55	84
3.	1065	24.20	12.75	231
4.	1281	17.20	11.86	52
5.	1399	16.30	8.40	58
E				
1.	1260	21.62	10.35	111
2.	1465	24.88	11.92	98
3.	1163	23.74	12.39	504
4.	1122	24.40	12.80	165
5.	1240	23.06	11.69	66
F				
1.	1230	16.90	10.35	85
2.	1125	17.20	11.93	90
3.	1095	18.90	9.94	83
4.	1105	19.10	10.76	97
5.	945	19.80	11.57	95

ANNEXURE E

SERUM VITAMIN A LEVELS OF THE SELECTED CHILDREN BEFORE AND AFTER SUPPLEMENTATION(ug/100ml)

Group	Initial	Final	Difference
A			
1.	15.00	21.90	6.90
2.	14.40	22.40	8.00
3.	18.00	24.10	6.10
4.	7.50	15.55	8.05
5.	14.00	22.75	8.75
B			
1.	11.25	20.25	9.00
2.	18.00	25.80	7.80
3.	13.75	22.00	8.25
4.	15.75	22.50	6.75
5.	7.50	16.40	8.90
C			
1.	15.00	23.70	8.70
2.	18.00	25.80	7.80
3.	11.30	19.20	7.90
4.	15.00	21.75	6.75
5.	8.13	14.00	5.87

Group	Initial	Final	Difference
D			
1.	15.00	23.90	8.90
2.	18.00	24.70	6.70
3.	11.30	20.30	9.00
4.	15.00	22.45	7.45
5.	8.13	16.95	8.82
E			
1.	18.00	26.10	8.10
2.	15.0	23.70	8.70
3.	13.85	22.60	8.75
4.	11.25	20.15	8.90
5.	15.00	23.70	8.70
F			
1.	7.50	5.80	1.70
2.	11.35	9.50	1.85
3.	15.00	13.75	1.25
4.	7.50	6.25	1.25
5.	11.30	9.80	1.50

ANNEXURE F

TOTAL PERCENTAGE ABSORPTION OF B CAROTENE IN THE SIX GROUPS OF CHILDREN

Group	B Carotene Intake (ug)			B Carotene excreted through faeces(ug)	B Carotene absorbed (ug)	Percentage of B carotene absorbed
	Home diet	Supplement	Total			
A						
1.	129	1200	1329	506	778	58.57
2.	141	1200	1341	497	793	59.11
3.	146	1200	1346	482	784	58.25
4.	122	1200	1322	501	766	57.83
5.	147	1200	1347	493	793	58.92
B						
1.	281	1200	1481	468	903	61.00
2.	97	1200	1297	464	795	61.33
3.	72	1200	1272	459	763	60.00
4.	704	1200	1904	462	1171	61.50
5.	119	1200	1300	466	803	61.17

Group	B Carotene Intake (ug)			B Carotene excreted through faeces(ug)	B carotene absorbed (ug)	Percentage of B carotene absorbed
	Home diet	Supplement	Total			
C						
1.	107	1200	1307	495	768	58.75
2.	59	1200	1259	515	719	57.08
3.	67	1200	1267	5U5	734	57.92
4.	52	1200	1252	518	712	56.83
5.	84	1200	1284	511	737	57.42
D						
1.	74	1200	1274	597	754	59.17
2.	84	1200	1284	615	749	58.35
3.	231	1200	1431	6U2	834	58.25
4.	52	1200	1252	609	724	57.83
5.	58	1200	1258	619	726	57.83

Group	B Carotene Intake(ug)			B Carotene excreted through faeces(ug)	B Carotene absorbed (ug)	Percentage of B carotene absorbed.
	Home diet	Supplement	Total			
E						
1.	111	1200	1311	137.2	1162	88.6
2.	98	1200	1298	191.5	1090	84.0
3.	504	1200	1704	171.5	1464	85.9
4.	166	1200	1366	163.0	1180	86.4
5.	66	1200	1266	183.0	1072	84.7
F						
1.	85	--	85	58.78	45	52.50
2.	90	--	90	39.09	52	57.70
3.	83	--	83	37.81	46	54.82
4.	97	--	97	29.96	54	55.82
5.	95	--	95	43.09	54	57.10

ANNEXURE G

HAEMOGLOBIN LEVELS OF THE SELECTED CHILDREN BEFORE AND AFTER SUPPLEMENTATION

No.	Initial levels (g/100ml)						Final levels (g/100ml)					
	A	B	C	D	E	F	A	B	C	D	E	F
1.	12.96	8.92	11.23	11.80	9.21	8.78	13.05	9.72	12.08	12.64	10.04	7.83
2.	9.21	9.94	8.50	12.53	10.94	9.07	10.21	10.86	9.52	13.40	11.73	8.14
3.	9.79	10.94	12.38	9.72	8.78	9.50	10.60	11.72	13.15	10.65	9.67	8.64
4.	10.51	12.09	10.87	11.59	12.74	10.22	11.55	13.00	11.64	12.68	13.53	9.50
5.	11.74	12.74	9.07	10.22	11.74	10.66	12.65	13.48	9.92	11.13	12.71	9.77