VITAMIN A STATUS AND THE EFFECT OF ORAL SUPPLEMENTATION IN PREGNANT NIGERIAN WOMEN

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A THESIS IN THE DEPARTMENT OF HUMAN NUTRITION SUBMITTED TO THE FACULTY OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICINE IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE

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C E R T I F I C A T I O N

I CERTIFY THAT THIS STUDY HAS BEEN CARRIED OUT BY

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ABSTRACT

This study was designed to investigate vitamin A nutritional status of pregnant and non pregnant non lactating Nigerian women. The beneficial effects of oral vitamin A supplementation was also investigated in the pregnant women.

The study was carried out in three phases: Phase one was the cross-sectional study carried out on 22, 88 and 61 pregnant women in the 1st, 2nd and 3rd trimesters. The controls were 35 non pregnant non lactating women in the proliferative phase of the menstrual cycle. Their ages ranged from 18 to 45yrs with a mean age of 27.8+/- 6.82yrs. The subjects were randomly selected from both the University teaching and Adeoyo hospitals, Ibadan and the study lasted for a period of nine months.

The result of the study showed that 11% of the subjects had plasma vitamin A levels in the deficient range (< 20ug/dl) while 60% had marginal values (20 - 29ug/dl). Plasma vitamin A levels was observed to decrease as pregnancy progressed (P<0.05). The levels of β-carotene were however observed to be in the normal range.

The second phase of the study was designed to determine the adequacy of vitamin A in the body using the relative

dose response test (RDRT) technique. Thirty pregnant women at different trimesters of pregnancy and ten non pregnant non lactating women were studied for a period of five weeks. 13.6% of the subjects had RDRT values greater than 20% which is indicative of liver store less than 20ug/g. This level is associated with deficiency of vitamin A.

The longitudinal study was the third phase of the study. This phase spanned a period of 18months. Twenty eight pregnant women were supplemented with either oral vitamin A or lactose in gelatin capsule (placebo) from the 14th week of pregnancy until 6weeks postpartum. Vitamin A supplementation maintained the packed cell volume (PCV), increased both the plasma vitamin A and retinol binding protein (RBP) levels in the vitamin A supplemented subjects. Though the neonates of mothers supplemented with vitamin A had higher higher birth weights, plasma and retinol binding protein levels when compared with the controls, the difference was however not significant. The levels of the plasma proteins were observed to decrease significantly during labour and immediately postpartum.

Proximate analysis of the meals (as consumed) of the

pregnant mothers in the longitudinal study revealed that the pregnant women in this environment met only 47% of their daily requirement for Vitamin A.

DEDICATION

THIS THESIS IS DEDICATED TO THE GLORY OF GOD AND TO MY LOVED ONE.

Iyabode Adeyefa, 1991.

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Iyabode, 1991.

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LIST OF ABBREVIATIONS

mm	millimetre
cm	centimetre
ug	microgram
<	less than
>	greater than
%	percentage
fig	figure
a	alpha
B	beta
Y	gamma
g	grammes
kg	kilogramme
min	minutes
hr	hour
ed	edition
inc	incorporated
yrs	years

LIST OF ACRONYMS

B-C	Beta carotene
a-C	alpha carotene
Y-C	gamma carotene
VIT.A	Vitamin A
ALB	Albumin
RBP	Retinol Binding Protein
TTR	Transthyretin
PCV	Packed Cell Volume
CSS	Cross Sectional Study
LS	Longitudinal Study
RDRT	Relative Dose Response Test
SD	Standard Deviation
S	Subjects
C	Controls
I.U	International Unit.

C H A P T E R - O N E

I N T R O D U C T I O N

Although the importance of Vitamin A in Nutrition has been known for almost a century yet its role in metabolism is poorly understood.

A number of metabolic roles have been ascribed to the Vitamin - these include its functions in vision, growth, reproduction, maintenance of epithelial cells and immune properties. The specific role it plays in each case however has not been fully explained except for its well elucidated role in vision (Wald, 1960).

The role of Vitamin A in reproduction has been the focus of attention by various workers (Moore, 1957; Thompson et al, 1964; Howell et al, 1964; Bodansky et al, 1942; Pullium et al, 1962). Severe deficiency of Vitamin A has been found to cause infertility in all vertebrate species studied.

The consequences of the deficiency ascribable to Vitamin A range from a) disruption of the oestrous cycle and b) permanent keratinization of the vagina c) necrosis of the functional zone of the placenta, and d) foetal mishaps such as resorption, stillbirths and various

congenital malformations. The failure of fertilization or implantation are uncommon features of vitamin A deficiency (Bates. 1983). At the other extreme hypervitaminosis A is also associated with an increased risk of congenital malformation (Conlan. 1953: Bates,1983).

Earlier. the function of retinol could not be ascertained until evidence was adduced to demonstrate that retinoic acid would correct the non-specific effects of vitamin A deficiency except those of vision and reproduction (Thomson et al. 1964: Howell et al. 1964).

Various mechanisms have been postulated to explain the observed reproductive functions of vitamin A. These include effects in steroid hormonogenesis. It has been demonstrated that vitamin A (1) improved the reproductive performance of female rabbits (Hay and Kendall. 1956) (2) helped in the conversion of pregnenolone to progesterone in female rats (Coward. et al 1966) (3) Increased the ovarian secretion of progesterone. 20α -hydroxy-preg-one-3-one and pregnenolone in pregnant rats (Ganguly et al: 1971ab) and (4) supported survival of fetus and lactation in the dams (Ganguly et al. 1971ab).

Thus retinol deprivation probably can cause a disturbance of steroid hormone production although the extent to which this leads to the overall impairment of reproductive capacity is not clear.

It has been observed that when pregnancy is allowed to proceed to term in rabbits and pigs which are receiving marginal amounts of vitamin A just sufficient to prevent total resorption of fetuses a high incidence of congenital malformations is observed. The type of defects depends on the timing and duration of the deficiency (Wilson et al 1953; Pallauah, 1966). O'Toole et al. (1974), in a study of the effect of hypovitaminosis A on eight Rhesus monkeys observed abortions, and Xerophthalmia at birth but not congenital malformation. It was then suggested that primate embryoes may present with milder forms of vitamin A deficiency signs when compared to other mammals. A few instances of congenital malformations possibly attributed to vitamin A deficiency have been reported in human subjects (Bates, 1983). The evidence that uncomplicated vitamin A deficiency can be teratogenic in human is however inconclusive.

Thus it is apparent that there is a critical amount of vitamin A required for successful reproduction. Hume and Krobe (1949) demonstrated that pregnancy improved an

increased demand for vitamin A. This observation has been confirmed by other groups (Howell et al. 1964; Thomoson et al. 1964). However the exact amount of vitamin A required in order to meet optimal needs varies with the different physiological states. The adequacy of vitamin A intake during pregnancy will depend on the prevalent food cultures as dictated by the geographical zones of the world.

MATERNAL STATUS OF VITAMIN A DURING PREGNANCY

A number of studies have described night blindness and impaired dark adaptation in pregnant women receiving a diet inadequate in its vitamin A content (Rodriguez and Irwin, 1972). Also, a decrease in plasma retinol levels during the course of pregnancy, and an increase post partum has been reported (Fulliam et al 1962, Venkatachalam et al 1962; McGanity et al. 1969, Edozien et al 1976). The observed increase in plasma vitamin A levels post partum however is not sustained and values may fall to deficient levels if intake is not increased (Lund and Kimble 1943).

Studies have also revealed the inadequacy of the intake of vitamin A and its precursors amongst women in developing countries (Rodriguez and Irwin, 1972; Bates, 1983; Le Francois et al. 1981).

The control of plasma vitamin A levels during pregnancy clearly differs from that of the other lipid soluble components of the blood. While vitamin A level decreases in pregnancy that of vitamin E and other lipid soluble substances increases (Knoop et al. 1978). This is by virtue of vitamin A association with retinol binding protein (RBP) and transthyretin (TTR). In pregnancy. the decrease in Vitamin A usually parallels the decrease in serum albumin (Hytten and Leitch 1971). It was therefore suggested that both albumin and RBP production may be controlled the same way. It is however not clear whether the drop in plasma Vitamin A during pregnancy is as a result of inadequate intake. haemodilution of pregnancy. increased demand in the foetuses or a conservation mechanism invoked to preserve the liver vitamin A for subsequent use during lactation.

TRANSFER OF VITAMIN A FROM MATERNAL STORES TO THE FOETUS

In the rat. the foetal liver accumulates a small but remarkably constant amount of vitamin A during gestation despite wide variations in maternal intake and stores (Moore, 1971).

Takahashi et al. (1975) found that the concentration of vitamin A in the liver of neonates increased 1.4 fold when maternal concentrations increased 100 fold.

However at very low maternal intakes, the liver A concentration in the neonates was observed to be substantially reduced.

Takahashi et al. 1977 also found that accumulation of vitamin A in the conception followed a complex pattern. During the early stages (day 7 - 9) the vitamin accumulated to a high concentration in the placenta. From day 9 - 11, the concentration fell abruptly to less than 20% of the initial peak and during days 11 - 14, both vitamin A and RBP accumulated in parallel. During days 16 - 20 the fetal liver started to synthesize RBP and accumulated vitamin A and foetal stores increased. Gal and Parkinson (1974) showed that there was a reduction in the plasma vitamin A levels in pregnancy in the early 5th to 9th weeks and after the 36th week of gestation. Though they attributed this to the effect of circulating sex hormones, the trend they observed follows the observation made by Takahashi et al (1971). It revealed that the timing of the drop in plasma vitamin A levels in the mothers corresponded to the period of increased concentrations of vitamin A in the foetus.

These studies are consistent with the observation that the supply of Vitamin A to the foetus is from the retinol RBP complex from maternal stores via the

maternal blood (Bates 1983).

Human cord plasma retinol levels have however been shown to be lower than the corresponding maternal levels (Lewis et al. 1947: Venkatachalam et al. 1962) except when maternal levels are very low in which case the relationship may be reversed (Mclaren and Ward, 1968. Rodriquez & Irwin. 1972).

EFFECT OF VITAMIN A SUPPLEMENTATION IN PREGNANCY

Several attempts have been made to solve the problem of vitamin A deficiency in pregnancy through oral supplementation with vitamin A ranging from 650 - 9000ug daily with varying degrees of successes.

Lewis et al. (1947) found that 3000ug vitamin A or the equivalent amount of carotene given daily during the last trimester of pregnancy, had no effect on plasma retinol levels in the neonates but significantly increased the levels in the mothers. In Cows and pigs, large doses of vitamin A supplied to the mother increased the extent of placental transfer to a moderate large doses of carotene in contrast were entirely without effect on foetal vitamin A stores and very little per se was transferred to the foetal plasma.

Venkatachalam et al (1962), gave 9000ug vitamin A per day throughout that last trimester of pregnancy to

twelve malnourished Indian women who apparently had very low dietary sources, and observed significant higher cord levels in them than in the unsupplemented controls. Lund and Kimble in 1943 also showed that the administration of 10,000iu/day of vitamin A brought about a maintenance of normal levels of the vitamin in the mother's blood throughout the course of pregnancy. They observed that an amount greater than this level was without benefit under normal conditions.

RATIONALE FOR THE STUDY

Vitamin A is an essential nutrient for normal growth and development. Since pregnancy is a time of active growth for both the mother and the foetus this period therefore deserves special attention.

Chronic vitamin A deficiency plagues many developing regions of the world with its tragic consequences such as increased morbidity and mortality, different stages of eye affectations and blindness observed mostly in children. Milder forms of vitamin A deficiency have also been observed in pregnant women (Venkatachalam et al. 1962). In the Nigerian situation, vitamin A deficiency has been identified in the Northern part of the country (Thompson et al, 1964; Oomen, 1971; Standford-Smith, 1979) and especially in children under 5 years of age. Olurin, (1970) and Animashaun (1978) also reported cases

of xeroophthalmia in children under 5yrs of age in Ibadan and Lagos respectively. All these studies were conducted with reference to the paediatric age group, hospital based and disease related.

Information on the vitamin A status of women in the reproductive age group in Nigeria is non existent. However studies from other countries have shown that pregnant women especially during the last half of pregnancy may be vitamin A deficient therefore requiring supplementation (Lund and Kimble, 1943; Venkatachalam et al. 1962).

The adequacy of vitamin A for both mother and foetus during pregnancy and for the neonate postnatally is dependent on the prepregnancy vitamin A status of mothers as well as intake during pregnancy.

Studies have shown that a diet adequate for nonpregnant non lactating women was also adequate during the first trimester of pregnancy. During the second half of pregnancy the diet became inadequate and needed supplementation in order to maintain the plasma levels of the vitamin in the mothers (Lund and Kimble, 1943).

There is also evidence in the literature to show that the transfer of vitamin A from the mother to the foetus is dependent on the maternal vitamin A status.

This observation derives from a comparative study of the plasma vitamin A levels of Swedish and Ethiopian mother and liver vitamin A of their foetuses at autopsy. The Swedish mothers had significantly higher plasma vitamin A levels than the Ethiopian counterparts. Also, the Swedish foetuses had liver vitamin A reserves sufficient for 2 months while the Ethiopian foetuses had levels sufficient for only 5 days (Gebre-Medhin and Vahlquist, 1984). This suggests both the capability of the early infant to build stores as well as the influence of the mother's vitamin A status on the infant.

It is highly tempting to speculate that the protection from childhood diseases which vitamin A seems to offer (Feachem, 1987) will be ensured if adequate liver reserves are maintained in utero. Thus this study is therefore addressed to determining the vitamin A status of pregnant Nigerian women taking into consideration the prevalent food cultures, the existing food items and the low purchasing power of a larger percentage of the population; and also to assess the possible benefits of supplementation in pregnancy.

Vitamin A status exists in a continuum between clinically evident deficiency and toxicity. Information on the intermediate level of habitual intake (especially marginal intake and status) is highly essential so as to

plan for adequate intervention programmes.

HYPOTHESIS

1. Plasma vitamin A levels below 20ug /dl is indicative of inadequate liver store and diagnostic of pre-clinical vitamin A deficiency.

2. The average Nigerian diet is inadequate in its vitamin A content and therefore will not provide the extra Vitamin A that pregnancy requires.

AIMS AND OBJECTIVES

1. To establish a range of values for plasma Vitamin A in a control population of apparently healthy normally menstruating non-pregnant non-lactating adult Nigerian women; and to assess the liver vitamin A status using the relative dose response test.

2. To assess the vitamin A status of healthy pregnant women in Nigeria by conducting:

(a) A cross sectional study in different trimesters of pregnancy;

(b) Vitamin A absorption test in pregnant women during different trimesters.

3. To study the effect of supplementation of pregnant mothers with Vitamin A as measured by plasma Vitamin A levels in both the mothers and the babies.

4. To assess the dietary intake of Vitamin A and its precursors in the pregnant Nigerian women.

C H A P T E R T W O

L I T E R A T U R E R E V I E W

HISTORICAL BACKGROUND

In 1913, McCollum and Davies observed "fat soluble A" growth promoting factor isolated from animal fats and fish oils. They showed that animals fed a diet consisting mainly of polished rice, casein and minerals did not develop normally unless this factor was added. Drummond (1920) suggested that the "fat soluble A" be named vitamin A.

Vitamin A activity was later found in plant materials and was associated with the yellow carotenes present in plants (Steenbock and Boutwell (1920).

Moore in 1930, demonstrated that the carotenes were structurally related to vitamin A and were converted in vivo to the vitamin. Thus the provitamin status of B-carotene and certain other carotenoids was established.

The structural formula of vitamin A and B-carotene were first proposed by Karrer et al in 1930-31 and reported by Goodhart and Shills, 1974. Isler et al, synthesized the first pure vitamin A in 1947 while B-carotene was synthesized in 1950 by Karrer et al. and Inhofen et al.

2.1 NOMENCLATURE AND PROPERTIES OF VITAMIN A AND PROVITAMIN

Vitamin A is a generic term used for all compounds, related chemically, that exhibit the biological activity of retinol. The major naturally occurring form is vitamin A alcohol (retinol) fig. 2-1. Other forms are Vitamin A acid (retinoic acid) (fig.2-2) and Vitamin A aldehyde (retinal) (fig.2-3). In general retinol is used synonymously with Vitamin A (Goodhart & Shills, 1974). Retinoids on the other hand include both the natural forms of vitamin A and synthetic analogs with or without biological activity of retinol. One I.U of vitamin A is 0.3 ug all-trans retinol.

Retinol equivalent is used to convert all sources of vitamin A and carotenoids substances in the diet to single units One ug of retinol is biologically equivalent to 6 ug of β-carotene (β-C and 12 mg of mixed dietary carotenoids.

Vitamin A occurs in two common forms. Vitamin A-1 or retinol, the most common in mammalian tissues and marine fishes, and vitamin A-2 or retinol-2 common in fresh water fishes. Both are isoprenoid compounds (terpenes) containing a six-membered carbocylic ring, and an eleven carbon side chain. Vitamin A activity in mammals is not only found in the retinols but also provided by certain

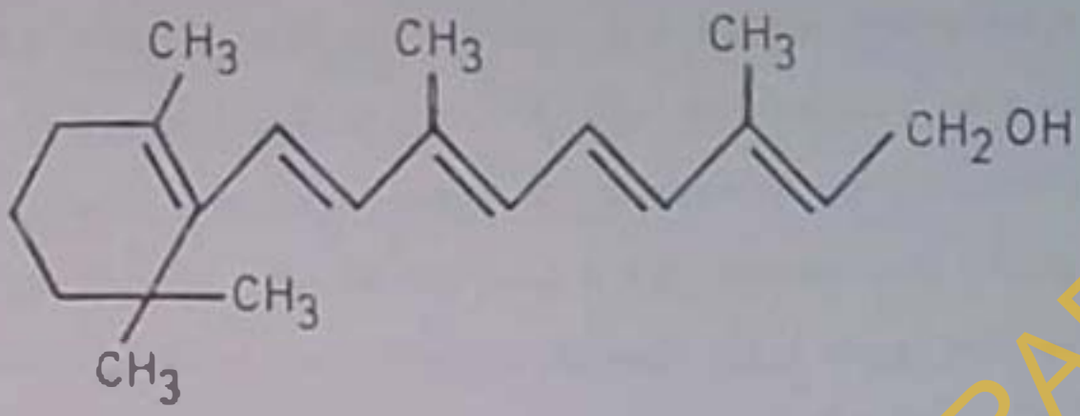


Fig. 2-1 Vitamin A alcohol (retinol)

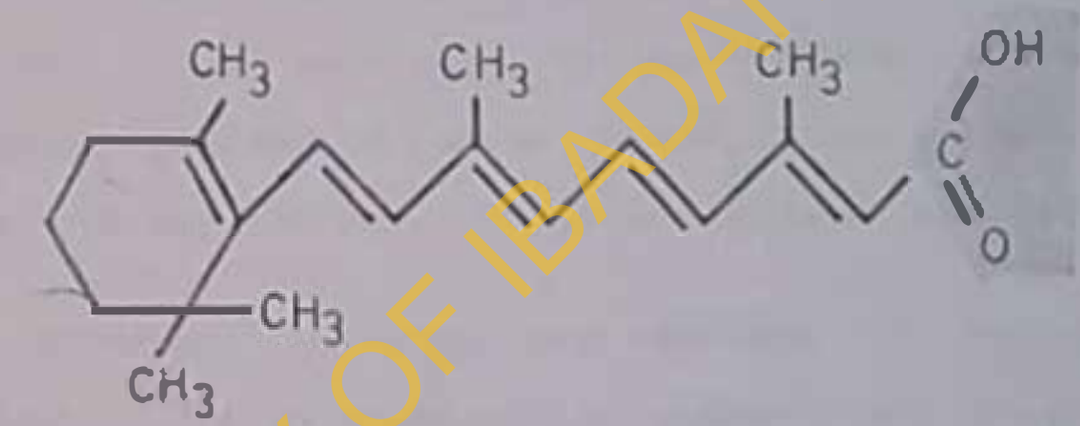


Fig. 2-2 Vitamin A acid (retinoic acid)

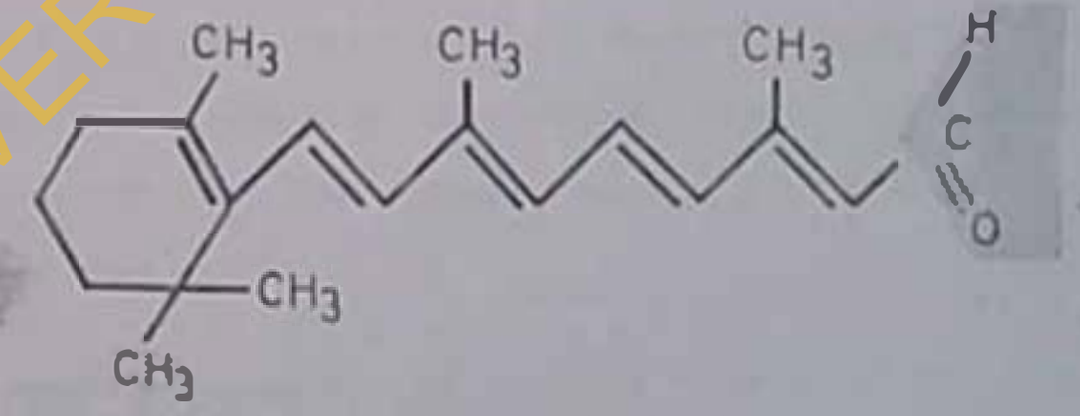


Fig. 2-3 Vitamin A aldehyde (retinal)

carotenoids widely distributed in plants, particularly α, β - γ carotenes. These carotenes have no intrinsic vitamin A activity per se but are converted into vitamin A by enzymatic reactions in the intestinal mucosa and the liver. β-carotene, a symmetrical molecule, is cleaved in its centre to yield two molecules of retinol. Retinol occurs in the tissues of mammals and is transported in the blood in the form of esters of long chain fatty acid (Goodhart and Shills, 1974).

Vitamin A, an organic compound found only in the animal kingdom, is a very pale yellow (almost colourless) substance composed of carbon, hydrogen and oxygen. The vitamin is soluble only in fat and organic solvents.

The carotenoid pigments are composed of carbon and hydrogen. The crystals of the pigments are a deep red colour but they are intensely yellow in solution (Steenbock, 1919).

Vitamin A exists naturally in several isomeric forms. A cis-trans isomerism resulting from configurational differences at the double bonds in the side chain illustrated in fig. 2-4.

The naturally occurring form of Vitamin A is the all-trans isomer. Neo-Vitamin A (13-cis) has about 83 per cent of the potency of the all-trans form, and the 11-

cis isomer (neo-b) has 75 per cent of the biological activity of the all-trans isomer (Goodhart and Shills, 1974). Dehydro-retinol has only about half the biological activity of retinol (Goodhart and Shills, 1974) also exists in various isomeric forms.

Retinol, Retinal and Retinoic Acid

Retinol (vitamin A alcohol) is the most important form of vitamin A. It performs all the known functions of vitamin A since it can be oxidised to the other forms of the vitamin. Retinol is therefore used synonymously with vitamin A.

Retinal (RCHO) is the aldehyde corresponding to retinol. It is the active form of Vitamin A required for vision (Wald, 1960) and certain other functions of Vitamin A (Goodhart and Shills, 1974). The blindness prevented by vitamin A is specific in that the early stages of the blindness can only be treated by the vitamin.

Retinoic Acid (RCOOH) is the corresponding acid to retinol. While it supports growth in Vitamin A deficient animals, it has no role in vision (Dowling and Wald, 1960). Retinoic acid elicits many biological and biochemical responses from cell in vitro including increasing the number of epidermal growth factor receptors on surfaces of cultured cells, stimulation of

differentiation of embryonal carcinoma cells. preventionof the expression of Epstein-Barr virus in virus infected cells and the reversible inhibition of growth of human breast cancer cell lines in long term tissue culture (Martin, 1980).

Retinal and retinoic acid also exist in cis-trans isomeric forms. The structural formulae of retinal and retinoic acid differ only from that of retinol by having another functional group on carbon atom 15 (figs. 2-2 and 2-3).

Carotenoids with Provitamin A activity

Carotenoids exists as a- B- and Y- carotenes and lycopene. B-carotene which is the most important provitamin A of all the carotenoids is a symmetrical molecule containing two B-ionone rings connected by a conjugated chain (fig. 2:5. In a and Y-carotenes, one of B-ionone rings is replace by the structures shown in fig. 2:6. The remainder of the molecules are identical (Goodhart and Shills, 1974).

The biopotency of a- and Y- carotene is about half that of B- carotene (Zechmeister et al. 1949). The biological activity of these carotenoids with provitamin A activity results from conversion to Vitamin A by the organism at carbon atom 15 and 15' with resultant splitting of the molecule (Olson, 1961).

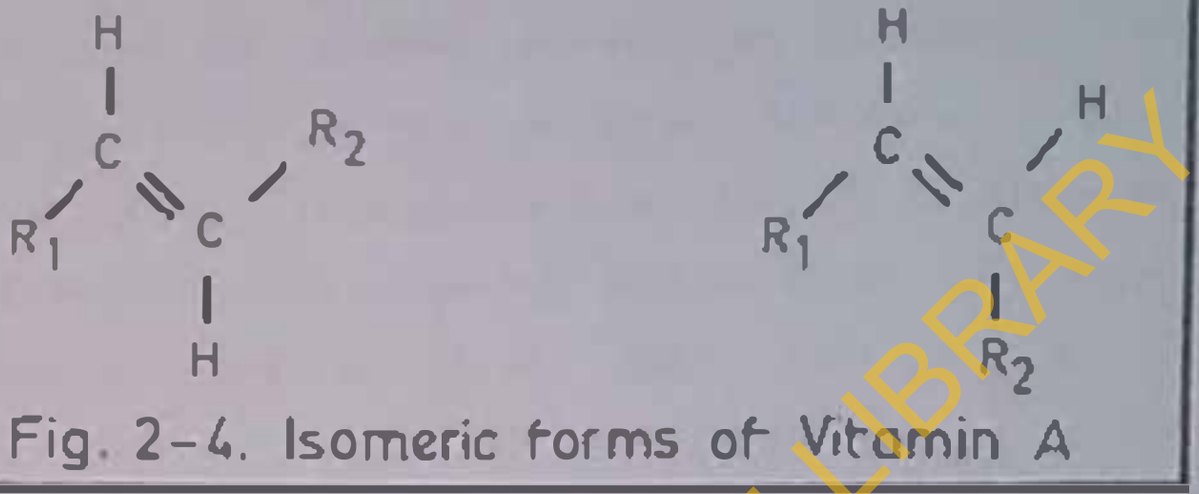


Fig. 2-4. Isomeric forms of Vitamin A

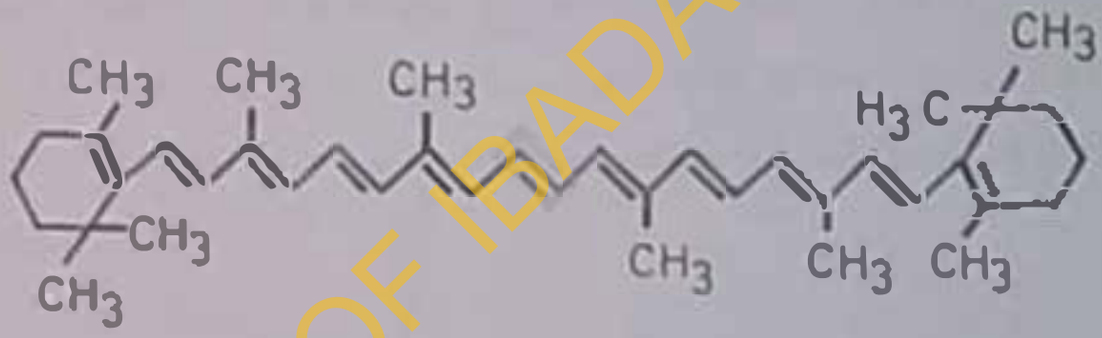


Fig. 2-5. β - Carotene

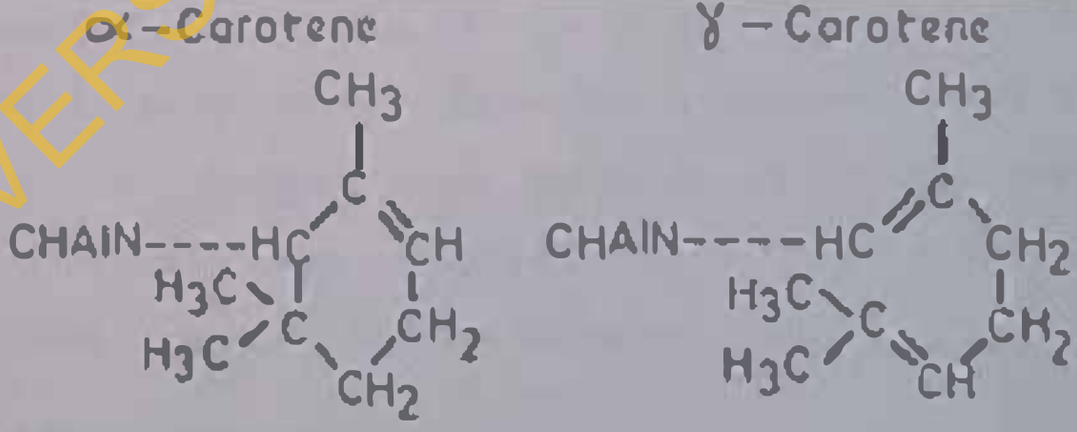


Fig. 2-6. Isomeric forms of β - Carotene

2(ii): GENERAL CHEMICAL PROPERTIES OF VITAMIN A AND PROVITAMIN A

Vitamin A

Retinol melts at 63 to 64° and has an absorption maximum in ethanol at 324 to 325nm (Baldingh et al. 1951). The vitamin is soluble in fats and in all the usual organic solvents. It is insoluble in water but may be dispersed in the aqeous phase by emulsification or by attachment to proteins (Baldingh et al. 1951). Retinol and its esters have a yellowish-green fluorescence. The fluorescence of retinyl esters in alcoholic solution increases rapidly followed by destruction of the retinyl esters (Sobotka et al. 1943). In the absence of anti-oxidants, Vitamin A is very unstable in oxygen (Embree and Shantz, 1943).

Oxidation

Potassium permanganate oxidation of retinol yields retinal (Morton, 1941). This has led to the use of manganese dioxide as an oxidant to convert allylic alcohols into the corresponding aldehydes (Ball et al. 1948). A petroleum ether solution of retinol, left in the dark at room temperature in the presence of manganese dioxide yields retinal.

Reduction

Lithium aluminium hydride reduces Vitamin A aldehydes.

acids, and esters to the corresponding retinol analog (Robeson, 1955), sodium and potassium borohydride have the same effect (Brown and Wald, 1956).

Isomerization

Retinal is isomerized by exposure to light. Each isomer gives a steady state mixture of all possible isomers with the all-trans retinal always dominant (Brown and Wald, 1956). Thermal isomerization of aqueous solutions also occurs (Wald et al. 1955).

Instability to acids

Vitamin A is extremely sensitive to acids: they can cause rearrangement of the double bonds and dehydration (Bentel et al. 1953).

Colour-reactions

Acidic reagents give transient blue colour reactions with Vitamin-A. These tests are useful for qualitative or comparative measurements. The purple color obtained with sulphuric acid was one of the first methods used to identify Vitamin A in liver oils. Later, arsenic trichloride and the Carr-Price reagent (antimony trichloride in chloroform) were used (Carr and Price, 1926). Other Lewis acid such as trifluoroacetic acid have been used for quantitative determinants of Vitamin A (Neeld and Pearson, 1963).

Provitamin A

B-carotene melts at 181 to 182oc. In Petroleum ether, all-trans B-carotene has absorption maxima at 453, 481 and 273nm (Zechmeister and Polgar, 1949). Pure synthetic, crystalline all-trans B-carotene, after drying in a high vacuum drying pistol, has absorption maxima in n-hexane at 468nm. (Goodhart and Shills, 1974). B-carotene is readily soluble in carbon disulphide, chloroform and benzene but partially soluble in. Carotene is rapidly oxidised in air giving a colourless product. This process is accelerated by light.

Like most carotenoids, carotene produces colours with various reagents, including sulphuric acid and nitric acid (Kramer and Jucker, 1950).

Vitamin A and carotene react with antimony trichloride, carotene yielding a blue colour. The reaction of carotene with with antimony trichloride is less rapid and less specific having two absorption maxima at 490nm and 1020nm as against 620nm for Vitamin A.

Cis-trans isomerism occurs in carotenoids (Zechmeister, 1962). It may be induced by refluxing the pigment in a solvent by illumination, by treatment with acids or iodine, or by melting the crystals (Zechmeister, 1962).

GENERAL METABOLISM OF VITAMIN A AND THE PROVITAMINS ABSORPTION

Carotenoids: In most mammals, most of the ingested provitamin A is converted to Vitamin A in the intestinal wall. There is, however, a great deal of species specificity in the ability of different mammals to absorb dietary carotenoid. Man and cattle can absorb both Vitamin A and the carotenoid, and convert carotenoids with provitamin A activity to the Vitamin. In contrast, the rat and the pig do not absorb significant amounts of carotenoid pigments. However, they can convert provitamin A to the vitamin in the gut (Thomoson et al. 1950). The small intestine is the most important organ involved in the conversion of provitamin A to the Vitamin. The liver and the kidney are also capable of carrying out this process (Goodhart and Shills,1974). The process of conversion involves two soluble enzymes, B-carotene 15-15' dioxygenase and retinaldehyde reductase. The first enzyme catalyzes the cleavage of B-carotene at the central double bond by a dioxygenase mechanism, to yield two molecules of retinaldehyde (Goodman and Olson, 1969). Retinadehyde reductase reduces the retinaldehyde to retinol. The absorption of dietary carotenoid is significantly reduced when the diet is unusually low in fat (Roels et al. 1958). The amount of carotene absorbed from raw

carrots is highest in the presence if low molecular weight and short chain fatty acids (Brown and Dinor, 1945).

Vitamin A: The major dietary form of vitamin A is all-trans retinyl esters. These esters are hydrolyzed in the intestine by pancreatic retinyl ester hydrolase and the resulting retinol is then absorbed into the mucosal cell. Retinol in the mucosal cell (newly absorbed or newly synthesized from carotene) is reesterified with long-chain, mainly saturated fatty acids, and the retinyl esters are absorbed into the body, mainly in association with lymph chylomicrons. During chylomicrons metabolism most of the retinyl esters remain with the chylomicrons "remnants", and are removed from the circulation almost exclusively by the liver (Goodman et al. 1965). A small portion of the absorbed retinol is oxidised to retinal and further to retinoic acid (Fidge et al. 1968).

retinol can be absorbed as such but is mainly reduced and converted to retinyl ester within the mucosa (Deshmuk et al. 1964), although a portion is converted to retinoic acid (Fidge et al. 1968).

Transport of retinol

Retinyl esters in chylomicrons formed in the intestinal

mucosal cells travel through the lymphatic system, via the thoracic duct, to the blood stream, and are stored in the liver. The uptake of the chylomicron retinol esters hydrolysis and reesterification occur in the liver, where the resulting retinyl esters (mainly retinyl palmitate) are stored. Vitamin A is mobilized as the free alcohol, retinol, bound to a specific plasma transport protein, retinol binding protein (RBP). The retinol then travels via the blood stream to the tissues. Only 10 to 17 per cent of the Vitamin A content of the blood in normal human subjects in the fasting state is in the ester form. However in postprandial state after Vitamin A intake, the percentage of the ester in the circulating blood increases rapidly. This is as a result of the Vitamin A ester arriving in the blood stream from the gut via the lymphatic system (Hoch, 1959).

The blood level of Vitamin A is independent of the liver reserves as long as there are very small reserves of Vitamin A present in the liver, the blood level remains normal. As soon as the liver is depleted of its Vitamin A reserves to a certain level, the blood Vitamin A level falls rapidly (Goodhrt and shills, 1974).

Retinol Binding Protein and Prealbumin

Retinol Binding Protein (RBP) is a single polypeptide chain with a molecular weight close to 20,000, and a

single binding site for one molecule of retinol. In plasma, most of RBP normally circulates as the retinol-RBP complex (holo-RBP); the usual level of RBP in plasma is about 40-50ug/ml (Goodman, 1980).

RBP interacts strongly with transthyretin and normally circulates as a 1:1 molar protein-protein complex. In addition to its role in Vitamin A transport, transthyretin plays a role in the binding and plasma of thyroid hormones. The formation of the RBP-transthyretin complex serves to reduce the glomerular filtration and renal catabolism of RBP (Goodman, 1980).

Plasma RBP levels are low in patients with liver disease but high in patients wit chronic renal disease. These findings reflect the fact that RBP is produced in the liver and mainly catabolised in the kidneys.

The transthyretin molecule is a stable tetramer, composed of four identical subunits with a molecular weight of 54,980. Transthyretin appears to contain four binding sites for RBP (Goodman, 1980).

Several studies have examined the retinol transport system in patients with protein-calorie malnutrition, who have been found to have decreased concentrations of plasma RBP, pre-albumin, and Vitamin A. Low intake of dietary protein and calories is frequently accompanied

by an inadequate intake of Vitamin A. However, even in cases of malnutrition where there is adequate Vitamin A intake, the plasma RBP and Vitamin A levels are low reflecting a functional impairment in the hepatic release of Vitamin A because of defective production of RBP (Goodman, 1980). RBP is responsible for the delivery of retinol from the liver to the extra-hepatic sites of action of the vitamin. Evidence is available that this delivery process may involve specific cell surface receptors for RBP (Chen and Heller, 1977). The retinol thus carried is delivered to the specific sites where it enters the cell for subsequent metabolism and action. The apo RBP returns to the circulation, where it shows a reduced affinity for transthyretin and is selectively filtered by the renal glomeruli. Studies in the rat and in humans have suggested that Vitamin A toxicity occurs in vivo. This occurs when the level of Vitamin A in the body is such that retinol begins to circulate in plasma and to be presented to membranes, in a form other than bound to RBP (Goodman, 1980). It has been suggested that the nonspecific and unregulated delivery of Vitamin A to biological membranes, in contrast to the specific and regulated delivery via RBP, leads to Vitamin A toxicity.

Vitamin A mobilization from the liver, and its delivery

to peripheral tissues, are highly regulated by factors that control the rates of RBP production and secretion by the liver. One factor that specifically regulates RBP secretion from the liver is the vitamin A nutritional status of the animal. Retinol deficiency specifically blocks the secretion of RBP from the liver, so that plasma RBP levels fall and liver RBP levels rises. Conversely, repletion of vitamin A deficient rats intravenously with retinol stimulates the rapid secretion of RBP from the expanded liver pool (in the deficient rats) into the plasma. This release of RBP is not blocked by inhibitors of protein synthesis indicating that it comes from the expanded liver pool of RBP, rather than from de novo protein synthesis (Goodman, 1979).

The block in RBP secretion seen after Vitamin A depletion is highly specific for RBP. Thus neither Vitamin A depletion and deficiency, nor retinol repletion of deficient rats significantly altered plasma levels of transthyretin. The secretion of RBP and that of transthyretin appear to be independently regulated processes with formation of the RBP-transthyretin complex occurring in plasma after secretion of the two proteins from the liver cells (Goodman, 1979).

RBP in the liver is mainly associated with the liver

microsomes, and is especially enriched in the rough microsomal fraction. The Golgi apparatus was found to contain a maximum of 22% of RBP in the liver in normal rats, and 9% in Vitamin A-deficient rats. The Golgi apparatus is therefore not a major sub-cellular locus for RBP in either normal or deficient rats. Inconclusive evidence that the microtubules are involved in the secretion of RBP has been obtained in studies with the drug colchicine. Smith et al.(1978) found two lines of differentiated rat hepatoma cells that synthesize RBP during culture in vitro. When the cells were incubated in a Vitamin A-free serum-less medium, a relatively large proportion of the RBP synthesized was retained within the cells. Addition of retinol to the medium (at levels of 0.1 or 1ug/ml) stimulated the release of RBP from the cells into the medium and also increased the net synthesis of secretion of rat serum albumin by these cells. Thus these cell lines appear to respond to vitamin A depletion and repletion in a similar manner as does the intact rat liver vivo (Godman 1979)

RETINOIC ACID AND ITS METABOLITES

Retinoic acid is a compound that demonstrates selective Vitamin A biological activity. It supports a normal rate of body growth, as well as normal differentiation

of epithelial tissue. Retinoic acid cannot, however, replace retinol as a visual pigment precursor, and does not support vision and reproduction (Goodman, 1979). Animals maintained on retinoic acid as the only source of Vitamin A activity are, hence, both blind sterile. In the normal animal, retinoic acid represents an extremely small proportion of the Vitamin A in the body. A very small proportion of the retinaldehyde formed from β-carotene cleavage is oxidized in the intestine to retinoic acid.

Retinoic acid is absorbed through the portal system and transported in plasma bound to serum albumin. It does not accumulate in the liver or other tissues in any appreciable amounts. It is metabolised rapidly mainly to more polar compounds, and then largely excreted in the urine and bile. The major biliary metabolite has been identified as retinoyl-β-glucuronide.

McCormic et al (1978), observed that 5,6-epoxyretinoic acid was isolated from intestines of Vitamin A deficient rats given retinoic acid. This metabolite has been shown to have biological activity, although the extent of its activity is not fully understood. The in vivo and in vitro metabolism of retinoic acid in hamster was investigated using both tracheal organ culture and subcellular preparations derived from intestinal

mucosa, liver, and testis. These studies revealed the production of several metabolites more polar than the parent compound. Two of the early products of this metabolic pathway were identified as 4-hydroxy and 4-ketoretinoic acid. The formation of these metabolites was maximal in Vitamin A-deficient hamsters that had been induced with retinoic acid than in Vitamin A-normal animals. In addition, the two metabolites showed decreased biological activity relative to retinoic acid in a tracheal organ culture assay. These findings suggest that oxidative attack at carbon-4 of the cyclohexenyl ring may be the first step in the elimination of retinoic acid from tissues.

VITAMIN A AND GLYCOPROTEIN AND MEMBRANE METABOLISM

Vitamin A is necessary for the maintenance of normal differentiation and of mucus secretion of epithelial tissues. The biosynthesis of some glycoprotein is decreased in Vitamin A deficiency and enhanced upon administration of excessive doses of the Vitamin (Goodman 1980). In order to explain these various observations it has been suggested that retinol or a derivative of retinol may serve as the lipid portion of a glycolipid intermediate involved in certain glycosylation reactions. Thus, in this hypothesis, retinol is thought to function in a manner analogous to

dolichol in specific glycosyl transfer reactions (Goodman 1980). It has been suggested further that these particular glycosylated reactions may be involved in the biosynthesis of specific glyco-proteins in vitamin A-requiring tissues. If this were true, then specific defects in glycoprotein synthesis would occur in vitamin A deficiency, and might explain the variety of abnormalities in cellular metabolism seen in vitamin A deficiency since glycoproteins are common constituents of membrane systems and are involved in a variety of biological functions (Wald et al. 1979; Goodman, 1980).

Retinyl phosphate has been shown to be formed in mammalian cells both in vitro and in vivo. The enzyme system that forms mannosyl retinyl phosphate is located primarily in the rough endoplasmic reticulum of rat liver cells. Under appropriate conditions, glycosyl transfer can be demonstrated from the retinol glycolipid to membrane glycoprotein. It is not known, however, whether this occurs in vivo under normal conditions (Goodman 1980). The hypothesis that retinoid containing glycoproteins are obligatory intermediates for specific glycosylation reactions (e.g. in the synthesis of specific glycoproteins in certain tissues) is an intriguing one. This hypothesis suggests credible biochemical effects and therefore requires further

studies.

INTRACELLULAR BINDING PROTEINS FOR RETINOL AND RETINOIC ACID

Evidence for the existence of a specific, soluble binding protein for retinol in rat tissues was first reported by Bashor et al. (1973). Subsequently, the existence of a similar but distinct cytosolic acid was also demonstrated. The intracellular binding proteins for retinol (CRBP) and for retinoic acid (CRABP) have both been purified to homogeneity from rat liver, rat testis, and bovine retina. The major properties of the purified preparations for each protein from different sources were quite similar to each other. Both CRBP and CRABP have molecular weights close to 14,600 and single binding sites for one molecule of retinoid ligand. The intracellular binding proteins differ from serum RBP with regard to molecular weight (the intracellular proteins are smaller), immunoreactivity (they are unreactive in the serum RBP radioimmunoassay) binding affinity for transthyretin (they show no affinity for transthyretin). The ultraviolet absorption spectra of CRBP and of CRABP are almost identical. The explanation for this phenomenon is not clear.

Very recent studies using a newly developed

radioimmunoassay for CRBP from rat liver showed that CRABP is immunologically distinct from CRBP (Chytil and Ong, 1979). CRBP from rat testis showed identical immunoreactivity as that from liver, suggested that the same CRBP molecule is found in different tissues.

Interest in these intracellular retinoid binding proteins has been stimulated by reports suggesting a relationship between the binding affinity of the proteins for various vitamin A-related compounds and the biological activity of the compounds. Furthermore, a number of retinoids with anticarcinogenic activity can associate with the tissue binding proteins, and it has been reported that the binding ability tends to correlate with the biological activity for given compounds. Accordingly, it has been suggested that the binding proteins might be involved in some way in the biological expression of Vitamin A activity within the cell (Goodman, 1980).

Many basic questions exist about the intracellular binding proteins. It has been suggested that these proteins may play a direct role in the biological expression of vitamin A activity (e.g. analogous to steroid hormone receptors). CRBP may be involved in facilitating the specific interaction of retinol with binding sites for retinol in the cell nucleus (Chytil

and Ong, 1979). Another possibility is that these proteins mainly serve as intracellular transport proteins, and act to transport specific retinoids in a directed way from one locus to another within the cell. Further studies are needed in order to explore these and other possibilities.

Retinol, taken up by the cell at the cell surface receptor, is released from RBP prior to subsequent translocation within the cell, metabolism, and or initiation of a biological effect.

EFFECTS OF VITAMIN A DEFICIENCY ON REPRODUCTIVE PERFORMANCE DURING PREGNANCY

Vitamin A deficiency has been found to be a cause of infertility or impaired reproduction in vertebrates (Moore, 1957). Various studies have shown that the oestrous cycle is disrupted, and the vagina becomes permanently keratinized. But the most characteristic feature of deficiency are foetal resorption, stillbirths, and congenital malformations rather than ovarian dysfunction or failure of fertilization or implantation. Retinoic acid supports other functions of Vitamin A activities not vision or reproduction. The complications of inanition, decreased resistance to infection, and other non-specific effects of Vitamin A deficiency made the effect of Vitamin A deficiency

difficult to interpret until it was shown that retinoic acid would correct the systemic abnormalities out failed to support vision and reproduction (Bates, 1983). In retinol deficient retinoic acid fed rats and guinea pigs, the oestrous cycle and conception seemed normal, but the foetuses were resorbed (Thompson et al, 1964, Howell et al, 1964). Necrosis of the junctional zone of the placenta was the earliest abnormality shown (Howell et al, 1964). The possible effects of vitamin A deficiency on steroid hormone production have been investigated (Hay and Kendall, 1956; Juneja et al, 1969; Ganguly et al, 1971a,b). Disturbances of steroid hormone production can probably be caused by retinol deprivation, but the extent to which this is responsible for the overall impairement in reproductive performance is not clear.

TERATOGENIC EFFECTS OF HYPERVITAMINOSIS A

The foetus is to some extent protected against the effects of excessive maternal intakes of Vitamin A by some homeostatic mechanism operating on circulating levels of retinol in the mother. However, abundant evidence exists that excessive intakes by mother can result in damage to the foetus, especially during the critical periods of organ and limb development (Bates, 1983).

This observation was first made by Cohlan (1954) who produced abnormalities of the skull and brain in 54% of the offspring of maternal rats who were given 10,000 ug Vitamin A from the second, third, or fourth day to the sixteenth day of pregnancy. The nature of the abnormality depended on the gestational age at the time of administration (Giroud, 1960). These abnormalities took the form of anacephaly, cleft palate, anophthalmia, spina bifida, or sydactyly. Ingestion of large doses of Vitamin A on days 17 - 18 of gestation have been found to produce abnormal behaviour in the offspring rather than gross malformations.

Malformations have also been produced by excessive intake of Vitamin A in the guinea-pig, rabbit, hamster, mouse and the pig. There are however clear differences in susceptibility between species and between variants of the same species in the response to high doses (Lorente & Miller, 1977; Seller et al. 1979). Extrapolation form these studies and deductions from fewer studies in humans may suggest that ingestion of large amounts during human pregnancy is not advisable, although there is little evidence or information to indicate the upper limit of safety. Gal et al. (1972) observed that the maternal serum levels of mothers of infants with central nervous system defects were higher

than in those with normal babies. This does not however imply a causal relationship.

In cows, sheep, goats, dogs and cats, the newborn have liver Vitamin A stores which is lower than those of their mothers (Moore, 1957). In cows and pigs, large doses of Vitamin A supplied to the mother increased the extent of placental transfer to a moderate extent. Large doses of carotene, in contrast, were entirely vague without effect on foetal Vitamin A stores, and very little carotene was transferred to the foetal liver.

These studies are in agreement with the idea that the supply to the foetus is mainly from the retinol-RBP complex in maternal stores, except when they fall to very low levels (Bates, 1983).

Values for foetal liver Vitamin A levels at autopsy are wide. The early studies were put together by Moore (1957). Gal et al. (1972) observed a range of values from less than 10 to more than 130ug/g in livers of British infants near term. A wide range was also observed by Montreewasuwat & Olson (1972) in Thailand, although in this study, and that of Iyengar & Rao (1972) on foetuses from poorly nourished Indian women, there were few values above 50ug/g, and mean values were

of the order of 20ug/g. Although foetal levels are generally lower than those of adults, it is difficult to be certain about the quantitative relationship between maternal status and intake and foetal liver in humans, and whether they are as tightly controlled as they are in the rat.

Human maternal values for plasma retinol usually tend to be higher than the corresponding cord plasma retinol levels (Lewis et al. 1947). Vahlquist et al. (1975) found that RBP and thyroxine binding transthyretin levels at birth, in a presumably well-nourished population are about half the adult levels. Preterm infants have been observed to have lower plasma retinol levels at birth than term infants (Brande et al. 1978; Shenai et al. 1981). However babies of low birth weight but not premature had plasma retinol levels similar to those of normal birth-weight (Bauer et al. 1977).

Lewis et al. (1947) found that 3000ug Vitamin A, or the equivalent amount of carotene, given daily during the final months of pregnancy, had no effect on plasma retinol levels in the neonates. Also Bates, (1983) reported that single large doses (up to 60,000ug given to the mother shortly before parturition did not increase cord plasma retinol levels. Venkatachalam et al (1962) gave 9,000ug Vitamin A/day throughout the last

trimester of pregnancy to twelve malnourished Indian women who apparently had a very low intake from dietary sources. and observed significantly higher cord levels in them than in unsupplemented controls: it seems likely that their stores were severely depleted in the absence of supplementation.

MATERNAL STAIUS DURING HUMAN PREGNANCY

Several studies have described night blindness, or impaired dark adaptation in pregnant women receiving a diet inadequate in its Vitamin A content.

Hirst and Shoemaker (1941) determined the concentration of serum Vitamin A in a series of 35 pregnant women and found that 40% of the values were below normal range. Bodansky et al (1962) on the other hand showed a significantly higher mean values in the first 6 months when compared to the last 3 months of pregnancy. They attributed this finding to the storage of Vitamin A in the foetal liver and utilization by the fetal tissues. They also suggested other reasons for a reduction in plasma Vitamin A levels in the 3rd trimester. These included possible interference with the release of Vitamin A from the liver associated with the conservation mechanism of the liver at this time. There is no evidence however to indicate interference with gastro-intestinal absorption during pregnancy.

Various estimates of the total Vitamin A content of the normal adult liver, the chief source of Vitamin A in the body have been reported. It ranges from about 5000,000 i.u. to 11,000,000 i.u. (Ralli et al. 1941; Abels et al. 1941). The foetus may make two kinds of demands upon this depot: storage of Vitamin A in the foetal liver and utilization of Vitamin A by the actively growing foetal tissues.

Foetal liver during the third trimester contains considerable amounts of Vitamin A and a total store of 12,000 i.u. of the Vitamin is found in the liver of the newborn infant (Lewis et al. 1941). They also suggested that the deposition of 12,000 i.u. in foetal liver may entail the release of several-fold that amount from the maternal liver. Vitamin A is not used economically. For example, when large amounts are fed, only a small fraction can be accounted for by fecal excretion, storage in the liver, and daily requirements (Le Page 1941). Also Lewis et al. (1942), indicated that during depletion, Vitamin A is released from the liver of the rat in amounts much greater than required by the animal.

The actively growing tissues of the foetus may also utilise considerable amounts of Vitamin A. The basal metabolic rate increases during the latter half of

pregnancy. There is general agreement that most of this increases is due to the growth of the foetus, although it may also be in part accounted for by a possible activator of the maternal endocrine glands (Du Bois, 1936).

Lund and Kimble (1943) observed that economic status and health education influenced the adequacy of the intake of Vitamin A in the pregnant population studied. They also observed a significant correlation between the intake of Vitamin A and the plasma values for the groups of subjects.

A diet which was adequate for non-pregnant women was also adequate for pregnant women during the first trimester except when such complications as hyperemesis gravidarum intervened. During the second trimester, only the best diet met the needs. By the third trimester there was a need for supplements of Vitamin A in addition to amount supplied by the diet. Gal and Parkinson (1974) also observed a significant effect of Vitamin A supplementation with as slow as 2,500 i.u.daily when treated subjects were compared with the untreated control group. Optimum plasma Vitamin A levels could be maintained during the last trimester of pregnancy by the addition of 10,000 i.u. of Vitamin A. Larger supplements of 20,000 i.u. had no additional

effect, (Lund & Kimble 1943).

Lund & Kimble (1943) gave some reasons for the pattern of plasma Vitamin A in pregnancy. Haemodilution which accompanies pregnancy might be considered as a possible cause of the progressive decline in vitamin A blood values. But since there was no decline in carotene value of the subjects during the same period this does not hold true.

The ingestion of large amounts of carotene, whether by diet or supplements, elevated the carotene without affecting the plasma Vitamin A. Carotene metabolism in pregnancy is however poorly understood.

Vitamin A mobilization into the blood stream postpartum was observed by Lund & Kimble (1943). A single common factor to every patient is emptying of the uterus. Labour, Caesarean section, anaesthesia & analgesic had no effect on the mobilization of Vitamin A postpartum (Lund & Kimble, 1943; Clausen et al. 1942). Puerperal diuresis was also eliminated in the possible causes of Vitamin A mobilization postpartum. Vitamin C and Carotene values did not change with Vitamin A. Mobilization of Vitamin A was noticed - as early as 6 hours postpartum and lasted up to 24 hours. Large doses of Vitamin A 6000,000 and 330,000 i.u. given to mothers

before delivery in an effort to produce very high plasma levels though succeeded but could not maintain the plasma levels in early puerperium. The puerperal values decreased unless the supplements were continued (Lund and Kimble, 1943).

There was no correlation between the toxaemia of pregnancy and plasma Vitamin A, even though low values might be anticipated because of the liver damage common in some toxaemias. Plasma Vitamin A could also not be correlated with the duration of labour.

Considering these various observations, Lund and Kimble (1943) advocated Vitamin A supplements of 5,000 i.u. of Vitamin A daily in the 2nd trimester and 10,000 i.u. in the third trimester and they also observed that amounts greater than this are not necessary under usual conditions. They further suggested that since the provitamin A cannot be efficiently converted and absorbed, a diet which supplies generous amounts of vitamin A itself rather than one which depends principally on conversion from carotene is preferable (Lund and Kimble, 1943).

Gal and Parkinson in 1974 showed that there was no difference in the plasma levels in the three trimesters though there was a reduction in the early 5th to 9th

weeks and after 36th week of pregnancy. They attributed their findings to the effect of circulating sex hormones. Studies have shown that both plasma progesterone and estradiol increase during pregnancy (Bates, 1983).

The initial decrease in the serum Vitamin A content in early pregnancy could therefore be related to the changing site of progesterone production from the corpus luteum to the placenta. Similarly the decrease in circulating levels of Vitamin A in the final stages of pregnancy could be related to a decrese in the circulting progesterone that precedes spontaneous labour.

VITAMIN A AND B-CAROTENE IN NON PREGNANT NON LACTATING WOMEN

Plasma retinol levels very considerably among apparently well-nourished individuals living in industrialised countries (Wald et al. 1980). Blood retinol level is regulated largely by the synthesis of RBP in the liver, which is the primary storage site for the retinyl ester (Goodman, 1984). Retinol is released into the blood only when molecules of both retinol and RBP are available. However, the factors that determine individual blood retinol levels are to a considerable extent unclear. Some regulation is likely mediated horeonally, inasmuch as males have higher levels than

females (Willett et al. 1983) and oral contraceptive users have higher plasma retinol levels than non users (Bamji and Ahmed, 1978).

Walter et al. (1984), administered daily doses of 10,000 i.u. Vitamin A daily to a sub population of well nourished women with marginal plasma Vitamin A levels in a placebo-controlled randomized study for a period of 4 weeks. A significant increase of 9% was observed in the plasma Vitamin A levels.

TRANSFER OF MATERNAL STORES TO MILK

Large variations in maternal intake of viatmain A affect milk levels and the transfer of Vitamin A to the young to a greater extent, than variations in the size of maternal liver reserves (Henry et al. 1949). However, although the transfer rate may be independent of the size of the maternal reserve over a wide range, the liver reserves are usually the major contributor to the milk and the offspring. This was demonstrated in cows by Bronstetter et al. (1973) who observed that retinol in preference to retinyl esters is transferred from the blood to the milk. Most of the retinol is reesterified in the mammary gland and occurs as retinyl esters in milk (Bates, 1983). Vahlquist & Nilsson (1979) studied the transfer of Vitamin A to the milk of rhesus monkeys and concluded that, unless their Vitamin A intake was

very high. 80 - 90% of the Vitamin in their milk was derived from the circulating retinol-RBP complex, the remaining 10-20% being transfered from lipoprotein complexes of Vitamin A or its esters.

Retinol esterification by isolated mammary gland microsomes from lactating rats was demonstrated. This further provided evidence that retinyl esters in milk are derived from non-esterified retinol in the blood (Bates, 1983).

The levels of both Vitamin A and of carotenoids are substantially higher in colostrum and early milk than in mature milk in all species studied. Bates (1983) also reported a rise in plasma retinol levels from 3.4ug/100ml to 15ug/100ml after the first day's suckling. In humans, the Vitamin A concentration in colostrum is two to five-fold higher than that in mature milk (Lasher et al. 1945; Kon & Mawson, 1950) while for carotenoids there is at least a five-fold difference. The carotenoid pigments present in human milk are, however, a relatively poor source of Vitamin A: Bates, (1983) showed that a- and B-carotene together contributed only 23% of the total pigment, with xanthophyll contributing 47% lycopene 9% and unidentified pigments 21%.

Recommended dietary allowances

The principal criterion used in determining the RDP for Vitamin A is the maintenance of liver retinol stores. This is stipulated at above 10 ug/day for the normotensive storage requirements (ACC SCN News 1990). The safe level of intake of retinol equivalents for adults was set at 500ug/day for women and 600ug/day for men.

For infants, pregnant and lactating women respectively, the safe levels of 350,500 and 850ug retinol Eq./day have been recommended (ACC SCN New 1990).

Dietary Sources

The carotenoid pigments are widely distributed in plant and animal tissues. They are characterised by their typical red, yellow and orange colors. Many of them have no Vitamin A activity. Therefore the occurrence of a pigmented carotenoid in food is not necessarily an indication of its value as a source of provitamin A. Table 2.1 gives the vitamin A and B-carotene content of a list of food items from the various food groups.

Fruits contain varying but generally low amounts of carotenoids. Cereals and cereal foods in general do not contain carotenoids or preformed vitamin A. The only exception is soyabean which contains traces of carotene.

Among the vegetable oils, the richest source of provitamin A is palm oil (oil extracted from the fruit coat of Elaeis guineensis). The provitamin A activity of the ripe red palm fruits varies from 65,000 to 113,000 I.U. of provitamin A per 100g (Goodhart and Shills, 1974) between 6,500 and 13,000 I.U for red palm oil).

Preformed vitamin A is found almost exclusively in animals. Human animals concentrate most of the vitamin A in the liver where it is stored. Other significant pools of vitamin A are found in the kidney, milk, and blood plasma (Goodhart and Shills, 1979).

Milk products and eggs are usually rich sources of vitamin A. In skim milk production practically all carotenoids and preformed vitamin A have been removed together with the fat.

Among the meats- pork, beef, chicken, lamb, rabbit, turkey and veal contain only traces of Vitamin A. Fish liver oils are extremely rich sources of vitamin A and the vitamin A content varies over a wide range according to the species of the fish. The highest values were found in red steenbras which contained up to 1,130,000 I.u of vitamin A per gm oil (Kapsin et al 1945).

TABLE 2.1

β-carotene and vitamin A contents of various foods consumed in the tropics

FOOD ITEMS/100g	β-CAROTENE (ug)	VITAMIN A (ug)
Group 1		
Cereals and Grain Products		
Maize (mature)		
white	5-20	-
yellow	100	-
Maize (immature)		
yellow	405	-
Millet (whole)	10	-
Sorghum	10-15	-
Group 2.		
Starchy Roots, Tubers, and fruits.		
Banana(ripe)	120-285	-
Cassava (raw)	30	-
Potatoes(raw)	540	-
Plantain (ripe)	780	-
Potatoes (irish)	25	-
Sweet potatoes		
" yellow	300-1255	-
" deep yellow	2400	-
Yam(raw)	10	-
Bambara nuts (dried)	10	-
Group 3		
Grains and legumes		
Cowpea (whole, dried)	70	-
Peanut (dried)	15	-
Soybean(dried)	55	-
Group 4		
Nuts and Seeds		
Kernel (Cocoplum sp.)	385	-
Ginger bread plum	210	-
Group 5		
Vegetables and vegetable products		
Amaranth (raw)	5710	-
Cabbage Chinese(raw)		
(Brassica sinnesis)	2280	-
Baobab leaves (dried)	9710	-
Cabbage(common)	100	-
Carrots(raw)	5480	-

Cassava leaves(raw)	11,775	-
(bitter)		
Coffee leaves(dried)	2360)	-
Cowpea immature seeds	150	-
" leaves raw	7970	-
Hares lettuce(raw)		
(leaves)	1430	-
Okra (raw)	185	-
Peanut (groundnut)		
(leaves)	7755	-
Peppers (hot) (raw)	330	-
" red raw	7140	-
dried	14250	-
" sweet raw		
" immature	180	-
" dried	2840	-
Pumpkin(raw)	3565- 3600	-
Sweet potatoes	2290- 7050	-
Cocoyam	1800	-
Tomatoes (raw)		
ripe whole	360-700	-

Group 6

Fruits

African locust bean	2430	-
Apricot	3145	-
Avocado	530	-
Bush mango	310	-
Cashew(raw)	740	-
Date(raw)	145	-
Grape	50	-
Guava	240	-
Mango	3200	-
Orange	250	-
Palm oil (purée)	6150	-
raw	42420-168800	-
Pawpaw(raw)	950	-
Watermelon	250	-

Group 7.

Meat- Poultry and Insects

Liver -beef	180	810
Eggs -raw	300	350
Fish Tiger-raw	550	2465
Cheese	170	-
Milk whole	345	400
Cow milk	630	629

Group 8

Oils

Butter	545	630

Others		
Chili	1060	-
Vinegar	3050	-

SOURCE:FAO. United Nation (1968). Food composition table for use in Africa complied by Woot-Tsuen WU Leung. U.S. Department of Health, Education and Welfare Public Health Service.

C H A P T E R T H R E E

M A T E R I A L S AND M E T H O D S

SUBJECTS

The study was carried out in three phases:

1) A cross-sectional (CS) study of pregnant women which involved the study of 171 pregnant women recruited during the three trimesters of pregnancy and 35 non-pregnant non lactating (NPNL) in the proliferative phase of their cycle.

2) Vitamin A absorption test carried out on 10 NPNL women and 30 pregnant women also from the different trimesters of pregnancy.

3) A longitudinal study of the effect of oral vitamin A supplementation carried out on 28 pregnant women from the 14th week of pregnancy to the 6th week post partum.

SECTIONAL CRITERIA

All subjects were seen by a physician at the begining of the study. Medical history and clinical examination were carried out on each subject. Furthermore biochemical indices such as Packed cell volume (PCV), Liver function test (LFT), urinalysis were also carried out on each patient at admission. This was necessary in order to exclude those patients with

abnormally low PCV and abnormal LFTs. Subjects were admitted into the study based on the following criteria:

1) Absence of any respiratory, cardiovascular, hepatic diseases, and other systematic diseases associated with pregnancy were also excluded.

2) Informed consent of the subjects.

On admission each subjects had the objectives of the study explained in detail to her. The details of her participation were carefully enumerated and the need for cooperation and compliance in the execution of the study was explained. Ethical committee approval was obtained before the commencement of this study.

At admission each subject was required to complete a questionnaire on her personal, educational, vocational, and nutritional status (Appendix 1).

At this initial stage the physical measurements of weight, height, and blood pressure were taken by the observer.

CROSS-SECTIONAL STUDY

The subjects in this phase were recruited from both the University College and Adeoyo hospitals, Ibadan. They were seen on every antenatal clinic day for a period of nine months. The subjects were classified into five socio economic groups using income, education and

occupation based on a modified method of Taylor and Akande (1975). Fasting blood samples were collected from each subject in this study group in the morning between 8 a.m. and 9 a.m. from the ante cubital vein into heparinized and E.D.T.A. bottles. These samples were kept in black polyethylene bags in a refridgderator for 2hrs on each occassion. This was to prevent light penetration and destruction of the Vitamin A content of the samples. The blood samples were separated and plasma samples were then stored frozen at -20° until analyzed.

RELATIVE DOSE RESPONSE TEST (RDRT)

This phase of the study spanned a period of 5 weeks. 40 subjects were admitted and were selected from the University College Hospital, Ibadan. The subjects comprised of 10 non pregnant non-lactating women and 30 pregnant women. The non pregnant non-lactating women were normally cycling women studied in the proliferative phase of their cycle. The pregnant subjects were recruited from the ante natal clinic of the department of Obstetrics and Gynaecology, U.C.H., Ibadan. The control subjects were healthy staff members of the U.C.H. Ibadan.

Three subjects at a time were admitted into the Metabolic Research Unit early in the morning on the day of the study and were kept for a period of

5hrs. They were put in the recumbent position on arrival at the Metabolic Research Unit. An indwelling canula was inserted in the ante cubital vein, which was kept patent with normal saline infusion. Blood was drawn at -15 and 0 minutes after which 450ug Vitamin A in oil was fed to each subjects. Blood samples were taken after 5 hrs of the dosing. The relative dose response was calculated from the following formula: A5 - A0/A5 X 100 where A0 is the fasting plasma Vitamin A. A5 - a second specimen taken after 5 hr.

LONGITUDINAL STUDY (b.S.)

A total of fifty two pregnant subjects in the first half of the second trimester were recruited into this phase of the study. This period was chosen because of the supposed teratogenic effects of large doses of vitamin A in early pregnancy during cell differentiation. The study lasted for a period of 18 months.

The subjects were divided into two groups and were seen from the 14th week of pregnancy and every 7 weeks thereafter until 6 weeks after delivery. The two groups were matched for Age, parity, and weight. They were required to have plasma Vitamin A level < 30 ug/dl and normal liver function tests. 7,000 IU (2000 ug) of Vitamin A in oil or lactose prepared in gelatin capsule was given to the supplemented and the placebo groups

respectively. The study was single blinded and the subjects were not given any dietary advice. They were however instructed not to take any other multivitamin preparations apart from folic acid and fersolate.

Blood samples were obtained from each subjects at every appointed visit. immediately after delivery and 6 weeks after delivery. Cord blood samples were also obtained at delivery. The subjects were informed to report any symptoms experienced during the study period.

Only 29 subjects and controls concluded the study but the results of 28 (14 in each group) were computed.

Weighed food samples (breakfast. lunch. supper) were collected from each subject in their various homes during the study period. Samples of each food item for each meal were collected as eaten and stored frozen at -20oC until analyzed.

24 hour dietary recall was also carried out on each visit to ensure that the dietary pattern of the subjects was not altered significantly during the weighed dietary procedures.

3.1 ANALYSIS OF SAMPLES

1. SERUM

B-carotene and Vitamin A

The procedure is a modification of the methods of Kimble (1939) and Kaser and Steikal (1943) except that Trifloroacetic Acid (TFA) is substituted for SbCl3 (Neeld and Pearson, 1963).

The principle of this method involves the reaction of ..-electrons in the conjugated double bonds of Vitamin A with trifluoroacetic acid to form a chemical compound with a blue colour.

Procedure for B-carotene and Vitamin A

1ml of serum was transferred in duplicate into 16 x 25 mm glass stoppered test tubes. With mixing 2ml of absolute ethanol/ascorbic acid in water 100g/l was added followed by 3 ml of petroleum ether (boiling range 30 to 40°C). Ascorbic acid was used to prevent the oxidation of Vitamin A during the extraction process (Driskell et al, 1985). The mixture was stoppered and shaken vigorously for two minutes to insure complete extraction of carotene and Vitamin A. The tubes were centrifuged slowly for three minutes. 2ml of the petroleum ether (upper) layer was pipetted into a Coleman 75 x 100 mm cuvette. The cuvette was stoppered immediately and the

carotene was read immediately at 450mu against a petroleum ether blank in the Coleman Jr. spectrophotometer. The cuvette was removed and the petroleum ether was evaporated to dryness in 40°C water bath. The residue was taken up immediately in 0.1ml of chloroform. The Coleman Spectrophotometer was set at 620nm and set to zero optical density with a blank consisting of 0.1ml of chloroform and 1.0 TFA reagent. The sample cuvette was placed in the spectrophotometer, and 1.0ml of TFA reagent added and the reading was taken at exactly 30 seconds after addition of the reagent.

B-carotene

Standardization

The B-carotene working standards obtained were run seven times. The inter assay coefficients of variation (CV) for B-carotene standards at 0.5, 1.0, 2.0, 3.0 and 4.0uG/ml were 9.7, 2.6, 0.7, 1.4 and 1.4 respectively.

Two separate control plasma were also run and the intra assay coefficients of variation for B-carotene were 5.5 and 4.4 while the inter assay CV for B-carotene were 5.2 and 4.1

Standard curves and calculations

The B-carotene intermediate standard was weighed and diluted with petroleum ether to given solutions

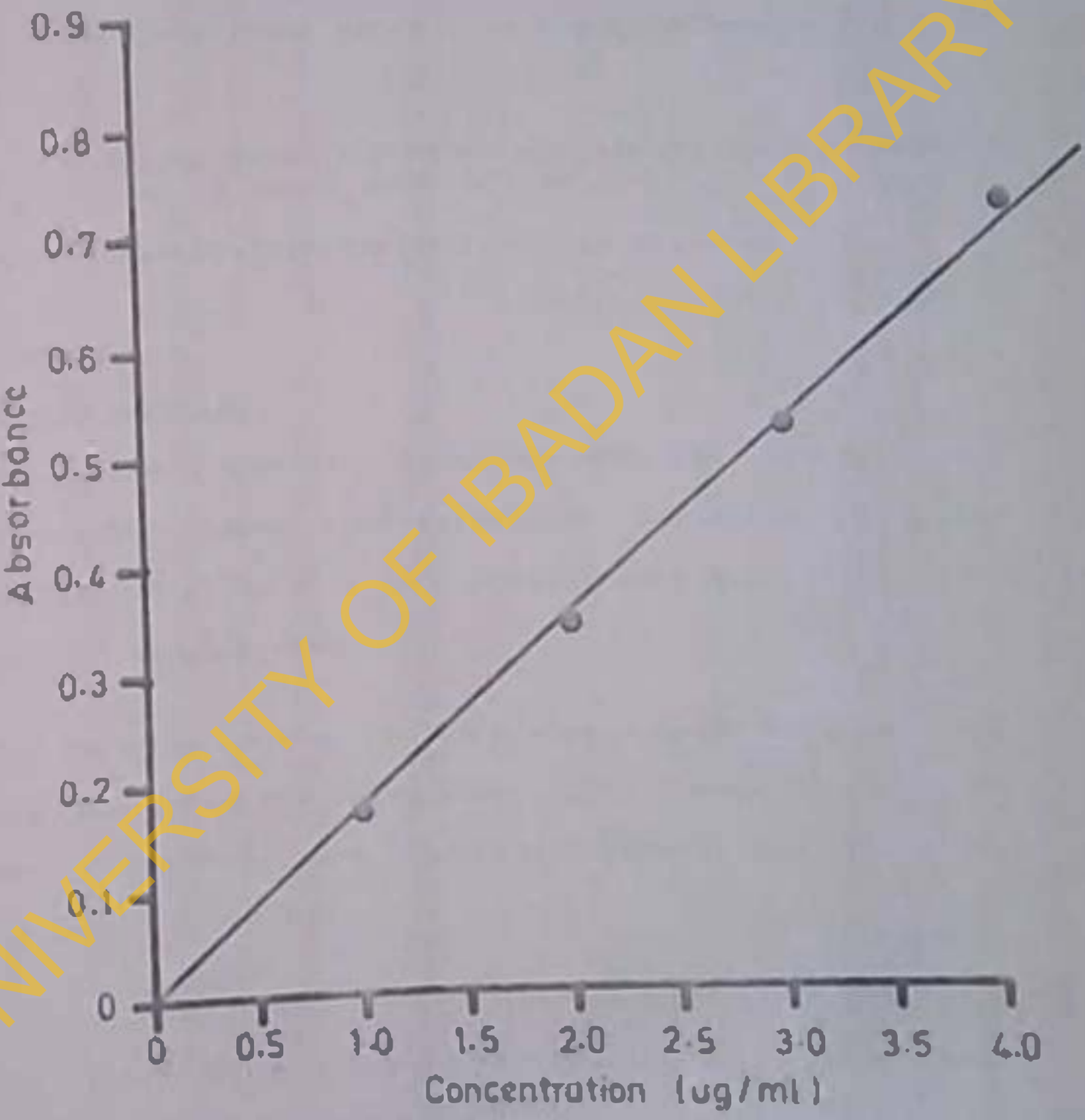
containing 0.5, 1.0, 2.0, 3.0 and 4.0ug of B-carotene/ml respectively. The optical densities of these solutions were read at 450nm against a petroleum ether blank and a standard curve plotted (fig.3.1). The F value was calculated based on the following formula:

$$F = \frac{\text{ug carotene/ml}}{\text{optical density}}$$

The F value obtained was 6.4 as compared to 3.8 in the literature (Neeld and Pearson, 1968). B-carotene concentration (ug/ml) was plotted against the absorbance.

Since B-carotene also reacts with TFA reagent to produce the typical blue colour it would normally produce with Vitamin A, carotene standards were run to permit calculations of a correction factor. For the purpose of calculation the carotene correction factor (cf) for the Vitamin A procedure was carried out. 4.0, 8.0, 10.0ug/ml of B-carotene was prepared and 2.0ml of each of B-carotene standards was treated as a sample, beginning with the evaporation step of the Vitamin A procedure. The average ratio of absorbance at 620nm/concentration of B-carotene (in ug/ml) was then calculated and used in the compilation of the B-carotene corrected factor CF.

FIG. 3.1
B-CAROTENE STANDARD CURVE



CALCULATION

B-carotene

B-carotene per ml of sample was obtained from the standard curve and the following calculations were carried out:

ug B-carotene/100ml serum = ug B-carotene/ml x 3.0 x 100

where

3.0 = volume petroleum ether containing the B-carotene from 1.0 ml of serum after extraction.

100 = concentration factor ug/ml to ug/100ml

Vitamin A

Standardization

The Vitamin A working standards were run seven times. The inter assay coefficients of variation (CV) for Vitamin A at 4, 8, 12 and 16ug/ml were 9.1, 3.3, 1.0, and 1.5 respectively.

Two separate control pooled plasma samples were run and the mean intra and inter assay coefficients of variation for Vitamin A were calculated using the following formula: $\frac{S.D}{MEAN} \times 100$

The intra assay C.V were 4.4 and 4.1 while the inter assay C.V. were 5.7 and 5.2.

Vitamin A standards were weighed and diluted to give

solutions containing 4.0, 8.0, 12.0 and 16.0ug/ml of Vitamin A. Standard curve was prepared (fig.3.2) by pipetting 0.1ml aliquots of these standards into the cuvette and reacted with TFA reagent. In the literature the F value was 4.99 while 4.44 was obtained from this assay calculated by the equation:

$$F = \frac{\text{ug vitamin A/tube}}{\text{optical density}}$$

From the foregoing calculations and the volume of reagents used, the amount of Vitamin A in the sample were calculated.

For accurate calculation of the Vitamin A content, it is necessary to correct the absorbance by carotene at 620nm:

$$A3 = A2 - (X\ A1)$$

where

A1 = absorbance of carotene at 450nm

A2 = absorbance at 620nm due to both carotene and Vitamin A

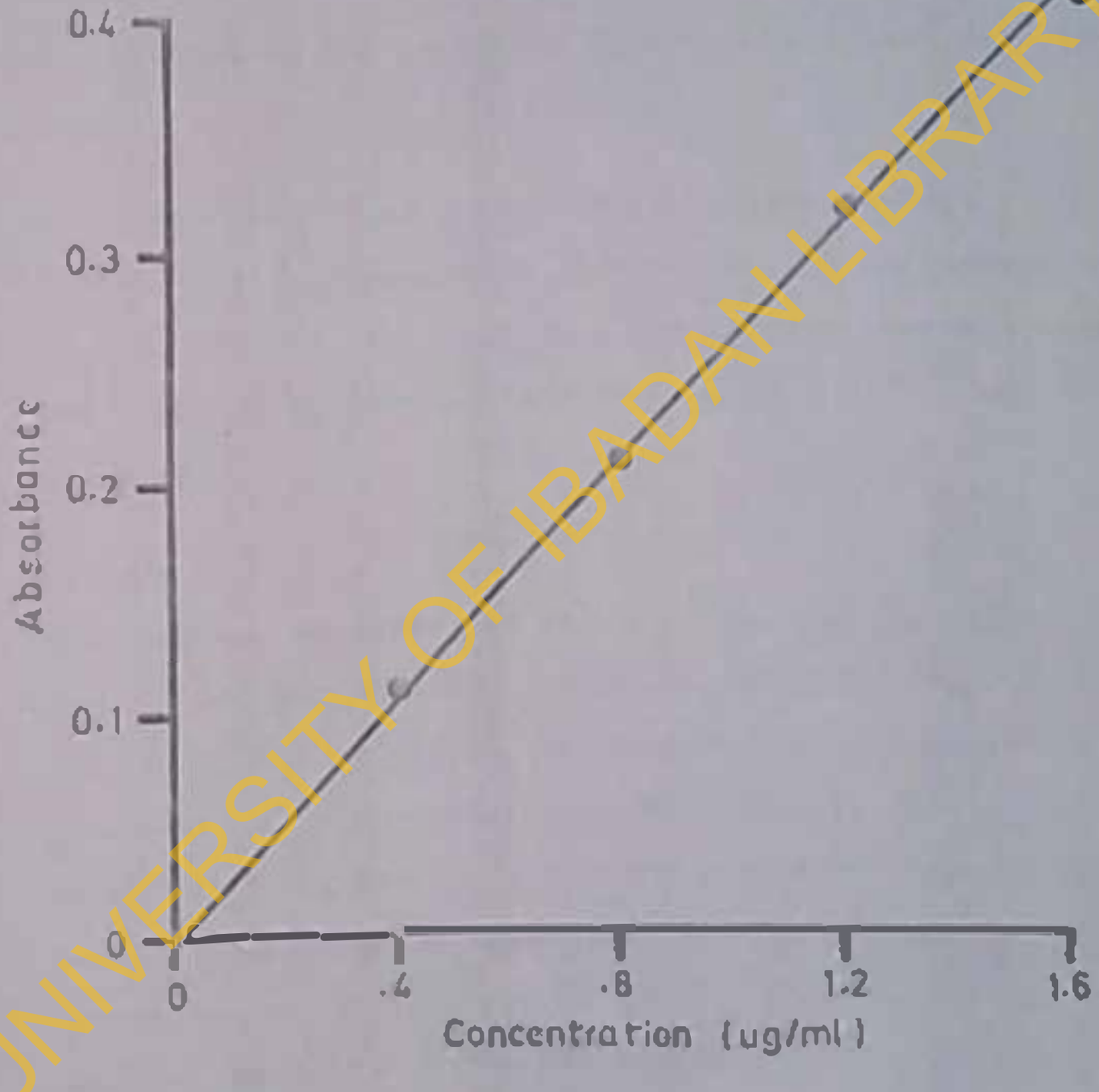
A3 = absorbance at 620nm of Vitamin A (corrected for absorbance contributed by b-carotene)

X is factor which converts the carotene absorbance at 450 nm into the equivalent absorbance at 620 nm in the color reaction.

$$\frac{\text{(F-value) of Carotene using Vitamin A procedure}}{\text{A450 of petroleum ether solution of carotene}}$$

ug Vitamin A (free alcohol)/100ml

FIG. 3.2
VITAMIN A STANDARD CURVE



$$= \frac{A3 \times \text{ug retinol standard/cuvet}}{A620 \text{ retinyl acetate standard}} \times \frac{3}{2} \times 100 \times 0.872$$

where

3 = volume of the petroleum ether extract of 1.0ml serum.

2= aliquot of the petroleum ether extract used for the assay.

100=convertion of ug retinol/ml to retinol/100ml.

0.872= ratio of molecular mass of retinol to molecular mass of retinyl acetate. Thus this factor corrects for the use of retinyl acetate instead of retinol as the standard.

Albumin

The method employed is the modification of that of Doumas and Biggs (1972) optimized for Sigma Catalog No 625 2. The principle of the method involves the reaction of human serum albumin specifically with bromocresol purple (BCP) to form a stable blue purple color complex with an absorption maximum at 600nm. The intensity of the colour is proportional to the serum albumin concentration in the sample.

Procedure

1 ml of albumin reagent (BCP) was added to tubes labelled blank, standard and test. To standard, test, and blank 0.01 ml albumin standard, serum, and saline

were added respectively. The solutions were mixed together and the absorbance of standard and test read against the blank at 500nm.

CALCULATIONS

Albumin concentration (g/dl) of sample =

$$\frac{A\ test}{A\ Std} \times \text{concentration of standard}$$

Retinol binding protein, and transthyretin

The method employed in the analysis of serum retinol binding protein, and transthyretin was a modification of the radial immunodiffusion procedure described by Mancini (1965). The commercial plates used were obtained from Calbiochem Behring Corp., Lajolla, Calif.

Procedure

The control serum for the different partigens was introduced into well 1 and serum samples into wells 2-12 of the different plates (fig. 3.3).

After a diffusion period of 2 days the diameters of the precipitates were measured to an accuracy of 0.1mm using the measuring template.

The concentration corresponding to the precipitate ring diameters measured were read from the table of calibration values. The accuracy of the result was

FIG. 3-3

RADIAL IMMUNODIFFUSION PLATE SHOWING CIRCLES OF ANTIGEN-ANTIBODY REACTION TO DETERMINE SERUM RETINOL BINDING PROTEIN AND TRANSTHYRETIN.

checked by means of control serum for Nor-partigen and the batch-dependent precipitate ring diameter was within the confidence range (D = +0.3mm).

Standards for each parameter were prepared at 25%, 50%, 75%, and 100% concentrations. The D values were read and plotted (figs. 3.4 and 3.5). The values for the samples were read off the calibration curves for the two parameters.

3.3 ANALYSIS OF FOOD SAMPLES

Each food sample was dried in an oven at 60 °C for 72 hours until a constant weight was obtained. Each sample was then analysed for lipid, nitrogen, calorie, B-carotene and Vitamin A.

TOTAL LIPID

The method employed was the ether extraction method. The principle of the method is based on the fact that non-polar components of samples are easily extracted into organic solvents.

Procedure

A soxhlet apparatus extractor with a reflux condenser and a round bottom flask was set up. 5g of each dried food sample was weighed into an oven dried fat-free extraction thimble of a known weight (W1). The thimble was placed in the [illegible] petroleum ether (BP, 40

FIG. 3-4 :
RETINOL BINDING PROTEIN.
Standard Curve.

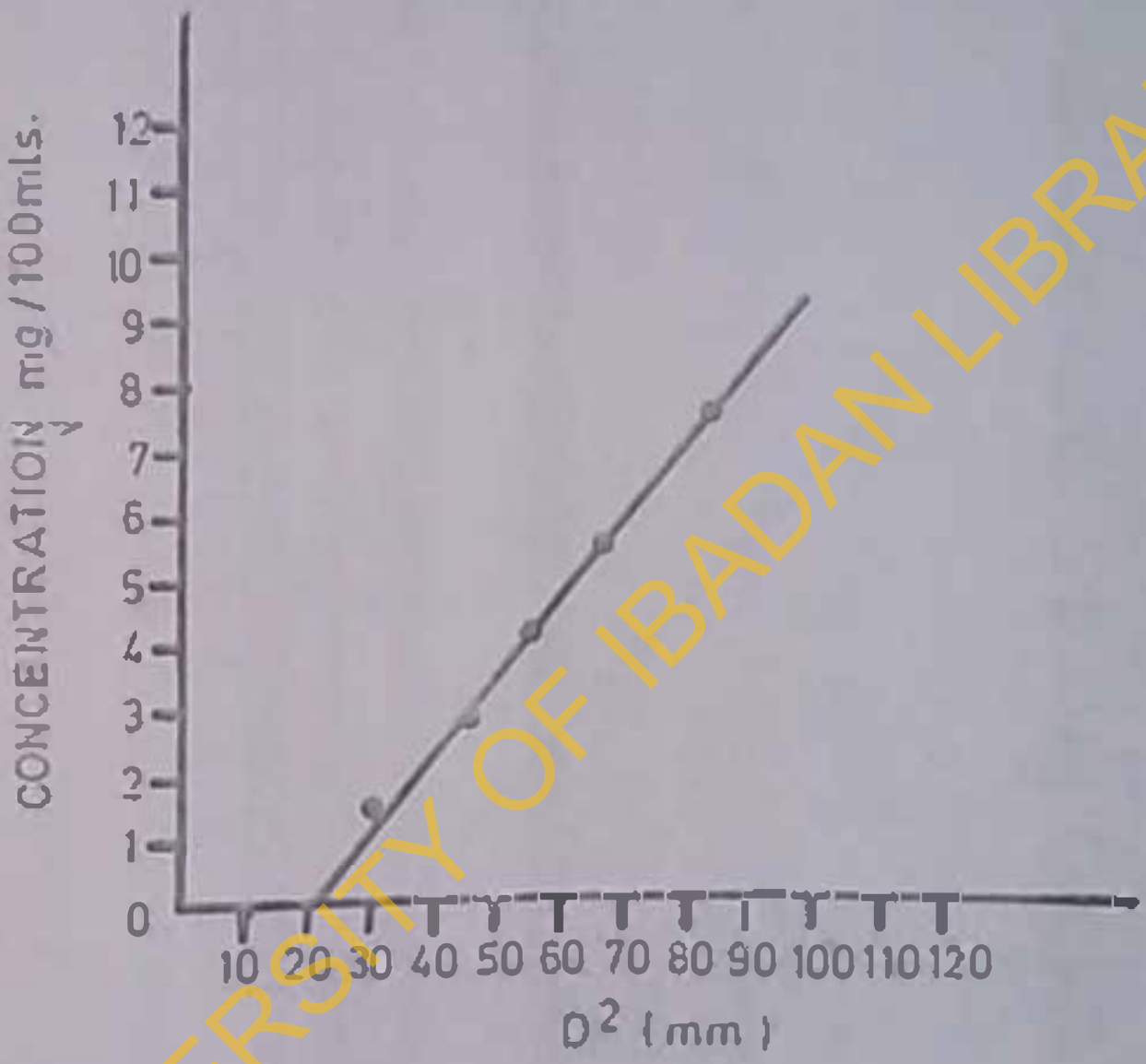
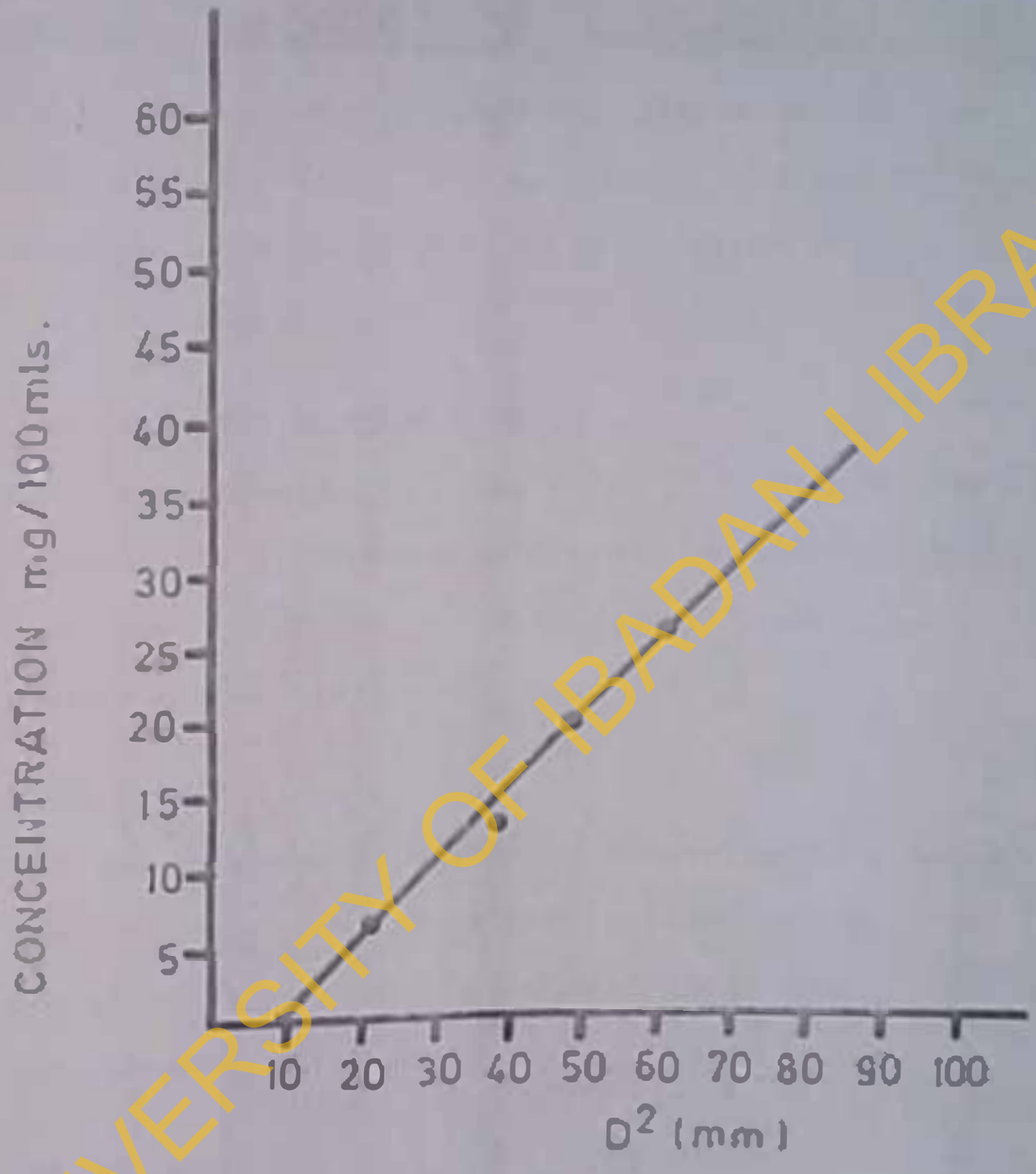


FIG. 3 - 5:

TRANSTHYRETIN STANDARD CURVE



checked by means of control serum for Nor-partigen and the batch-dependent precipitate ring diameter was within the confidence range (D ± 0.3mm).

Standards for each parameter were prepared at 25%, 50%, 75%, and 100% concentrations. The D values were read and plotted (Figs. 3.4 and 3.5). The values for the samples were read off the calibration curves for the two parameters.

3.3 ANALYSIS OF FOOD SAMPLES

Each food sample was dried in an oven at 60°C for 72 hours until a constant weight was obtained. Each sample was then analysed for lipid, nitrogen, calorie, β-carotene and Vitamin A.

TOTAL LIPID

The method employed was the ether extraction method. The principle of the method is based on the fact that non-polar components of samples are easily extracted into organic solvents.

Procedure

A soxhlet apparatus extractor with a reflux condenser and a round bottom flask was set up. 5g of each dried food sample was weighed into an oven dried fat-free extraction thimble of a known weight (W1). The thimble was placed in the extractor and petroleum ether (B.P. 40°

- 60°C) was filled half way through. The condenser was then replaced, the connecting joints tightly fixed, the extractor was placed on the heater. The source of heat was adjusted to allow the solvent boil over gently and left to siphon until the barrel in the extractor was empty. The condenser was detached and the thimble was removed, placed in a fat-free dry beaker and dried in the oven at 50° to a constant weight (W3).

Calculation:

Wt of fat in the sample =

Weight of extracted sample

Weight of sample

$$= \frac{W2 - W3}{W2 - W1} \times 100$$

TOTAL PROTEIN

The method employed was the modified Kjeldahl method (A.O.A.C. 1977)

Principle

The principle of the method involved the reduction of organic nitrogen to ammonium sulphate and carbon. The carbon is then oxidised to carbon dioxide while the ammonium sulphate is distilled to produce ammonia which is then reacted with NaOH.

Procedure

Macro-Kjeldahl

2g dry sample was weighed into a macro-kjeldahl flask together with 6 glass beads, 1 tablet each of Cu, Se, and Sodium Sulphate, and 40ml concentrated sulphuric acid. This was digested on low heat for 30 min, then on medium heat for the next 30 min, and finally on full heat until digested. Frothing was prevented by using low heat to start the digestion process. The heat was maintained just below boiling point for 45 min after the digest has been cleared. The sample was cooled and diluted to a total volume of 50ml after cooling to room temperature. sample was transferred into a 100ml volumetric flask and made up to volume. 20ml aliquots was distilled using Markham distillation procedure. 0.1ml HCl was employed in the receiving flask to trap the liberated ammonia. The distillate was mixed with 20 parts methyl red and then titrated with 0.1 NaOH.

Calculation

The Nitrogen content of the sample was calculated in percentage based on the dilution factor:

1ml 0.1M HCl = 1ml 0.1M NaOH

1ml 0.1 NH_3 = 1.4 mg N

Total protein (mg) = Volume of acid neutralized by titration x 1.4 factor

$$\% \text{ protein (dry matter)} = \frac{\text{mg protein}}{\text{mg sample}} \times 100$$

Recovery

Standard ammonium solution was dried in an oven at 105° overnight. A 2% solution of ammonium sulphate was prepared and 2 ml of it was treated like the sample. The percentage nitrogen recovery was calculated. The recovery in this case was 96.8%.

ENERGY

The calorie content of the food samples was analyzed using the Bomb Calorimetry method.

Procedure

The dried food samples were thoroughly mixed before weighing to ensure a homogenous and representative sample. 0.5g of the dried food sample was weighed on a balance into a dry labeled crucibles of known weights. 0.5 of benzoic acid was used as the standard and put into another crucible while an empty crucible was used for blank reading.

The crucibles were placed on the pillar of the base of the bomb calorimetry one after another and a length of 5 centimetre serving thread that was supplied with the apparatus was inserted between the coils of the platinum

wire and the other end was dipped into the centre of the sample in the crucible. Same cotton length was used for all the samples standard and blank. The bomb calorimetre was then lowered and locked by rotating it for the thread to engage firmly. The thermo couple was plugged into the hole and the bomb body and the valve on the bomb calorimetre was closed while the inlet on the front panel of the control box was opened by 1/4 turn. The pressure was allowed to rise to 25 atmosphere by opening the oxygen cylinder for about 20 seconds and the valve was then closed and the cylinder locked. It is assumed that the pressure is enough to ensure adequate combustion of any biological materials. By means of the Calvo Zero Knobs of the control box the light spot index of the galvanometre was brought to zero and the firing button of the bomb was then released.

Maximum deflection of the galvanometre was recorded after each bombing. The used gas was then released from the bomb by opening the pressure release valve at the right side of the base of the bomb. The body of the bomb was released and placed in cold water to cool so that heat is not transferred to the next sample bombed. After cooling, the body of the bomb was then wiped with a clean piece of cloth and the next sample treated as the first.

Calculation

E value of benzoic acid standard = 0.596 kCal/0.58

E value of food = kcal/0.58

Correcting for standard X (0.596

B-Carotene and Vitamin A

50mg of each powdered food sample was homogenized with 2ml of water. Saponification of the lipids was carried out using 4ml ethanolic potash (1ml 50% salt solution and 3ml ethanol) for 10 min. Vitamin A was extracted from the unsaponifiable fraction with diethyl ether and washed with water by adding anhydrous sodium sulphate and the clear yellow colour was measured spectrophotometrically at 450nm for its carotene content. Aliquots (4ml) were dried in the water bath at 37°C, they were dissolved in 0.1 chloroform and trifluoroacetic acid was added for the development of the Vitamin A colour complex which was read at 620nm.

$$\frac{\text{ug B-carotene (per 100ml)}}{\text{mg of tissue used}} = \text{ug B- carotene per mg of tissue}$$

$$\frac{\text{ug Vitamin A (per 100ml)}}{\text{mg of tissue used}} = \text{ug Vitamin A per mg of tissue}$$

STATISTICAL ANALYSIS

All data collected were fed into an IBM compatible personal computer and analysed using the Oxstat and packages. Mean +/- Standard deviations, Unpaired t-test, paired t-test, Analysis of variance, Pearson's correlation and Regression methods of data analysis were employed as appropriate.

C H A P T E R F O U R

R E S U L T S

This study was carried out in three phases and the results will be presented as such under the following headings:

1) Cross Sectional study (C S S)

2) Relative Dose Response Test (R D R T)

3) Longitudianl Study (L S)

All the subjects investigated in this study were made up of healthy pregnant women in the three trimesters of pregnancy while their healthy controls were non pregnant non lactating (NPNL) women in the proliferative phase of the menstrual cycle.

CROSS SECTIONAL STUDY: A CASE STUDY OF 206 SUBJECTS AND CONTROLS.

171 pregnant subjects and 35 (NPNL) controls were studied in this phase. The subjects were classified into three groups (according to the gestational age) into trimesters 1, 2 and 3 with 22, 88 and 61 subjects respectively.

The ages of the subjecys and the controls ranged between 18 and 47 yeare and 21 and 48 years respectively. Table 1 shows tthe mean +/- S.D of the ages. The age

distribution of the subjects and the controls within the different age range is shown in Table 2.

Parity for the subjects and the controls ranged between 0 and 8. The means +/-S.D are shown in Table 1.

TABLE 1

Mean (+/- SEM) age and parity of subjects and controls

	N	AGE	PARITY (yrs)
C	33	29.46+/- 7.5	1.17+/- 1.48
T1	22	26.95+/- 7.0	1.40+/- 1.22
T2	88	27.02+/- 6.4	1.65+/- 1.3
T3	61	27.75+/- 6.4	1.92+/- 1.59

T1 - 1st Trimester
T2 - 2nd "
T3 - 3rd "

From the analysis of the questionaire, the subjects and the controls were further classified into the different socio-economic classes based on profession, educational attainment and income using the method by. Table 3 shows the different classes in each group. The husbands of the subjects and the controls were traders (both petty and large scale), carpenters, farmers, teachers, medical

TABLE 2

Age distribution of the subjects and the controls in the cross-sectional study.

	Subjects			controls
Age range	T1	T2	T3	C
18-24	9	34	23	14
25-31	10	37	22	9
32-38	1	11	13	8
39-45	1	6	3	3
46-51	1	-	-	1

T1 - 1st trimester of pregnancy
T2 - 2nd " " "
T3 - 3rd " " "
C - Controls

---

practitioners, architects, administrators, and civil servants of various caders. The women on the other hand were engaged in various profit making activities such as those of the men but majority were teachers, civil servants and petty traders.

DIET

The analysis of the dietary patterns and habits of the subjects and the controls revealed that 59% of the study population consumed 3 meals per day and all of these belonged to the social classes one to three. 30% ate twice and the remaining 13% once daily. The reasons

given for this attitude varied and it ranged from lack of fund to such symptoms as nausea. Table 4 shows the various reasons given for missed meals. A list of B-carotene and vitamin A rich foods and the consumption pattern is given in Table 5.

TABLE 3

Socio-economic classification of subjects and controls in the cross sectional study

CLASS	SUBJECTS			CONTROLS
	T1	T2	T3	C
1	5	10	11	6
2	6	20	14	6
3	3	19	9	10
4	4	22	11	6
5	4	17	16	7
Total	22	88	61	35

Class 1- Academic professionals, senior administrators, proprietors.

Class 1- Non academic professionals, Nurses, Secretaries and Teachers.

Class 3- Non manual skilled workers, Clerks, Typists, Police officers.

Class 4- Manual skilled workers, Drivers, Carpenters, Goldsmith.

Class 5- Semi-skilled (unskilled workers) labourers, small-scale farmers.

TABLE 4

The reasons given for missed meals by the pregnant women.

Reasons	Number
Lack of fund	72
Lack of time	105
Nausea	44
Loss of appetite	41
Other illnesses	25
Weight reduction	20

TABLE 5

A list of β-carotene and Vitamin A rich foods and their consumption pattern.

	T1	T2	T3	C	X
N	22	88	61	35	
Food items					
Palm oil	20	76	56	32	86
Carrots	12	28	17	16	37
Mangoes	14	52	33	22	58.5
Liver	3	10	6	9	14
Milk	11	39	25	11	41.7
Eggs	14	32	23	8	37.4

The consumption of B-carotene rich foods was highest for red palm oil followed by mangoes and lastly carrots. The intake of vitamin A containing foods on the other hand was highest for milk followed by eggs with liver coming last.

86% of the total population used red palm oil more than four times a week, whilst only 14% consumed any form of liver once a week. 37% took carrots and 58.5% had mangoes twice a week.

Analysis of the vitamin A deficiency signs and symptoms showed that 6(3.5%) subjects and 2(5.7)controls had night blindness. Old corneal scars were observed in 23(11%) of the total population.

BLOOD AND PLASMA

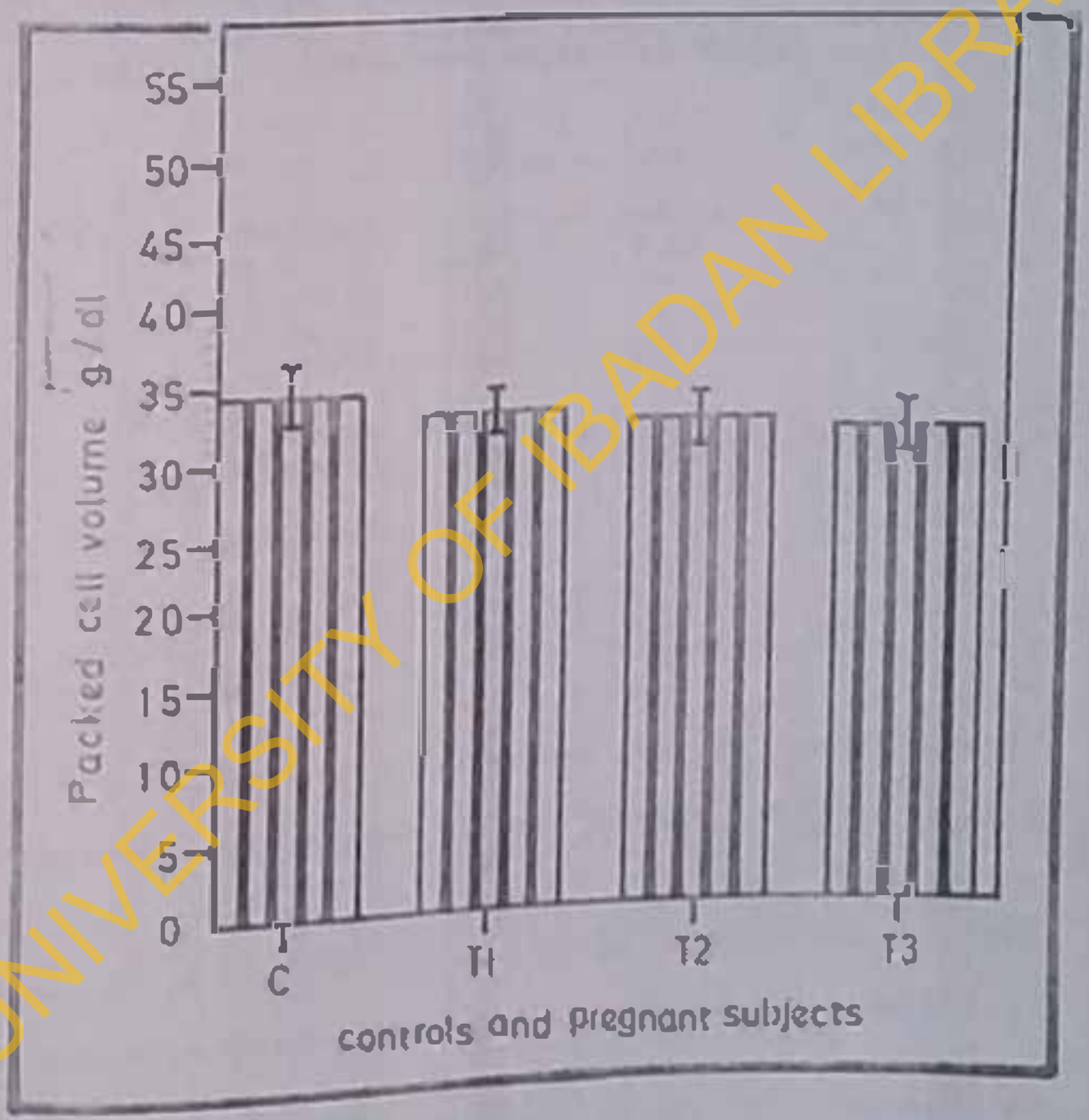
The mean packed cell volume (PCV). B-carotene (BC). Vitamin A (VITA) and albumin (ALB) values +/- SD for the subjects and the controls are shown in Table 6.

The pcv values for the controls ranged between 32% and 40% while those of the subjects was between 28% and 38%. Fig. 4.1 shows the pattern in the subjects and the controls. 2% of the subjects in the 3rd trimester of pregnancy had PCV levels less than 30%. Analysis of variance as shown in Table 7 revealed that the subjects in the different trimesters had lower plasma values than

FIG. 4-1

MEAN PACKED CELL VOLUME VALUES OF SUBJECTS.

& Controls in the Cross-Sectional Study



the controls (P< 0.01). The PCV of the subjects decreased as pregnancy progressed (P < 0.05) despite the purported regular in take of folic acid and ferrous sulphate in 82% of the subjects throughout pregnancy.

TABLE 6

Mean packed cell volume (PCV) vitamin A, (VITA) b-carotene (BC) and albumin (ALB) of the subjects and controls in the cross sectional study

	N	PCV (%)	BC (ug%)	VITA (ug%)	ALB (gm%)
C	35	34.6+/- 1.8	73.6+/- 16.89	29.0+/- 4.25	3.6+/- 0.3
T1	22	33.1+/- 1.6	71.5+/- 17.1	29.7[illegible]+/- 7.44	3.5+/- 0.3
T2	88	32.5+/- 1.8	70.0+/- 16.7	25.37+/- 5.61	3.4+/- 0.4
T3	61	31.9+/- 1.7	70.8+/- 17.0	23.11+/- 6.5	3.3+/- 0.4

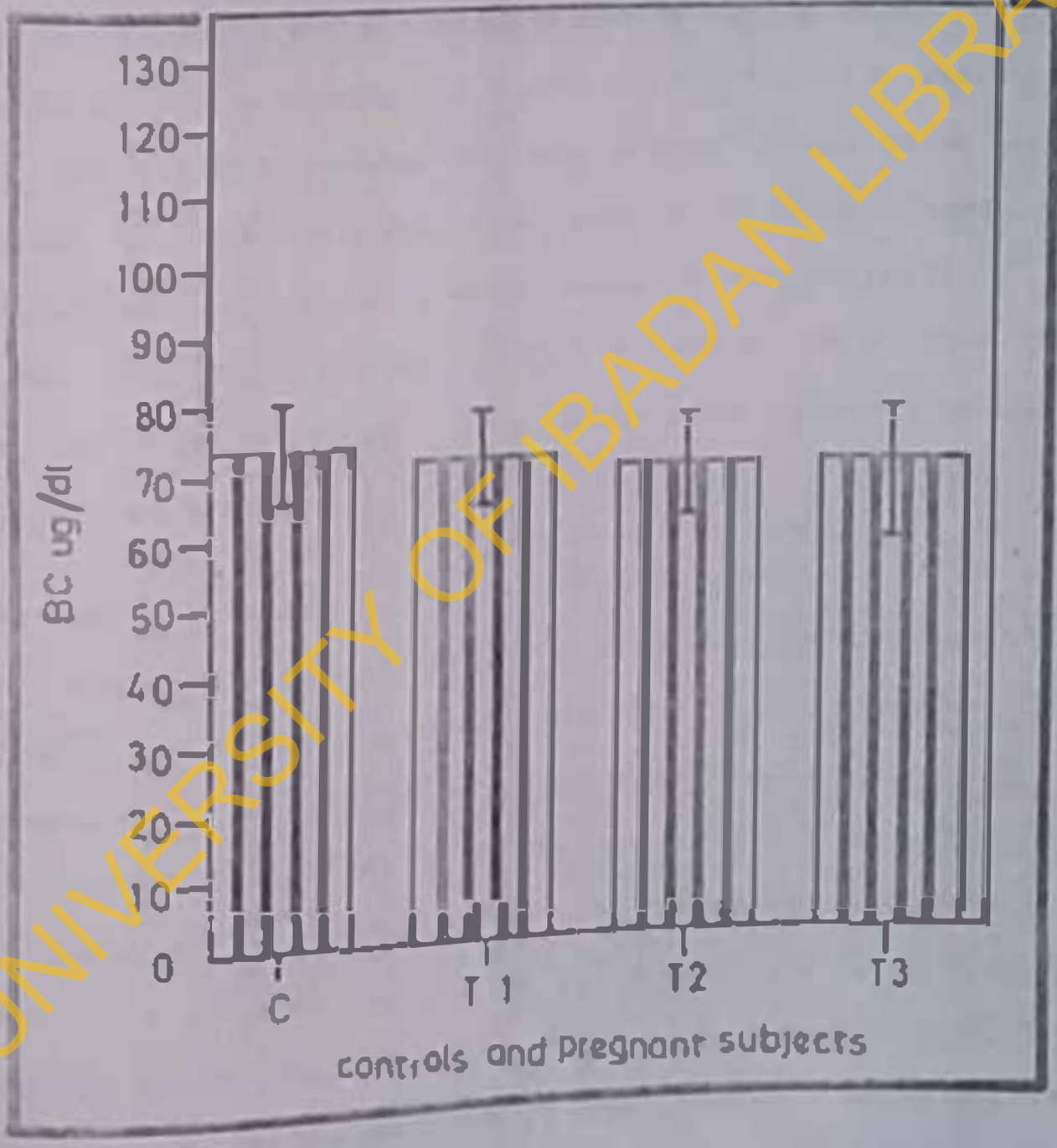
T1 - 1st trimester of pregnancy
T2 - 2nd " " "
T3 - 3rd " " "
C - Controls.

The PCV values were observed to be significantly correlated with plasma albumin levels in the subjects and the controls (P< 0.05; r > 0.3). Analysis of variance of the PCV values showed no difference within the subjects and between the subjects and the controls.

B-carotene levels were between 45 ug% and 120 ug% for both the subjects and the controls. Table 6 shows the mean +/- S.D while Fig. 4.2 shows the pattern of plasma

FIG. 4-2

Mean Plasma B Carotene values of subjects and controls in the Cross-Sectional study



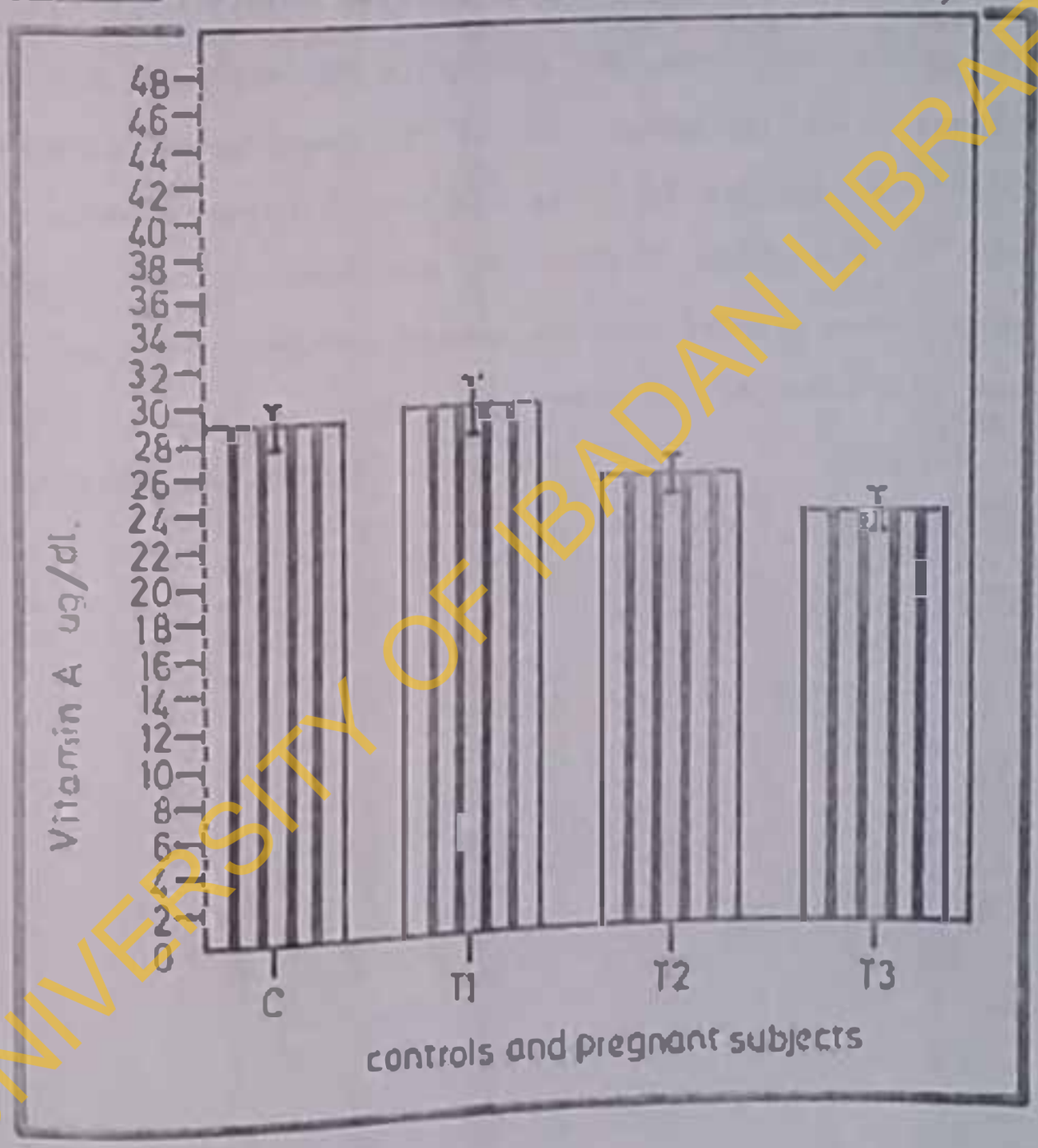
B-carotene levels in the subjects and the controls. Only 2% of the total population had B-carotene levels less than 50ug/dl.

The mean plasma vitamin A levels ranged from 19.1ug to 41.9ug/dl for the controls and 9.1 to 52.3 for the subjects. The controls had vitamin A levels higher than those of the subjects ($P < 0.01$) except for the subjects in the first trimester who had vlaues comparable with those of the controls. The level of plasma vitamin A decreased as pregnancy progressed in the subjects ($P < 0.01$) Fig.4.3. Plasma vitamin A levels were observed to be significantly correlated with plasma albumin levels ($r > 0.5$; $P < 0.05$).

Further analysis of the data showed that only 1 (0.5%) of the total population had plasma vitamin A less than 10ug/dl and she belonged to the 3rd trimester of Pregnancy. 24 (11.7%) had values in the range of 10 - 19.6ug/dl. The latter group belonged to the 2nd and 3rd trimesters of pregnancy. 14 (23.3%) of the subjects in the 3rd trimester had plasma vitamin A levels between 10 and 19.6ug/dl. 60.2% of the pregnant subjects had plasma vitamin A levels between 20 and 29ug/dl majority of which belonged to the 3rd trimester of pregnancy. The cut off levels used are based on the WHO criteria for identifying both clinical and subclinical vitamin A

FIG. 4-3

Mean Plasma Vitamin A Values of Subjects and controls in the Cross-Sectional study



deficiency in any population.

Plasma albumin levels ranged between 2.9 and 4.2g/dl in the controls while in the pregnant subjects it was 2.2 to 4.0g/dl. The mean +/-S.D are shown in Table 6. Plasma albumin levels were significantly higher in the controls than the subjects (P< 0.01) and the values decreased as Pregnancy progressed (P< 0.05). Plasma albumin levels were significantly correlated with vitamin A in both the subjects and the controls (P< 0.05; r >0.5). 47% of the Pregnant subjects had plasma albumin levels lower than 3.5g/dl. Fig.4.4. shows the levels in the controls and the subjects.

TABLE 7

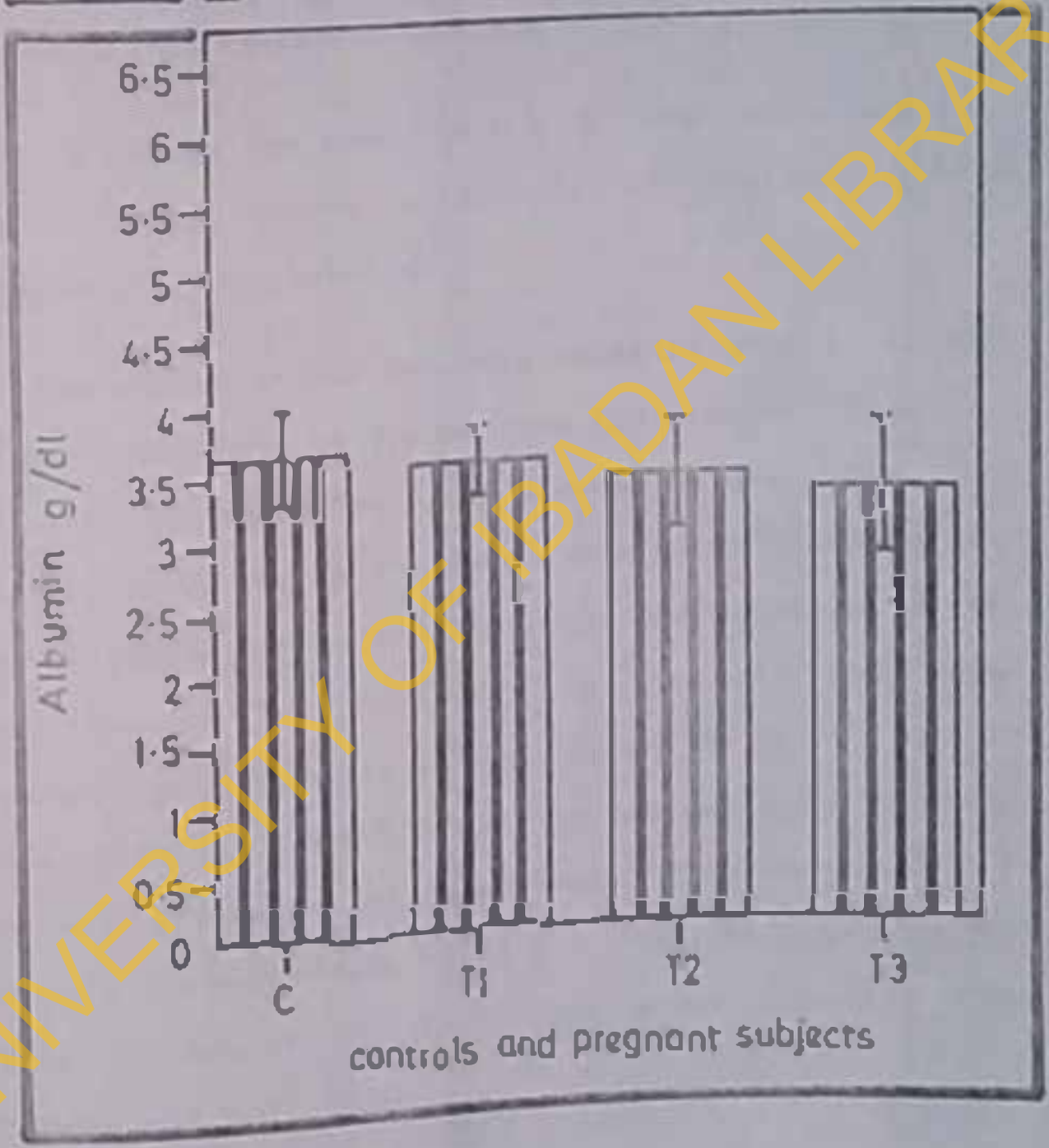
Analysis of variance between controls and subjects and within subjects in each trimester.

PARAMETERS TESTED	CALCULATED F VALUE	TABULAR F VALUE	LEVEL OF SIGNIFICANCE
Weight			
C vs S	6.82	2.68	0.01
Bet S	6.38	3.07	0.01
PCV			
C vs S	21.53	2.68	0.01
Bet S	3.71	3.07	0.05
BC			
C vs S	0.97	2.68	N.S
Bet S	0.78	3.07	N.S
VIT A			
C vs S	13.53	2.68	0.01
Bet S	12.36	3.07	0.01
ALB			
C vs S	6.57	2.68	0.01
Bet S	3.98	3.07	0.05

N.S - Not significant at P < 0.05
C - Non pregnant non lactating mothers
S - Pregnant subjects in the different trimesters
PCV - Packed cell volume
B-C - B-carotene
VITA- Vitamin A
vs - versus.

FIG. 4-4

Mean Plasma Albumin values of subjects and controls in the Cross-Sectional study



RELATIVE DOSE RESPONSE TEST

Vitamin A status of women was determined using fasting plasma vitamin A levels and relative dose response (RDR) procedure. The RDR was calculated by obtaining a fasting vitamin A level (A0), feeding 450ug retinol equivalent and obtaining a second specimen after 5hr (A5). The RDR was calculated as RDR = A5 - A0/ A5 X 100.

Table 8 shows the mean +/- S.D of the ages, weight, height, basal plasma vitamin A, RDR values of the subjects and the controls.

The RDR values of the subjects revealed that 4 (13.3%) of the 30 subjects studied had RDR greater than 20% and all of these had basal plasma values less than 20ug/dl. Three out of the four subjects belonged to the 3rd trimestery while the remaining one belonged to the 1st trimester of pregnancy. 12 subjects had basal plasma vitamin A levels between 21 and 29 ug/dl. Out of these 2(16.7%) had RDR >20%. The two subjects were in the 3rd trimester of pregnancy. The remaining 14 subjects who had plasma vitamin A >30ug/dl also had RDR values < 20ug/dl. In the control group none of tahe subjects had RDR > 20%.

TABLE 8

Mean age weight height basal plasma vitamin A and RDR values of pregnant subjects and the NPNL controls.

	N	AGE	WT	HT	BASAL	RDR
C	10	30.2+/- 2.9	57.5+/- 3.4	155.6+/- 4.0	28.6+/- 3.3	15.2+/- 3.2
T1	10	29.7+/- 4.2	59.2+/- 4.4	156.7+/- 4.0	27.0+/- 4.8	14.8+/- 3.7
T2	10	28.9+/- 3.4	63.7+/- 4.0	156.9+/- 2.2	26.3+/- 4.7	16.1+/- 3.2
T3	10	29.1+/- 4.0	67.6+/- 2.8	157.4+/- 2.0	23.3+/- 3.5	19.4+/- 4.0

WT - Weight

HT - Height

RDR - Relative dose response

T1 - 1st trimester of pregnancy

T2 - 2nd " "

T3 - 3rd " "

C - Non pregnant non lactating controls

LONGITUDINAL STUDY: VITAMIN A: SINGLE BLIND PLACEBO-CONTROLLED STUDY OF ITS SUPPLEMENTATION EFFECT ON HEALTHY PREGNANT NIGERIAN WOMEN

29 pregnant subjects and controls with normal liver function tests were carried to the end of the study bringing the drop out to 23 (44.2%). 15 subjects and 14 controls were taken through the study but a report will be presented on 28 (14 subjects and 14 controls).

The controls were the placebo group while the subjects were the treated group fed 7,000iu vitamin A in oil daily from the 14th week of pregnancy to 6weeks post partum. They were properly matched for age, age of pregnancy, parity, weight, height. (table 9 shows the means +/-S.D). Blood samples were obtained from each subject and control from the 14th week of pregnancy before supplementation until 6weeks post partum and also from their babies at paturition. They were bled six times during the course of the study. The 1st and the 2nd bleeds represented the 1st and the 2nd halves of the 2nd trimester; the 3rd and the 4th represented the 1st and 2nd halves of the 3rd tirmester; and the 5th and 6th bleeds represented immediate post partum and 6 weeks post partum periods. The plasma samles were analysed for B-carotene, Vitamin A, albumin, retinol binding Protein (RBP) and transthyretin (TTR). The food intake of 14 of the subjects and the controls was assessed

TABLE 9

Mean (+/- S.D) age, parity, weight, of the subjects and the controls (14 subjects in each group

	AGE (yrs)	PARITY (kg)	WEIGHT
controls	27.8+/- 5.44	1.07+/- 1.21	56.92+/- 7.81
subjects	28.5+/- 1.38	1.57+/- 0.34	59.86+/- 1.95

Using the method outlined in the chapter on materials and method.

COMPLIANCE AND SIDE EFFECTS

We assessed compliance of the subjects and the controls by Pill count. Overall 98% and 94% of the subjects and the controls respectively took their pill regularly but only those that had 100% compliance are included in this report.

Side effects were almost non existent except for one subject who complained of persistent headache and was immediately removed from the study.

PACKED CELL VOLUME

The PCV values of the subjects were maintained with oral vitamin A (7,000 I.U) in oil throughout the study

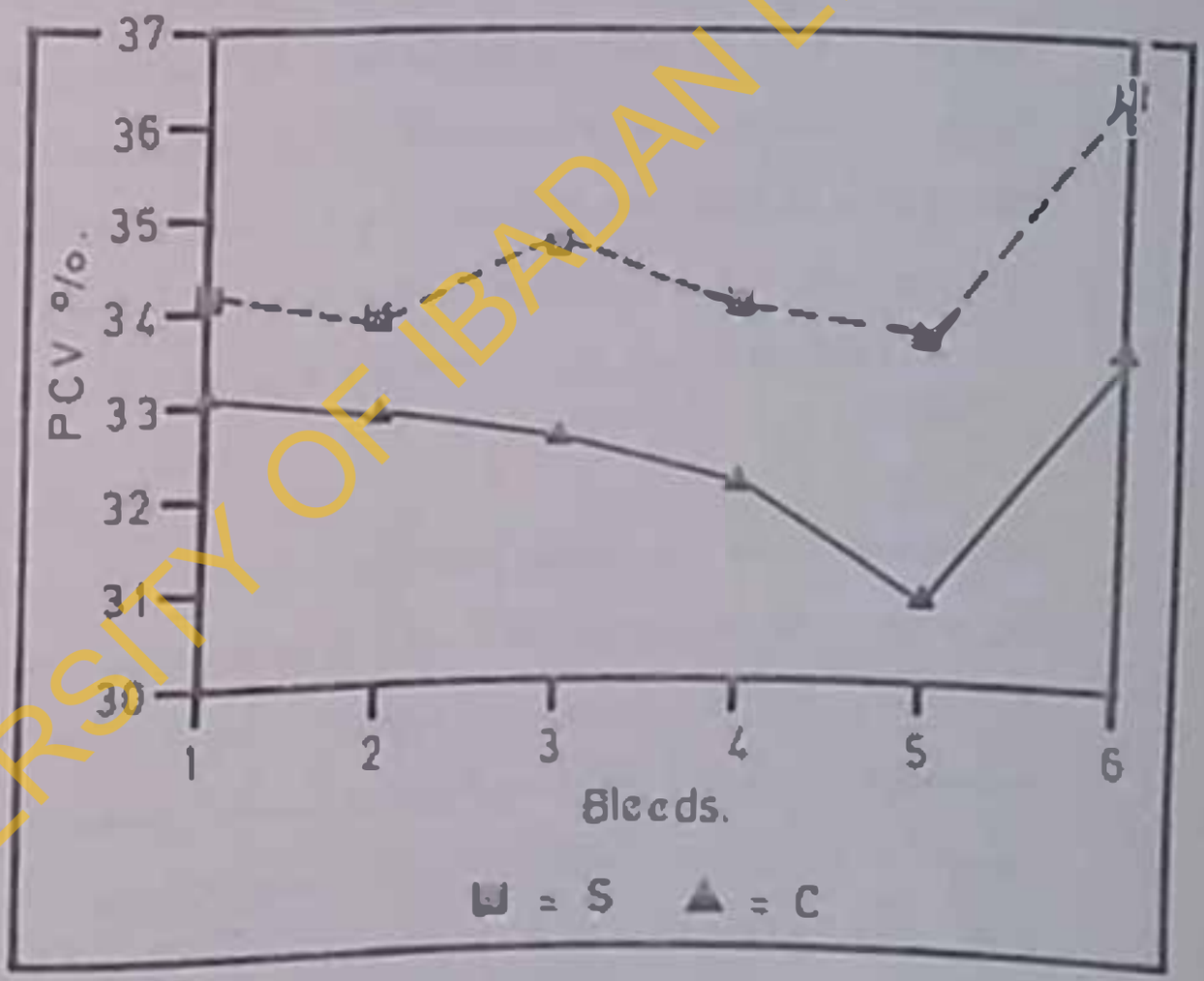
period. Those of the placebo controls decreased ($P < 0.05$) as pregnancy progressed and went up again at 6 weeks post partum. However their values were still significantly lower than those of the controls. The PCV values correlated significantly with plasma albumin and TTR levels throughout the study period ($P < 0.05$). The change in the PCV levels throughout pregnancy is as shown in Fig.4.5.

PLASMA VITAMIN A

The plasma vitmain A levels in the supplemented subjects increased progrssively throughout the study period while those of the controls decreased with increasing age of pregnancy ($P < 0.05$). The levels in the subjects and the controls did not differ in the 1st and the 2nd halves (1st and 2nd bleeds) of the second trimester. The values in the subjects were significantly higher than those of the control by the 1st half (3rd bleed) of the third trimester and the difference was maintained until the 6th week postpartum ($P<0.001$). The mean values are shown in Table 10.

In the subjects there was a 7ug% increase in the plasma vitamin A mean value at the 1st half of the third trimester as compared to the level at admission. The difference decreased to 3ug% with the progression of pregnancy but went up to the previous level at 6 weeks

FIG. 4-5 Mean Packed Cell Volume (PCV) values of subjects and controls in the Longitudinal study



post partum. This difference though small was observed to be significant ($P<0.05$). Fig. 4.6 shows the change in plasma vitamin A levels as pregnancy progressed. Plasma vitamin A levels correlated significantly with RBP in both the controls and subjects and to ALB and TfR in the controls ($P<0.05$). The plasma levels of vitamin A in the controls however remained the same.

PLASMA B-CAROTENE

The B-crotene levels did not change throughout the study period neither in the controls nor in the placebo group. B-C was observed to correlate significantly with vitamin A in the controls at the 3th and 6th bleeds and to RBP at the 4th to the 6th bleeds. Fig.4.7 shows the pattern of change in the subjects and the controls.

PLASMA ALBUMIN

Plasma albumin levels did not change in the subjects but decreased significantly in the controls as pregnancy progressed ($P< 0.05$). In the vitamin A supplemented subjects, plasma albumin levels were significantly correlated with PCV and TfR ($P<0.05$). Table 11 shows the mean +/-S.D of plasma albumin levels in the supplemented and placebo groups . The pattern of change in plasma albumin for the two groups are shown in Fig. 4.8

FIG. 4 – 6

Mean Plasma Vitamin A (VIT. A) values of subjects and controls in the Longitudinal Study

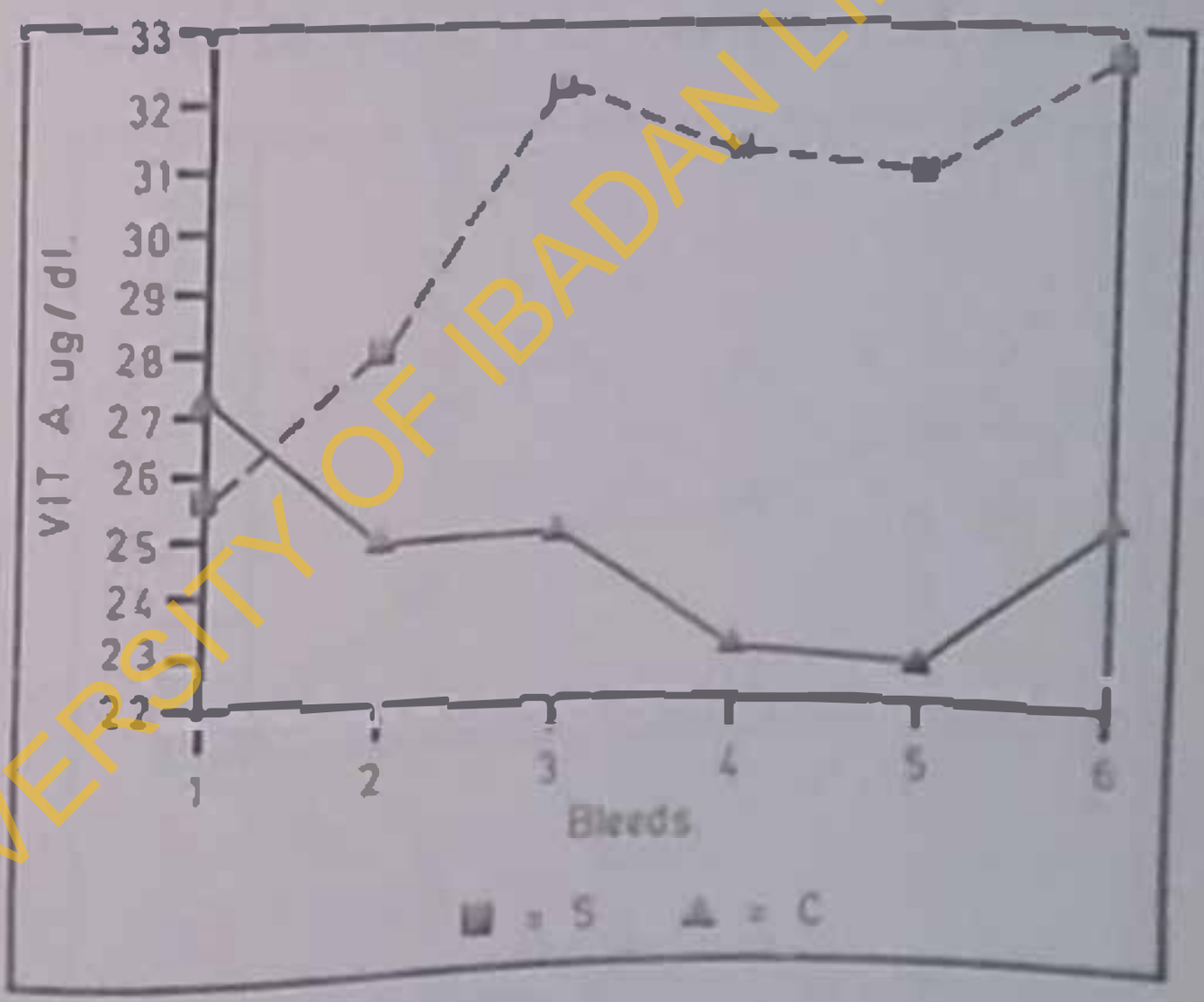


FIG. 4 – 7

Mean Plasma β-Carotene (BC) values of subjects and controls in the Longitudinal study

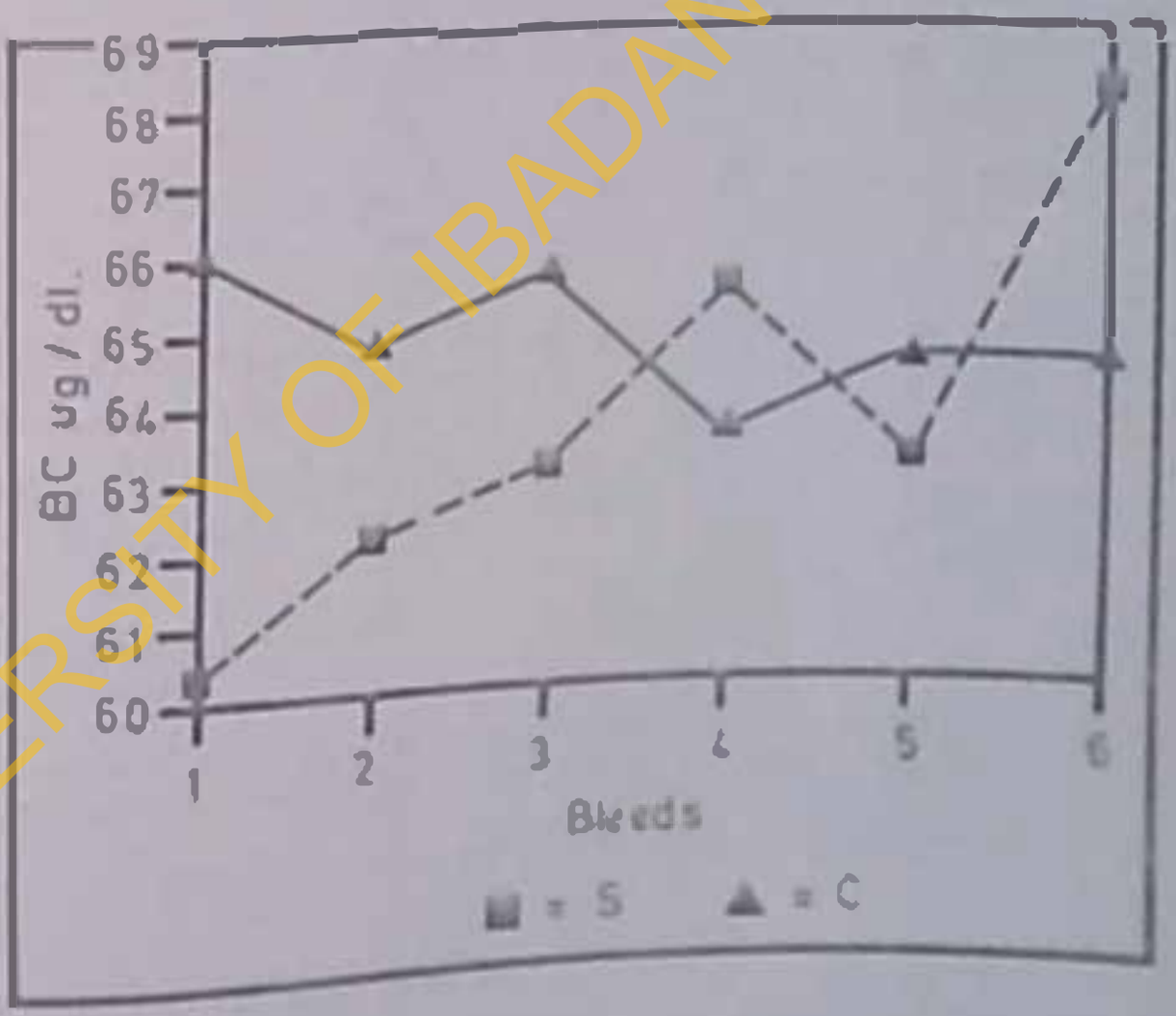


TABLE 10

Mean +/-S.D, PCV, VIT, and B-C, levels in the supplemented subjects and placebo controls (14 subjects in each group

No of bleeds :	1	2	3	4	5	6
PCV(%)						
S	34.1+/- 2.3	33.3+/- 2.3	34.7+/- 1.4	34.0+/- 1.6	33.7+/- 1.9	34.3+/- 1.7
C	33.1+/- 3.8	32.9+/- 3.1	32.6+/- 3.03	32.1+/- 2.97	30.9+/- 2.3	33.6+/- 1.86
VITA(ug/dl)						
S	25.6+/- 6.1	27.9+/- 5.7	32.3+/- 4.4	31.2+/- 4.9	30.8+/- 4.6	32.8+/- 4.4
C	27.2+/- 6.9	24.7+/- 5.1	24.8+/ 5.6	25.8+/- 6.2	22.3+/- 5.5	25.0+/- 5.8
B-C(ug/dl)						
S	60.2+/- 16.9	62.1+/- 17.7	63.0+/- 18.9	65.4+/- 13.7	63.0+/- 13.8	68.1+/- 13.1
C	66.0+/- 14.8	64.7+/- 12.4	65.6+/- 11.3	63.4+/- 10.3	64.3+/- 10.1	64.3+/- 10.1

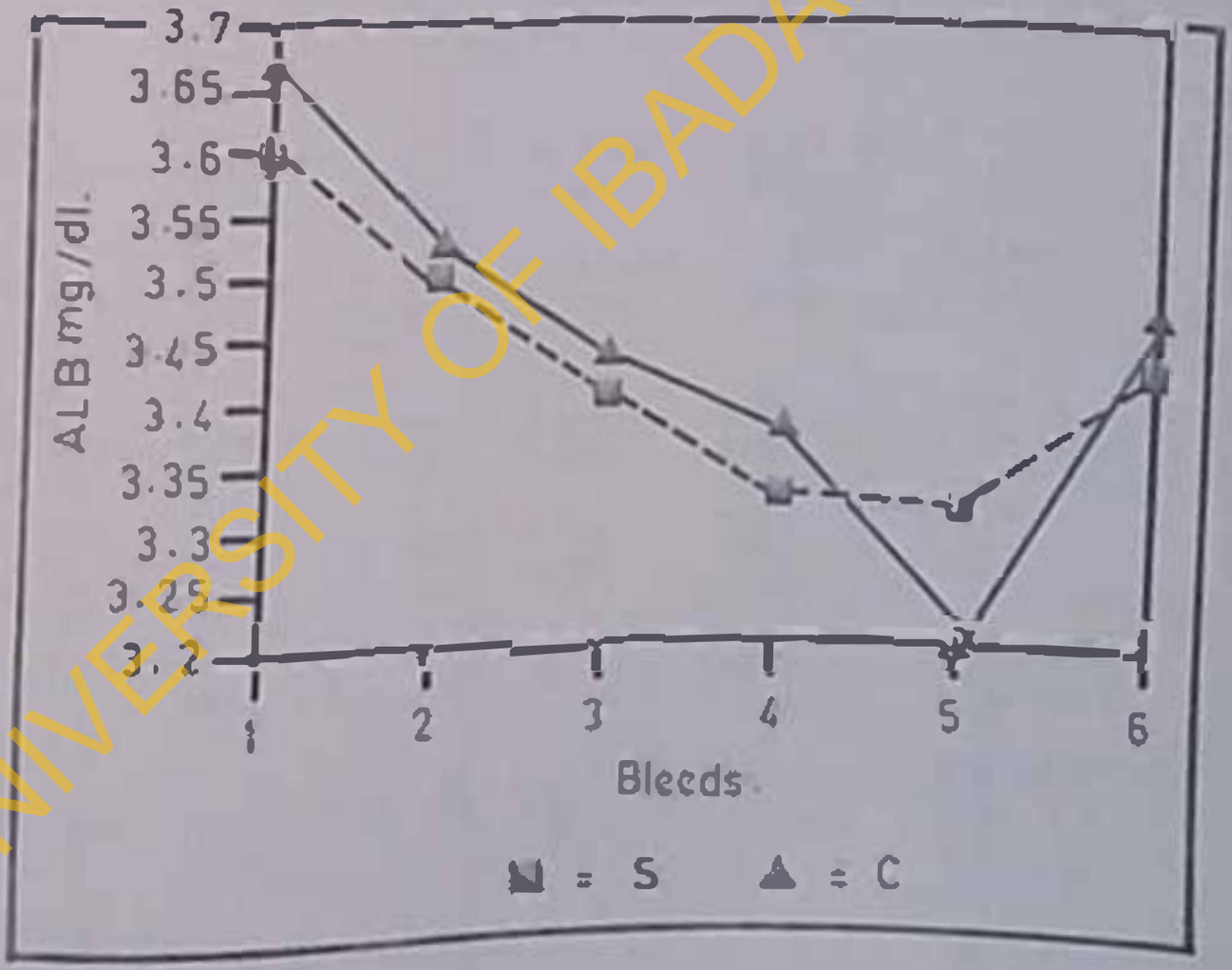
PCV -Packed cell volume
B-C -B-carotene
VITA -Vitamin A
S -Pregnant subjects supplemented with oral vitamin A
C -Pregnant women supplemented with oral placebo

RETINOL BINDING PROTEIN (RBP).

The RBP levels in the supplemented subjects did not change throughout the pregnancy period while those of the controls decreased progressively. The subjects had significantly higher levels than the controls (P<0.05)

FIG. 4–8

Mean Plasma ALBUMIN (ALB) values of subjects and controls in the Longitudinal study



from the 3rd to the 6th bleed. Fig. 4.9 shows the pattern of RBP in the subjects and the controls throughout the study period. Table 11 shows the mean +/-S.D of RBP in both the controls and the subjects. In the subjects and the controls plasma RBP levels correlated significantly with plasma vitamin levels (P< 0.05).

TABLE 11

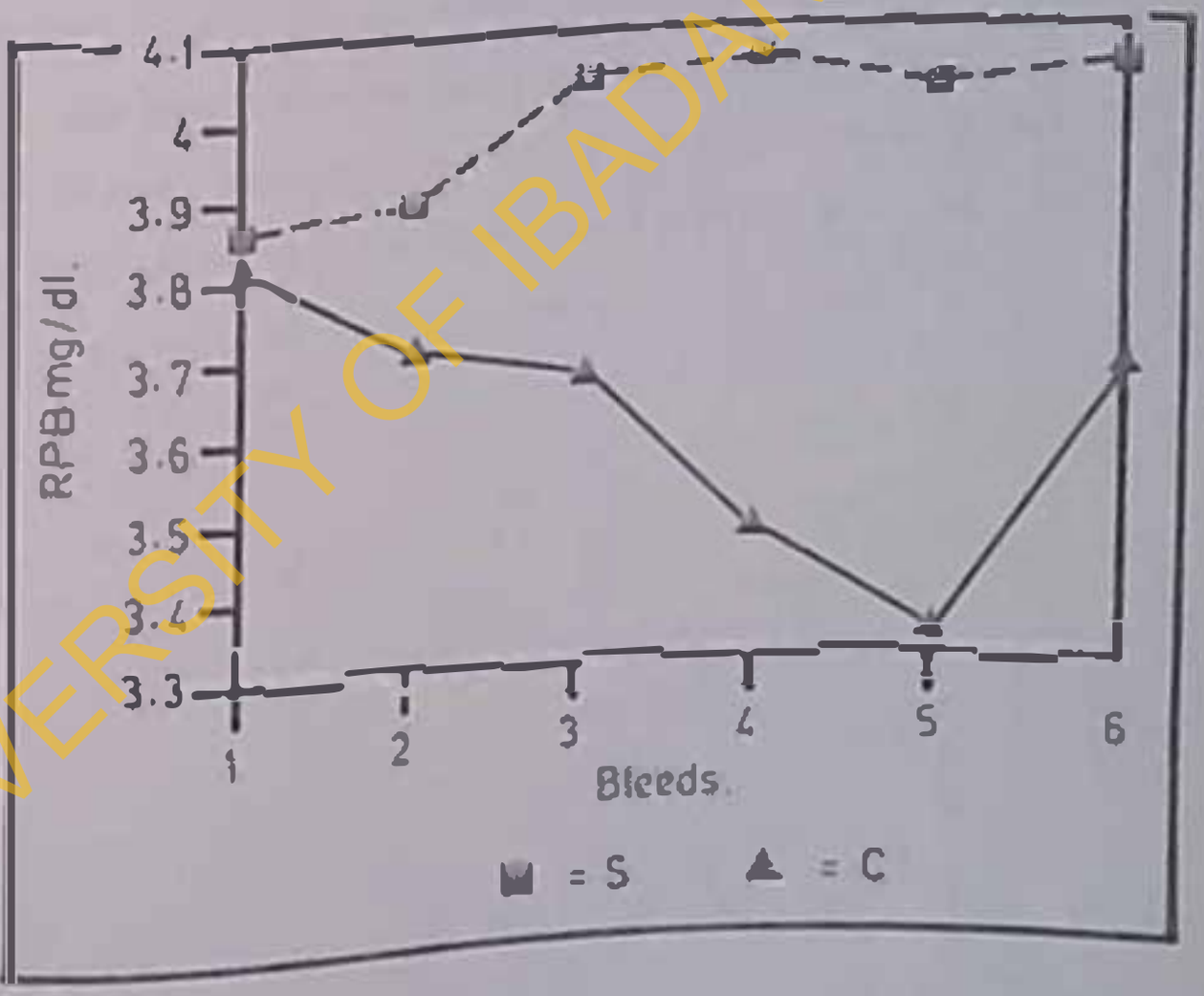
Mean +/- S.D ALB, RBP AND TTR levels in the supplemented and the placebo groups (14 subjects in each group

No. of bleeds	1	2	3	4	5	6
ALB (g/dl)						
S	3.6+/- 0.46	3.5+/- 0.42	3.4+/- 0.40	3.32+/- 0.40	3.31+/- 0.5	3.42+/- 0.43
C	3.67+/- 0.41	3.52+/- 0.39	3.43+/- 0.39	3.37+/- 0.35	3.20+/- 0.37	3.46+/- 0.33
RBP (mg/dl)						
S	3.86+/- 1.03	3.89+/- 0.98	4.04+/- 0.87	4.06+/- 0.99	4.02+/- 0.88	4.05+/- 0.91
C	3.81+/- 0.72	3.70+/- 0.74	3.66+/- 0.71	3.46+/- 0.59	3.32+/- 0.56	3.67+/- 0.41
TTR (mg/dl)						
S	19.1+/- 3.02	18.6+/- 3.4	18.8+/- 3.0	18.6+/- 3.2	18.2+/- 2.4	19.7+/- 2.4
C	19.2+/- 3.3	18.1+/- 3.2	17.7+/- 3.1	17.7+/- 3.0	16.05+/- 2.68	19.43+/- 2.4

ALB -Albumin
RBP -Retinol Binding Parotein
TTR -Transthyretin
S -Vitamin A supplemented pregnant women
C -Placebo supplemented pregnant women

FIG. 4 – 9

Mean PLASMA RETINOL BINDING PROTEIN (RBP) values of subjects and controls in the Longitudinal study



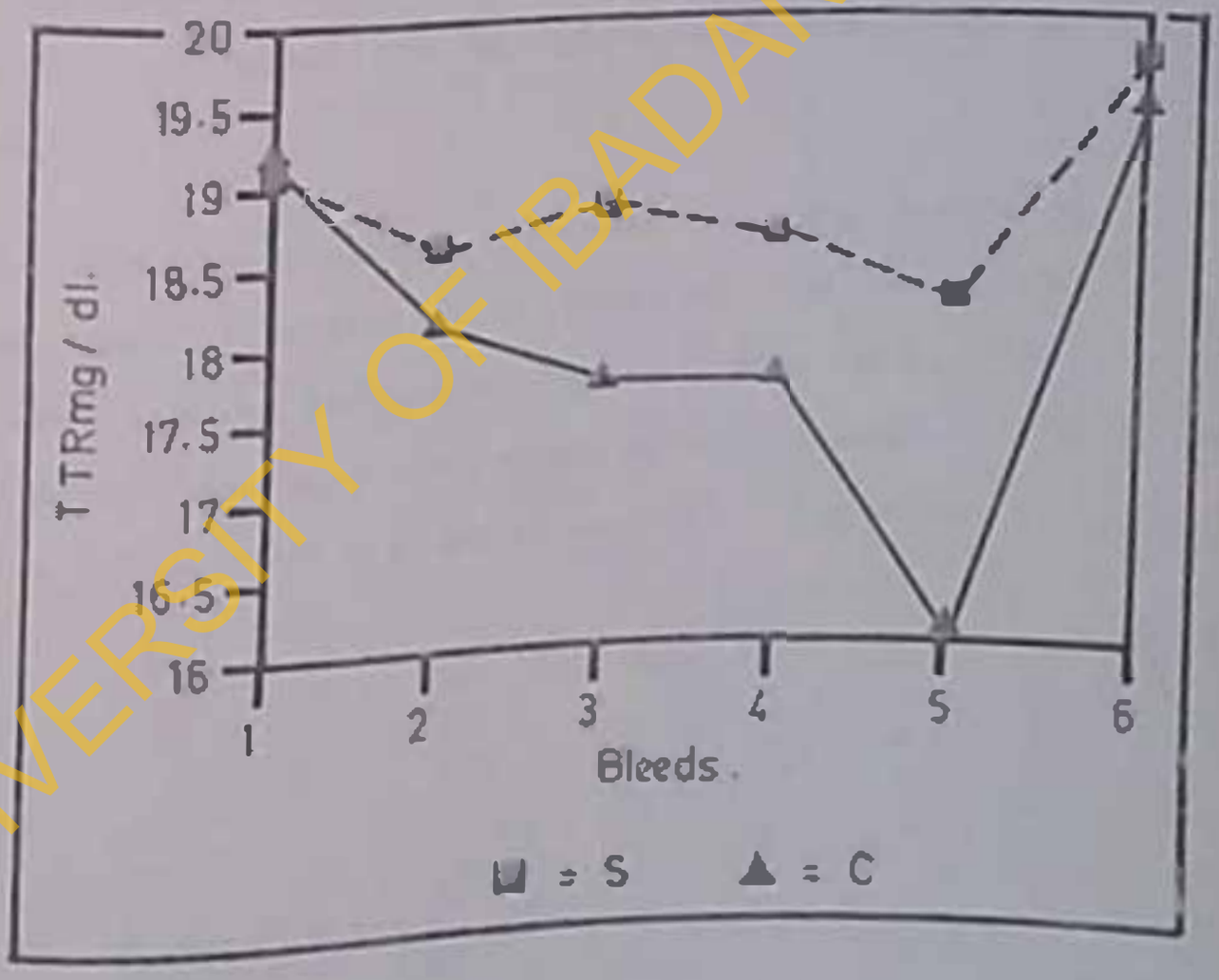
PLASMA TRANSTHYRETIN

Table 11 shows the means +/-S.D of the TTR levels in both the subjects and the controls. There were no differences between the TTR levels of the subjects when compared to those of the controls except at the 5th bleed when the subjects had significantly higher values than the controls ($P < 0.05$). Fig. 4.10 shows the trend of the TTR in the subjects and the controls throughout the study period.

The plasma protein levels (RBP, TTR, ALB) in both the subjects and the controls were significantly lower at the 5th bleed than all the other times. This point corresponded with labour but the levels went up to either predelivery or higher levels.

FIG. 4-10

Mean Plasma Transthyretin (TTR)(PCV) values of subjects and controls in the Longitudinal study



VITAMIN A STUDIES IN NEONATES

The babies born to the women were in this study phase made up of 12 males and 16 females. There was no difference in the mean apgar score (5mins) of the babies of the subjects as compared to that of the controls (9 vs 8.86). Birth weight of the subjects and the controls were similar in both groups (3.20+/- 0.05 vs 3.09 +/- 0.06) though the vitamin A supplemented group recorded a higher mean birth wieght. Table 12 shows the mean +/- S.D . apgar score, birth weight and PCV levels in both the subjects and the control neonates.

The PCV and plasma vitamin A levels in the subjects neonates were also similar to those of the controls. There were also no difference in the plasma B-C. TTR, and RBP of the subjects when compared with those of the Controls. The mean+/- S.D are presented in Table 12.

TABLE 12

Mean +/- S.D Apgar score, birth weight, PCV levels of the neonates (14 subjects in each group)

	APSCORE	B.W (KG)	PCV (%)
S	9.00+/- 0.78	3.20 +/- 0.05	53.07+/- 2.46
C	8.86+/- 0.68	3.09 +/- 0.05	51.86+/- 2.85

APSCORE - Apgar score
B.W - Birth wight
PCV - Packed Cell Volume
S - Babies of the vitamin A supplemented mothers
C - Babies born to the placebo treated mothers

TABLE 13

Mean +/- S.D VITA, B-C, ALB, TTR, RBP of the neonates (14 subjects in each group)

	VITA (ug)	B-C (ug)	ALB (g)	TTR (mg)	RBP (g)
S	21.02+/- 2.02	59.36+/- 11.67	2.93+/- 0.16	12.71+/- 3.77	3.36+/- 0.22
C	18.12+/- 3.52	59.36+/- 9.46	2.93+/- 0.38	11.61+/- 3.15	3.34+/- 0.32

VITA -Vitamin A
B-C -B-carotene
ALB -Albumin
TTR -Transthyrotin
RBP -Retinol Binding Protein
S -Neonates of the vitamin A group
C -Neonates of the placebo group

FOOD ANALYSIS

The food consumption pattern of the pregnant women in the longitudinal study revealed the the during the week-day 3 (21.5) of all the subjects surveyed consumed 2 meals daily on a regular basis this was reduced to 2 over the weekend. The others ate 3 times daily. Appendix 4.1 shows the typical menu of the population. The food samples were analysed using chemical analysis and Food composition Table (FAO, United Nations. 1968). Diet history and 24 hour dietary recall were carried out to validate the data on food habits of the subjects. The procedure is outlined in the chapter on materials and methods.

The mean+/- S.D of the various nutrients are shown on Table 14. The results showed that the calories and protein intake of the women ranged between 1452 and 2655 calories and 33 to 1319 per day respectively. The fat intake of the subjects varied from 30 to 74 g/day and majority of it was provided by palm oil and groundnut oil.

B-carotene intake of the women ranged between 298 and 1399 ug/day. The foods that contributed high B-carotene values were mostly palm oil containing foods such as vegetable soup, bean portage, yam portage, okro soup, moinmoin and akara. The cereals and tubers contained no

B-carotene at all.

The intake of vitamin A was from 0 to 385ug per day with a median of 168ug. The foods that contributed to the vitmain A intake of the women included milk and eggs. None of the women consumed liver throughout the survey period. The different levels of intake for the different nutrients are shown in Table 14.

TABLE 14

Mean +/- S.D Calories, protein, fat, B-carotene and vitamin A intake of pregnant subjects in the longitudinal study (14 subjects in each group)

	CALS (cals)	PRO (g)	FAT (g)	B-C (ug)	VITA (ug)
S	2115.64+/- 309.02	66.1+/- 17.80	47.29+/- 7.89	697.36+/- 123.69	46.79+/- 177.4
C	2029.59+/- 239.58	43.14+/- 11.29	53.45+/- 5.74	438.14+/- 130.51	116.71+/- 124.80

Cal -Calories
PRO -Protein
FAT -Fat
B-C -B-carotene
VITA -Vitamin

D I S C U S S I O N

C H A P I E R F I V E

CROSS-SECTIONAL STUDY

SUBJECTS

The ages of the subjects and controls were observed to be in the range of 18 to 48 years with a mean value of 28.6yr. Majority of the women were in the range 25-35 years indicating that the peak of reproductive life is between this age range. The parity of the women shows that parity among Nigerian women is as high as 8 children.

The result of the body weight indicates that the controls had lower mean body weights when compared with the subjects, and the subjects weight increased with age of pregnancy ($P < 0.01$). This observation shows the expected trend in weight gain during pregnancy.

Most of the women were unskilled and had minimum educational background which in turn dictated their profession and income.

The various economic activities in which the women and thier husbands were engaged in is representative of what obtains in the environment.

The dietary pattern and habits of the study population show that only half of the population studied consumed 3 meals a day and about 15% ate one good meal daily with occasional snacks. This observation shows the pattern of food consumption in the population.

Judging by the various reasons given for this observation, it is clear that apart from income, lack of time also contributed to their pattern of eating.

The consumption of β-carotene rich foods was highest for palm oil containing foods. This observation is in agreement with the findings of Domen (1970). They attributed this to the fact that in the Southern Savanah Zone, oil palm is cultivated and therefore consumed regularly. Voorhoeve (1966) on the other hand observed six cases of eye affectation in Ibadan in children suffering from Protein Energy Malnutrition (PEM) despite the availability of red palm oil. On the one hand this could be attributed to ineffective mobilisation of vitamin A from the liver. PEM has been shown to reduce the production of RBP and TTR which are required for the mobilization of vitamin A (Arroyave et al, 1961). On the other hand the intake of palm oil might be grossly inadequate due to faulty preparation methods of palm oil containig foods or for economic reasons.

The intake of carrots and mangoes were dependent on their availability. Carrots were available during the dry season while mangoes were present during the rainy season. On the average about 1/3 and 2/3 of the total population consumed carrots and managoes respectively during the study period. The study spanned a period of nine months and thus the two seasons prevailing in the country were considered in the cosumption pattern of the two sources of B-carotene. 60% and 40% of the study population were studied in dry and wet seasons respectively.

Though majority of the pregnant women were not vitamin A deficient as revealed by plasma vitamin A levels more than 60% of them had marginal levels. This therefore indicates that the two sources of B-carotene cannot be depended upon to provide the needed vitamin A in the diet though they are rich sources because they are not consumed on a regular basis. Palm oil is the only B-carotene rich source that was consumed regularly by at last 85% of the population. The preparaion methods used may reduce the amount of B-carotene that ultimately become available from palm oil. Bleaching and repeated heating of stews reduce the available ~-carotene in a meal. On the average, palm oil may supply between 50 - 75% of the required Vitamin A in this environment.

The PCV levels of the subjects were observed to decrease with increasing age of pregnancy and were significantly lower than those of the controls. This observation is consistent with that of Abudu and Sofola (1985). They observed a significant downward trend in the PCV values of pregnancy subjects studied. They attributed the decline to the increase in plasma volume from about the 8th week of prenancy to term. Donovan (1967) also observed a progressive drop in the PCV of pregnant subjects up to the 24th week of gestation. In the present study, the progressive decline in the PCV levels was observed until term. The common factor in all these studies is haemodilution and therefore the progressive decline in PCV can be attributed to increased plasma volume in the pregnant women. The difference in this study and that of Abudu and Sofola (1985) when compared with that of Donovan (1967) is attributed to the fact that black women start off with a higher plasma volume than the caucasians. This may explain the delayed drop in the PCV of the group studied by Donovan (1967).

The PCV vaues observed in our study are much lower than thsoe observed by Abudu and Sofola (1985). This may be explained by the irregularity of food intale observed in the subjects in this study.

The plasma B-carotene levels were maintained throughout

pregnancy and the controls had similar levels as the subjects. This observation is in agreement with that of Venkatachalam et al (1962) who observed that the plasma levels of B-carotene did not change with increasing age of pregnancy. This observation suggests that plasma B-carotene levels are not affected by haemodilution. An increase in the absorption of B-carotene might have been responsible for the maintainance in pregnancy. This suggestion may be explained by the fact that the serum level of carotene usually reflects the nutrient intake of carotene (Marrow et al. 1952). The level of B-carotene observed in this study is within the normal range (50-150 ug/dl) and is comparable to that obtained by Venkatachalam et al. 1962).

Plasma Vitamin A levels observed in this study are within the normal range (20 - 50 ug/dl) for the controls as observed by Tiez (1986) but 13(6.3%) of the subjects had values below the normal levels.

Venketachalam et al. (1962) and Baier et al. (1977) observed mean plasma Vitamin A levels similar to those observed in this study but Gal and Parkinson (1974) observed higher levels in their subjects. The difference may be attributed to the difference in the socio-economic background of the subject population. Whereas Venkatachalam et al. (1962) studied women in

India. Gal and Parkinson (1974) studied women from Queen Charlottes Hospital, London. The subjects in this study were from the high, intermediate and low socio economic classes. The mixture of the social classes might explain the similarity of the findings in this study and those of Venkatachalam et al (1962).

The plasma Vitamin A levels decreased as pregnancy progressed. This finding agrees with that of Venkatachalam et al. (1962) and Gal and Parkison (1974). Venkatachalam et al. (1962) observed a gradual and progressive fall in Vitamin A concentration of the serum from the 1st to the 3rd trimester of pregnancy. Gal and Parkinson however observed a fall in early pregnancy, followed by an increase and a few weeks before term the levels dropped but not significanly. The significant progressive fall in vitamin A from the 1st to the 3rd trimester observed in this study may be due to the fact that half of the population studied belonged to the intermediate and low socio economic classes. The rise observed by Gal and Parkinson, (1974) was attributed to the circulating progesterone levels.

The findings of a progressive drop in plasma Vitamin A as pregnancy progressed is further validated by the observation of other workers.

Hiret and Shoemaker (1943) found that 40% of 35 pregnancies had plasma Vitamin A levels below normal range.

Bodansky et al (1943) also showed a significant difference between the mean values of the first 3 months and the last 3 months of pregnancy. They attributed this to the storage of Vitamin A in the foetal liver and utilisation by the foetal tissue.

Lund and Kumble (1943) also suggested that actively growing tissue may also utilise considerable amounts of Vitamin A. The basal metabolic rate increases during the latter half of pregnancy and there is general agreement that most of this increase is due to the growth of the foetus. This may also be in part accounted for by a possible activation of maternal endocrine gland (by foetal effect).

The other reason given for a reduction in plasma Vitamin A levels in the 3rd trimester include the possible interferences with the release of Vitamin A from the liver associated with a probable derangement of the liver during pregnancy (Bodansky et al, 1943). It has been observed that when the liver Vitamin A reaches marginal levels, there is a conservation mechanism involved to protect the remaining Vitamin A and the

release of Vitamin A is greatly reduced (Underwood, 1990). Bodensky et al (1943), suggested that the foetus may make two kinds of demands upon the depot of Vitamin A in the normal adult liver (500,000 IU to 11,000,000) These include the storage of Vitamin A in the foetal liver and utilisation of Vitamin A by actively growing foetal tissues. Foetal liver during the 3rd trimester contains considerable amount of Vitamin A while a total store of 12,000 IU is found in the liver of the newborn infant (Lewis et al, 1941). They suggested that the deposition of 12,000 IU in foetal liver may entail the release of several fold that amount from the maternal liver. Though the fate of blood Vitamin A, arriving either from ingestion or from liver release is not precisely known. There is however evidence indicating that Vitamin A is not used economically. For instance there is evidence that when large amounts are fed only a small fraction can be accounted for by fecal excretion, storage in the liver, and daily requirements (Le Page, 1941). Lewis et al (1942) indicated that during depletion, Vitamin A is released from the liver of the rat in amount greater than that required by the animal which might support the hypothesis that in pregnancy there is conservation mechanism involved to preserve liver Vitamin A for subsequent use probably during lactation.

Lund and Kimble (1943) also observed that economics and health education influenced the adequacy of Vitamin A in the pregnant population studied. They also observed a significant correlation between the intake of Vitamin A and the plasma Vitamin A values for the group of subjects. It was also found out that during the 1st trimester, a diet which was adequate for non pregnant women was also adequate for pregnant women except when such complication as hyperemisis gravidarum intervened. During the second trimester only the best diet met the needs of the pregnant subjects and during the 3rd trimester there was a need for supplements of Vitamin A in addition to amounts supplied by the diet.

In the present study, only about 122(59%) of the total population had heard about Vitamin A. 64% of these knew what function the vitamin performs or what disease it prevents and they all belonged to social classes 1 and 2. This observation agrees with that of Lund and Kimble (1943) that health education influenced the adequacy of Vitamin A.

Hemodilution that usually occurs in pregnancy has also been suggested as a possible cause of the progressive decline in plasma vitamin A values. Lund and Kimble, (1943) did not agree with this statement. They observed that there was no decline in the B-carotene levels

throughout pregnancy as was also observed in the present study. They therefore suggested that since B-carotene levels do not fall during pregnancy the haemodilution theory cannot hold true for the progressive fall in plasma Vitamin A levels observed.

Also Vitamin A excretion in the urine has been observed to increase during pregnancy (Gaehtgens, 1937). Vitamin A is a fat soluble substance and for it to be excreted through the kidneys, it has to be made polar. It is either that the renal threshold of pregnant women decreases to such an extent that Vitamin A dispersed in aqsous phase is excreted or its metabolism to the more polar compounds is increased, thereby increasing its excretion. If either of the above is true, this might explain the fall in plasma Vitamin A levels during pregnancy.

Plasma Vitamin A levels have been observed to increase spontaneously as early as 6 hrs to 24 hrs postpartum (Lund and Kimble, 1943). This observation has been used to support the effect of hemodilution on plasma Vitamin A levels. However it has been reported that the increases in plasma Vitamin A level are not sustained and may even go as low as the deficient levels in some cases if additional Vitamin A is not fed (Lund and Kimble, 1943). It was therefore concluded that at the

time of delivery, some mechanism as yet unknown release the Vitamin from store (probably set in order to preserve Vitamin A) and mobilises it into the blood stream where it is available for lactation (Lund and Kimble, 1943).

All the reasons given above taken in concert may have contributed to the progressive fall in the plasma vitamin A levels during pregnancy. Judging by the obsevations in this study supported by those of Lund and Kimble, (1943) and Underwood, (1990) it may be suggested that a conservation mechanism was invoked to conserve vitamin A levels in the liver of the pregnant women in the face of apparent inadequate intake.

0.3% of the total population studied had plasma Vitamin A below 10 ug/dl. This may suggest apparent Vitamin A deficiency but it does not indicate a public health problem because it does not meet the 5% WHO criterion for diagnosis. Night blindness was reported 6(3.5%) subjects and 2(5.7%) of the controls had night blindness, old corneal scars were observed in 23(1.2%) of the total population. This might have been overreported since none of the controls had plasma Vitamin A values less than 20 ug/dl which is the point at which night blindness is usually reported. The observation in the subjects however may be true since

0.5% had values below 10 ug/dl and 11% had values ≤ 20ug/dl. 60% of the study population had plasma Vitamin A levels between 20 and 29 ug/dl. This observation suggests that the status of Vitamin A in a larger percentage of the pregnant as well as the control women may be marginal. There is evidence to suggest that plasma Vitamin A levels between 20 and 29 ug % may indicate marginal liver stores (Underwood. 1990).

Plasma albumin levels decreased as pregnancy progressed and were significantly correlated to plasma Vitamin A levels. This observation is consistent with the findings of Hytten and Leitch (1971). They reported that Vitamin and albumin follow the same trend in pregnancy and suggested that albumin and retinol binding protein may be controlled the same way. The decrease in plasma albumin levels may be attributed to either hemodilution, increased requirement in pregnancy (Ventakachalam et al. 1962) or a deficiency of protein (Aroyave, 1969).

The intake of Vitamin A and B-carotene rich foods using semiquantitative 24 hour recall and diet history methods showed tha about 31% of the subjects and controls consumed Vitamin A rich foods at least twice a week and these belonged to the high socio-economic classes. The consumption of B-C rich foods on the other hand cut

across all classes and the only constant source was palm oil but the amount consumed daily on the average can only be estimated. It was gathered that a bottle of red palm oil (about 800 mls) lasted 1 week for a household. The level of B-carotene in 100g of palm oil is about 10,000 IU. Considering a household of 6 members the available B-carotene per week from palm oil is 8000 IU. The available B-carotene per head per day is 1905 IU. Taking into consideration the biological activity of B-carotene, the utilisable B-carotene per head per day is about 190 Retinol equivalent. Also the preparation methods of foods may destroy some of the B-carotene. Assuming that other sources of B-carotene such as mangoes, green leafy vegetables and carrots contributed the same amount provided by oil palm. the available Vitamin A is about 400 IU retinol equivalent. The recommended dietary allowance for pregnant women is 600RE for the 1st two trimesters and 800 RE in the last trimester (Olson, 1987). The per capita availability of Vitamin A to the consumer in the U.S is 7,800 IU/day (2364 RE) while the observation in this study suggests 4000 IU (400 RE) in this environment.

The observation in this study therefore suggests that Vitamin A intake in this environment may be marginal or inadequate for the pregnant women especially in the 3rd

agrees with the observation of Olusanya et al. (1989). They observed that Vitamin A intake of lactating mothers in Oyo local government area is 3059 IU per capita per day. This figure is about half the RDA for lactating women indicating an inadequate intake. The observation in the present study may therefore suggest that the diet of pregnant and lactating women require additional sources of vitamin A to ensure adequate liver store for the foetuses and infants.

5.2 RELATIVE DOSE RESPONSE TEST

The plasma Vitamin A levels were correlated with RDR levels in the pregnant Nigerian population for the 1st time.

All the subjects that had RDR </= 20 ug/dl has basal plasma vitamin A </= to 20ug/dl. 16.7% of the total population with initial plasma Vitamin A levels between 21 and 29 ug/dl had RDR >/= 20%. None of the subjects with positive RDR (i.e. RDR >/= 20%) had plasma vitamin A levels below 15ug/dl.

This observation agrees with the findings of Flores et al (1984). They showed that all the subjects with initial serum retinol </= 20ug/dl had positive RDR (They used 20% as cut off, same as was used in this study). They also observed that 84% of those with initial plasma

vitamin A between 21 and 29 ug/dl had positive RDR. In this study 16.7% with initial plasma vitamin A levels between 21 and 29ug/dl had positive RDR. The difference in percentages may be attributed to the population groups studied. Whereas Flores et al (1984) studied children under 7 years of age from the low socio economic background, the present study was composed of pregnant women in the three trimesters of pregnancy from the low and intermediate socio economic classes. The difference in observation may therefore be as a result of the difference in physiological state of the populations studied.

Amedee Manesme et al. (1984) also studied vitamin A status of 12 adult generally well nourished surgical patients using Liver vitamin A concentration and RDR values. They observed that the subjects with the highest RDR values also had the lowest liver levels.

The findings in this present study therefore suggests that about 20% of the pregnant women studied had liver vitamin A store </= 20% ug/g using the same cut off point of liver storage for vitamin A observed by Amedee Manesme et al. (1984) in their subject population.

RDR is defined as the percentage increase in plasma retinol levels relative to the plasma retinol levels 5

hr after the oral administration of a standard dose (450 ug) of retinyl acetate (Loerch Maresse et al. 1984). The principle behind this test has been described. As liver reserves of Vitamin A become progressively depleted due to chronically inadequate dietary supply conservation mechanisms are invoked to increase the efficiency of Vitamin A utilisation among tissues and to maintain the level that is circulating to the target tissues (Underwood, 1990). When the liver reserve is depleted below a critical threshold, the rate of release of the remaining reserve is diminished. Synthesis of the carrier protein RBP continues and results in the accumulation of a pool of preformed RBP. Providing an exogenous source of Vitamin A causes the release of holo-RBP at a rapid and in a characteristic time course relative to the amount of accumulated preformed carrier protein (Loerch et al. 1979.

In the light of the available information in the literature and the findings in the present study it is evident that RDR will predict Vitamin A reserve in an individual with normal liver functions. It can therefore be concluded from the present study that 13% of the pregnant population examined had liver reserve < 20 ug/dl and majority were in the third trimester of pregnancy.

5.3 LONGITUDINAL STUDY

The significant effect of Vitamin A supplementation observed on the haematocrit levels of the subjects is in agreement with observation of Mejia and Chew (1988) and Bloem et al. (1990). They observed an increase in the haematocrit levels as early as two weeks after a single oral massive dose (20,000 IU) of Vitamin A. They attributed this to the increased mobilisation of iron from available store and increased iron utilisation for haemoglobin formation. Consequently the iron store decreased and this may trigger off absorption of iron. These studies were carried out in children. In the present study, pregnant women were studied. Apart from the Vitamin A supplementation, the subjects were also taking ferous sulphate and folic acid. While this may explain the reason for the maintenance of the PCV levels in the subjects, this cannot be acceptable because the controls also took ferous sulphate and folic acid but they still had significant lower PCV levels.

Mejia and Chew (1988) also observed that when Iron and Vitamin A were administered simultaneously the response was better than for Vitamin A or Iron alone. The improved PCV in the subjects may be explained by the Vitamin A supplements provided for the subjects in combination with the ferous sulphate and folic acid

supplements.

The subjects and the controls in this study had PCV levels >30% and therefore none of them was anaemic at the start of the study unlike the children studied by Bloem et al. (1990) who had lower than normal haemoglobin levels. The observation in this study suggest a true beneficial effect of Vitamin A on PCV levels because the controls had a progressive decline in their hematocrit levels while those of the subjects were maintained.

The haematocrit levels were significantly correlated to the albumin levels throughout the study period. The reason for this association is not clear. It may however be attributed to increased need for albumin in the synthesis of heme for Hb formation and subsequent increase in the red blood cells.

The effect of supplementation was not observed on the haemotocrit levels of the neonates. There was no difference in PCV levels of the neonates born to the subjects when compared to those born to the controls. The factors that cause anaemia in neonates are two-fold haemolytic or haemorrhegic (Behrman and Vaughan, 1987). None of the neonates suffered from any of these disorders. Also the synthesis of red blood cells in the

foetus is well controlled and buffered against dietary assaults (Hehrman & Vaughan, 1987).

The plasma vitamin A levels in the subjects increased sigificantly than those of the controls from the 1st half of the 3rd trimester until 6 weeks post partum. This observation is consistent with the findings of various other workers.

Wald et al. (1985) observed a small but significant increase in the plasma Vitamin A level after 3 months of supplementation with a daily dose of 10,000 IU. This group consisted of 57 non pregnant, non lactating women with normal basal Vitamin A levels.

Earlier than this study, Willet et al. (1983) compared the increase in serum vitamin A concentration in 15 supplemented subjects who took 25,000 IU of retinyl palmitate per day with that in 15 controls subjects who took a placebo. They observed an increase of 23 ug/l after 8 weeks and 6 ug/l after 16 weeks.

In the present study the supplemental Vitamin A level fed was 7,000 IU per day with resultant increase in the subjects over the placebo group of 7 ug% in the 1st half of the 3rd trimester and 5ug by the 6th week post partum.

In another study, Willet et al. (1984) gave 10,000 IU of Vitamin A daily in the form of fish oil extract and observed an increase of 45 ug/l in 16 subjects over and above 19 subjects who took a placebo. Garett-Laster and his coleagues (1981) observed an increase in the mean serum retinol of 7ug/l after 3 weeks of supplementation in 10 subjects given 30,000 IU compared to a slight fall over the same period of time in 8 unsupplemented subjects.

Urback et al (1952) administered 47,000 IU of Vitamin A acetate to 5 subjets. They estimated an average serum retinol level of the 26 weeks of supplementation and found a level of serum retinol which was 16% higher than that in an unspecified number of unsupplemented controls.

Van Bruggen and Straunfjord (1948) administered 100,000 IU of Vitamin A daily to 36 subjects. Serum retinol levels in these subjects were compared to levels in 36 unsupplemented controls. After 18, 24, 36 months of supplementation the mean difference between the groups was 1020, 1220, 1690 IU/L respectively equivalent to 321, 366 and 507 ug/l.

All these studies taken together make it clear that Vitamin A supplementation increases serum retinol levels. These studies also show that there is a dose -

response relationship as the largest doses of Vitamin A observed the largest increases in serum retinol.

Willet et al (1984) concluded that supplementation was more effective among subjects with initially low serum retinol concentrations. This observation agrees with the findings in this study. The pregnant women studied were those who had low levels (between 20 and 30 ug/dl) of Vitamin A to start with and these levels have been associated with marginal deficiency.

Most of the studies carried out on the effect of supplementation in pregnancy are consistent with observation in the non pregnant non lactating women. Lewis et al (1943) observed a significant increase (29 IU or 8.7 ug/dl) and also a maintenance of plasma Vitamin A levels in pregnant women receiving 10,000 IU of Vitamin A in the 3rd trimester of pregnancy.

More recently, Villard and Bates (1983) observed a significant (19%) increase in the plasma Vitamin A levels of supplemented group when compared with the unsupplemented group.

The observation from this study and other studies show that Vitamin A supplementation increases the plasma Vitamin A levels either in pregnant or non pregnant

women. Its beneficial effect is also evident from the fact that it improved the haematocrit values of the pregnant mothers.

The supplemental Vitamin A in this study (7,000 IU is about 3.5 times higher than the recommended dietary allowance (2000 IU or 600 ug/day). Lewis et al (1983) observed that supplemental Vitamin A higher than 10,000 IU is only of little additional benefit and this level is adequate to maintain the plasma levels throughout pregnancy.

The finding in this study confirms the fact that an additional Vitamin A over and above the normal intake will maintain normal plasma levels throughout pregnancy without the fear of toxicity. Evidence has shown that intake between 25-50,000 IU/day for periods of several months can produce multiple adverse effects (Hathcock et al. 1990). However daily dose as low as 10-20,000 IU over a period of 2 years has been found to produce intracranial hypertension in an 18 year old male (Vollbracht and Gilroy, 1976).

In pregnancy, various levels of supplemental Vitamin A have been associated with birth defects. Vitamin A between 18 - 500,000 IU taken acutely or chronically have been associated with several toxic symptoms

(Mounoud et al. 1975, Von Lennepet et al. 1985; Bernhardt and Dorsey, 1974).

The U.S. RDA for pregnant women is +/- 8000 IU and many prenatal vitamin formulae contain 8000 IU (Hatchcock et al. 1990). The level fed in this study was 7,000 IU. This level is safe in this environment where the intake is inadequate or marginal as observed by the level of intake of 248 - 1399 ug B-carotene and 0 - 385 ug vitamin A per day.

The LD_{50} for Vitamin A has been calculated. LD_{50} is the index of acute toxicity to the amount of substance in a single dose required to kill 50% of a population of animals. An LD_{50} expressed in mg/kg is calculated from a dose response curve and is dependent on various factors such as route of administration, species, strain, sex, age, nutritional status and environmental conditions.

LD_{50} values for retinol, all-trans -retinoic acid (RA 13-Cis RA and etretinate given orally to mice are 2570 (8.6 x 106 IU), 1100 - 4000, 3389 - 26,000 and >4000 mg/kg respectively. In the rats the LD_{50} values for retinyl palmitate, all-trans RA, 13-Cis RA and etretinate given by oral intubation are 7910 (14.4×10^6 IU), 2000, >4000, and >4000 mg/kg respectively (Kamm et

al. 1984). The LD_{50} for monkeys was estimated to be 168mg (0.56 x 10^6 IU retinol/kg.

The highest dosage for each specie was 250 times higher on a body weight basis than the human RDA for Vitamin A which is approximately 0.06 mg (110 IU) retinyl palmitate per kg/day. However this dose should not be construed to indicate that an intake of 250 times the RDA is safe for humans because there is difficulty in extrapolation between species.

In the light of all these findings, it is safe to advocate supplementation of Vitamin A in pregnancy to the tune of 10,000 IU per day for pregnant women especially in the 3rd trimester. This suggestion is firmly supported by the autopsy findings in the Swedish and Ethiopian infants.

The Ethiopian infants were observed to have liver reserves sufficient for 5 – 6 days compared with two months available to the Swedish group (Gebre-Medhin and Valquist, 1984). This suggested both the capability of the early infants to build stores as well as the influence of the mothers Vitamin A status on the infant. Plasma retinol binding proteins (RBP) increased significantly in the supplemented subjects when compared with the placebo treated controls. This increase is in

response to the increase in the plasma Vitamin A levels in the subjects. This observation is substantiated by the evidence of Gebe-Medhin and Velquist. (1984). They observed that Swedish infants' livers at autopies had higher Vitamin A levels than Ethiopian infants. Furthermore the RBP levels in the Ethiopian women were significantly lower than those in the lactating Swedish women. This indicates that RBP increases in response to an increase in the Vitamin A levels in a given population.

RBP has also been observed to be reduced in protein malnutrition (Aroyave. 1969). The plasma albumin levels in the treated subjects were maintained throughout the study period as compared to those of the controls which declined with the age of pregnancy. Plasma albumin levels affected the levels of RBP. It may be therefore be inferred that the significant increase in the RBP was influenced by the levels of the albumin. This increase may be required for de novo synthesis of RBP if that was a limiting factor for the mobilisation of vitamin A from the liver to the blood.

Transthyretin (TTR) levels did not change throughout the study period except at delivery when it dropped suddenly and rose again 6 weeks post partum. This may be explained by the evidence that Vitamin A

supplementation does not affect TTR the same way it affects RBP (Mourey et al. 1990). RBP has only one binding site for retinol whereas TTR has four binding sites for RBP. This suggests that RBP will be secreted in response to the available vitamin A in the liver but TTR will respond to the presence of RBP in the blood and the secretion may be much slower. TTR levels were significantly correlated with albumin levels in the controls and the subjects. This relationship also suggests that albumin may be necessary for the synthesis of TTR.

Plasma albumin levels were maintained in the subjects throughout the study period, while it decreased significantly from the 1st half of the 3rd trimester to 6 weeks post partum in the controls. Plasma albumin levels were significantly correlated with TTR and FCV in the subjects. Plasma albumin levels have been observed to follow the pattern of Vitamin A in pregnancy (Hytten and Leitch, 1971). Since retinoic acid which is one of the oxidative products of retinol is transported by albumin, the maintenance of albumin levels in the supplemented subjects may be in response to increased retinol in the blood. Retinoic acid is required for maintenance of growth and epithelial cells, and since pregnancy is a time of active growth, it may be

suggested that oxidation of retinol to retinoic acid increased in order to provide adequate retinoic acid for this purpose. This in turn increased the levels of albumin required for the transport of the retinoic acid. Also increased albumin levels may be required for the synthesis of RBP when there is a constant supply of Vitamin A.

The levels of plasma Vitamin A observed in the neonates born to supplemented mothers were higher than those of the unsupplemented group but the difference was not significant.

This observation agrees with that of Lewis et al. (1947). They observed that when monkeys were supplemented with Vitamin A, the plasma levels did not change but a high amount was observed in the liver of the foetuses. They therefore suggested that supplementation improves the liver storage of Vitamin A in the foetuses rather than increase the plasma levels.

Bates (1983) reported that the supply of Vitamin A to the foetus is mainly from the retinol-RBP complex from maternal stores via the blood to the foetus, except when the stores fall to very low levels. This suggests an increased transplacental transfer of Vitamin A from the supplemented mothers to the foetuses especially for

storage in the liver.

There were no differences in the RBP, Albumin, TTR levels in the subjects and the controls. Also Vitamin A supplementation had no effect on the Apgar Score, sex, birth of the neonates.

The levels of all the plasma proteins decreased at the 5th bleed in both the subjects and the controls. This may be explained by the fact that these proteins are negative acute phase reactants which are normally reduced during stress (Silverman et al, 1986). The 5th bleed corresponded to labour and since it is a stressful event, the plasma proteins might have dropped in response to the stress.

5.4 FOOD CONSUMPTION SURVEY

The mean calorie intake of the pregnant women suggests that the energy intake of pregnant women in this environment may be marginal but there is apparent inadequate intake in about 50% of the population.

This observation agrees with that of Olusanya et al. (1989). They observed that pregnant women in some parts of Oyo local government area consumed about 82% and 71% of the recommended dietary allowance (RDA) for calories and protein respectively. In the present study the mean calorie intake was 2115 +/- 309 calories per

day which is approximately 88% of the RDA of 2400 calories.

The mean protein intake in this study was 66 +/- 27g per day. This amount is 86.5% of the recommended intake (70g/d) for a reference pregnant women. However the major sources of this nutrient in the diet of majority of the women were cereals and legumes. These protein sources are of low biological value. The observation in this study agrees with that of Oluwanya et al. (1989). They found that pregnant women met about 71% of their daily protein requirement.

The intake of protein has been observed to affect plasma vitamin A levels (Arroyave, 1969). The level of protein intake observed in this study may be adequate to maintain the plasma levels of vitamin A in the normal range if vitamin A intake is not the limiting factor.

The intake of B-carotene and vitamin A in the pregnant population studied showed gross inadequacy especially on the part of vitamin A intake. On the average, only 38% and about 10% of the RDA for B-carotene and vitamin A respectively were met. Oluwanya et al. (1989) observed a similar trend in the consumption pattern of B-carotene and vitamin A in lactating women in this environment. They showed that the available B-carotene per caput per

day was 3059 IU which is about half the RDA for pregnant women.

The finding in the present study therefore suggests that the diet of pregnant women in this environment may be highly deficient in its vitamin A content and the consequences may be grievous for both mother and child. The level of consumption in this study therefore explains the progressive drop in the levels of plasma vitamin A observed in the subjects.

C H A P T E R S I X

S U M M A R Y

The findings from the study can be summarised as follows:

1) The intake of preformed and provitamin A may be inadequate in pregnant women in this environment.

2) Plasma Vitamin A levels decreased as pregnancy progressed suggesting a need for additional intake in pregnancy.

3) 11% of the pregnant women had plasma Vitamin A levels </= 20ug/dl suggesting apparent deficiency.

4) 60% had plasma Vitamin A levels between 20 - 29ug/dl indicative of marginal status

5) Relative dose response test was positive for 4(13.6%) indicating depleted liver store or liver store </= 20ug/g.

6) Vitamin A supplementation of pregnant women with 7,000 IU oral Vitamin A/day from the 14th week of pregnancy to 6th week post partum maintained the packed cell volume and also increased the plasma Vitamin A levels in the subjects suggesting a beneficial effect.

CHAPTER SEVEN

RECOMMENDATION

The observation in this study suggests that vitamin A deficiency may be a problem in this environment and it is therefore suggested that

1. An intervention programme in the form of supplementation in the short term be carried out to improve the vitamin status of both pregnant women and their infants.
2. Nutrition education of both the Agriculture extension workers and the housewife be undertaken to ensure on the long run the production and selection of good sources of vitamin A.
3. A comprehensive field survey be carried out to determine the incidence and prevalence of vitamin A deficiency in the pregnant women and neonates in Nigeria.

This action will go a long way in maintaining the vitamin A levels in the mothers and ensuring adequate store in the foetus.

In addition to the beneficial effects of vitamin A supplementation shown in this study, studies have also

shown high correlation between the prevention of cancers and vitamin A levels in adult population (Wald et al. 1980).

Also studies have shown that children with adequate plasma vitamin A levels and liver store may be protected against some childhood diseases (Feachem, 1987). Though the available evidences are inconclusive especially in the area of vitamin A and cancers in humans there is incontrovertible evidence based on adequate field and biochemical surveys to confirm the beneficial effects of vitamin A in childhood diseases. It is therefore justifiable to deduce from the foregoing that vitamin A supplementation of mothers during the prenatal period will be highly benoficial to both mother and child.

It is therefore recommended that pregnant women in Nigeria be supplemented with at least 7000 IU vitamin A daily from the 28th week of pregnancy until 6weeks post partum.

R E F E R E N C E S

1. Abels J.C. Gorham A.T. Pack G.T. and Rhoads C.P. (1941) Metabolic Studies In Patients With Gastro Intestinal Cancer : Hepatic Concentrations of Vitamin A. Proc. Soc. Exper. Biol and Med. 48. 488.

2. Abudu O.O. and Sofola O.A. (1988) Plasma Volume In Normal Pregnant Nigerian Primigavida. Int. J. Gynaecol Obstet 23(2) 137 - 142.

3. Acc Scn News (1990) In 1988 FAO/WHO Report OFN Requirement of Vitamin A, Iron, Folate and Vitamin B12 - Reviewed by C.J. Bates pp. 53.

4. Amedee-Manesme O. Anderson I. and Olson J.A. (1984) Relation of the relative dose response test to liver concentrations of vitamin A in generally well nourished surgical patients. Am. J. Clin. Nutr. 39 (6) 898 - 902.

5. Amodee-Manesme O. Furr H.C. and Olson J.A. (1984) The Correlation Between Liver Vitamin A. Concentrations In Micro (Needle Biopsy) and Micro Samples Of Human Liver Specimens Obtained By Autopsy. Am. J. Clin. Nutr. 39 (2) 315 - 319.

6. A.O.A.C. (1970) Improved Kjeldahl method for nitrate containing samples; - official final Action in Official methods of analysis of the Association of Official Analytical Chemists. 11th ed. p. 17.

7. Arroyave G. (1969) Interrelations Between Protein and Vitamin A and Metabolism. Am. J. Clin. Nutr. 22. 1114 - 1128.

8. Arroyave G. Wilson D. Mendez J. Beher M and Scrimshaw N.S. (1961) Serum and Liver Vitamin A and Lipids In Children With Severe Protein Malnutrition. Am. J. Clin. Nutr. 9, 180 - 185.

9. Baker H. Thind I.S. Frank O. De Angelis B. Caterini H. and Louria D.B. (1977). Vitamin Levels in Low-Birth Weight Newborn Infants and their Mothers. Am. J. Obstet. Gynaecol. 29 521 - 523.

10. Ball S. Godwin T.W. and Morton R.A. (1948) The Preparation Of Retina - Vitamin A Aldehyde. Biochem. J. 42 516 - 523.

11. Bamji M.S. and Ahmed F. (1978) Effect Of Oral Contraceptive Steroids On Vitamin Status Of Women and Rats. World Rev. Nutr. Diet 31, 135 - 140.

12. Bashor M.M. Toft D.O. Chytil F. (1973) In Vitro Binding Of Retinal To Rat - Tissue Compounds. Proc. Natl. Acad. Sci. USA 70 : 3483 - 3487.

13. Bates C.J. (1983) Vitamin A In Pregnancy and Lactation Proc. Nutr. Soc. 42. 65 - 79.

14. Behrman and Vaughan (1987) Anaemia. Nelson Textbook Of Paediatrics 13th Ed. W.B. Saunders. Int. Ed. 409 - 411.

15. Bernhardt I.B. and Dorsey D.J. (1974) Hypervitaminosis A and Congenital Renal Anomalies In A Human Infant. Obstet. Gynaecol. 43: 750 - 755.

16. Beutel R.H. Hinkley D.F. and Pollard P.I. (1955) Conversion Of Vitamin A Acetate To Retrovitamin A Acetate. J. Am. Chem. Soc. 77, 5166 - 5167.

17. Bloem M.W. Wedel M. Egtmaal E.F. Speek A.J. Sowalontha S. and Schreurs W.H. (1990). Vitamin A Intervention: Short-Term Effects Of A Single, Oral Massive Dose On Iron Metabolism. Am. J. Clin. Nutr. 51: 1. 76 - 79.

18. Bodansky O. Lewis J.M. and Lilienfeld M.C. (1962) The Concentration Of Vitamin A In The Blood Plasma During Pregnancy. J. Clin. Invest. 22 643 -647.

19. Golding Cama Collins Morton Bridgeman Isler Hofler Taylor Willand and Bracbury (1951) Pure All-trans Vitamin A Acetate and The Assessment Of Vitamin A Potency by Spectrophotometry. Nature, 168, 598.

20. Brandt R.B. Mueller D.G. Schroeder J.R. Guyer K.E. Kirkpatrick B.V. Hukcner N.E. and Enrlich F.E. (1978). Serum Vitamin A In Preemture and Tera Neonates. Paediat. 92(1) 101 - 4.

21. Branstetter R.F. Tucker R.E. Mitchell G.E.J. Boling J.A. and Brasley W.R. (1973) Vitamin A Transfer From Cow To Calves. Int. J. Vitamin. Nutr. Res. 2: 43, 143.

22. Brown. E.F. and Bloor W.R. (1945) Nutritive Value Of Fatty Acids Of Butter Including Their Effect On Utilization Of Carotene. J. Nutr. 29 349 - 360.

23. Brown P.K. and Wald G. (1956) The Neo-B Isomer Of Vitamin A and Retinene. J. Biol. Chem. 865 - 877.

24. Carr F.H. and Price E.A. (1926) Colour Reaction Attributed To Vitamin A Biochem. J. 20. 497 - 501.

25. Chen C.C. and Weller J. (1977) Uptake Of Retinol and Retinoic Acid From Serum Retinol Binding Protein by Retinal Pigment Epithelial Cells. J. Biol. Chem. 252 : 5216 -5221

26. Cohlan S.Q. (1953) Congenital Anomalies In The Rat Produced By Excessive Intake Of Vitamin A During Pregnancy. Paediatrics 12. 549 - 556.

27. Coward W.A. Howell J.W.C. Pitt G.A.J. and Thompson J.M. (1966) Effects Of Hormones On Reproduction In Rats Fed A Diet Deficient In Retinol (Vitamin A Alcohol) But Containing Methyl Retinoate (Vitamin A Acid Methyl Ester). J. Repro. Fertil. 12. 309 - 317.

28. Deshmuk D.S. Malathi P. and Ganguly J. (1964)
Studies On Metabolism Of Vitamin A: Dietary Protein Content and Metabolism of Vitamin A
Biochem. J. 90: 98.

29. Doumas B.T. and Biggs H.G. (1975)
Determination Of Serum Albumin. In Standard Method Of Clinical Chemistry D.A. Coper Ed.
New York: Academic Press Inc. Vol. 7 p. 175.

30. Driskell W.J. Lackey A.D. Hewitt J.S. Bashor M.M. (1985)
Stability Of Vitamin A In Frozen Sera.
Clin. Chem. 31: 6, 871 - 872.

31. Drummond W.B. (1920)
A Binet Scale For The Blind.
Edinb. M.J. 1920 N. S. 24 (a): 91.

32. DU Bois E.F. (1936)
Basal Metabolism In Health and Disease
Lea and Febiger, Philadelphia 2nd

33. Edozien J.C. Switzer B.R. and Bryan R.B. (1976)
Medical Evaluation Of The Special Supplemental Food Program For Infants and Children.
Vol. II. pp. 409. 411. N. Carolina.
University of N. Carolina.

34. Embree N.D. and Shantz E.M. (1943)
A Possible New Member Of The Vitamin A1 and A2 Group.
J. Am. Chem. Soc. 65. 905 - 909.

35. Feachem R.G. (1987)
Vitamin A Deficiency and Diarrhoea - A Review Of Their Interrelation and Their Implication For The Control Of Xerophthalmia and Diarrhoea Tropical Disease Bulletin 84. 3, R1 - R15.

36. Fridge N.H. Shirston I. Ganguly J. Goodeen D.S. (1968)
Pathways Of Absorption Of Retinol and Retinoic Acid In The Rat. J. Lipid. Res 9 1 103.

37. FNB. United Nations (1968)
Food composition Table for use in Africa - Compiled by Hoot-Iowen Mo Leung. U.S.

Department of Health. Education and Welfare Public Heath Service.

38. Flores H. Campos F. Araujo C.N.M. Underwood B.A. (1984)
Assessment Of Vitamin A Deficiency In Brazilian Children Using The Relative Dose Response Procedure.
Am. J. Clin. Nutr. 39 : 1898 - 1902.

39. Gauhtgens G. (1937)
Excretion Of Vitamin A During Pregnancy Klin. Wchnschnr. 16 : 52 - From Nutrition Abstr. and Rev. 7 : 171.

40. Gal I. and Parkinson C.E. (1974)
Effects Of Nutrition and Other Factors On Pregnant Women's Serum Vitamin A Levels.
Am. J. Clin. Nutr. 2. 688 - 694.

41. Gal I. Sherman I.M. and Pryse-Davies J. (1972)
Vitamin A Relation To Human Congenital Malformations. Adv. Teratol. 5. 143.

42. Ganguly J. Pope G.S. Thompson S. Toothill J. Edwards-Webb J.D. and Haynforth H.B. (1971)
Studies On The Metabolism Of Vitamin A: The Effects Of Vitamin A Status On The Secretion Rates Of Some Steroids Into The Ovarian Venous Blood Of Pregnant Rats.
Biochem. J. 122 225 - 234.

43. Ganguly J. Pope G.S. Thompson S. Toothill J. Edwards-Webb J.D. and Haynforth H.B. (1971)
Studies On The Metabolism Of Vitamin A: The Effects Of Vitamin A Status On The Secretion Rates Of Some Steroids Into The Ovarian Venous Blood Of Pregnant Rats.
Biochem. J. 123, 669 - 670.

44. Garrett-Laster M. Jake M. Russel R.M. Bate R.M. (1981)
Lowering Effects Of Pharmacological Dose Of Vitamin E On Serum Vitamin A In Normal Adults.
Nutr. Res. 1 : 359 - 364.

45. Gebre-Medhin M. and Vahlquist A. (1984)
Vitamin A Nutrition in the Human Foetus: A Comparison Of Sweden and Ethiopia.
Acta. Paediat. Scand. 73. 3. 353 - 360 From Assignment Children. Vitamin A Deficiency and

Xerophthalmia. Madi. P. E. ed. 1988.

46. Giroud A. (960)
In Ciba Foundation Symposium On Congenital Malformations. p.199 (G.E.W. Wolstenholme and CM O' Connor, Editors). London Churchill.

47. Goodhart R.S. and Sallis M.E. (1974)
Vitamin A: In Nutrition In Health and Disease 5th Ed
Philadelphia. Lea and Febiger. 142 - 157.

48. Goodman D.S. (1979)
Vitamin A and Retinoids Recent Advances, Introduction, Background and General Overview.
Fed. Proc. 38: 2501 - 2503.

49. Goodman D.S. (1980)
Vitamin A Metabolism
Fed. Proc. Vol. 39 No. 10, 2716 - 2722

50. Goodman D.S. (1984)
Vitamin A and Retinoid in Health and Disease
N. Engl. J. Med. 19. 310 (6). 1023 - 31.

51. Goodman D.S. and Olson J.A. (1969)
Conversion Of All-Trans B-Carotene Into Retinol
R.B. Clayton Ed. Method in Enzymology Vol. 25
Steroids and Terpenoids.
New York Academic Press 462 - 475.

52. Goodman D.S. Huang H.S. Shiratori T. (1965)
Tissue Distribution and Metabolism Of Newly Absorbed Vitamin A In The Rat.
J. Lipid. Res. 6: 390 - 396.

53. Harris P. Amoo S.R. and Drinkman J.H. (1951)
Biochemical Studies On Vitamin A. II. Biopotency Of Neovitamin A In The Rat.
J. Amer. Chem. Soc. 73. 1252 - 1254.

54. Hathcock J.N. Hattan D.G. Jenkins M.Y. McDonald J.T. Sundaresan P.R. and Wilkening V.L. (1990)
Evaluation Of Vitamin A Toxicity.
Am. J. Clin. Nutrition 52 : 183 - 202.

55. Hays R.L. and Kendall F.A. (1956)
The Teratogenic Effects Of Hypervitaminosis On Pregnancy In The Vitamin A Deficient Rabbit
J. Nutr. 59 : 337.

58. Hirst J.C. and Shoemaker H.G. (1941)
Vitamin A In Pregnancy Comparison Of Dark Adaptation and Serum Tests.
Am. J. Obstet and Gynae, 42. 404.

59. Hoch H. (1959)
State Of Vitamin A In Human Serum
Nature ed. London 158.

59. Howell J.H. Thompson J.N. and Pitt G.A.J. (1967)
Histology Of The Lesion Produced In The Reproductive Tract Of A Male Rat A Diet Deficient In Vitamin A Alcohol But Containing Vitamin A Acid. II. The Female Rat.
J. Reprod. Fertil. 7: 251 - 259.

60. Hume E.M. and Krebs H.A. (1949)
Vitamin A Requirement Of Human Adults. An Experimental Study Of Vitamin A Deprivation In Man. Medical Research Council Spec. Rep. Ser. No. 264 London HMSO.

61. Hytten F.E. and Leiten I. (1971)
The Physiology Of Human Pregnancy
pp. 49 Oxford : Blackwell Scientific Publications.

62. Inhofen Bohlmann Bartram Rupert and Fonner (1950)
Synthesis In The Carotenoid Series.
Ann. Chem. 570. 54.

63. Isler V.O. Huber W. Ronco M. and Kofler M. (1947)
Synthesis Of Vitamin A
Helv. Chim. Acta. 30 1911 - 1927.

64. Iyengar L. and Apte S.V. (1971)
Nutrient Stores In Human Foetal Livers
Br. J. Nutr. 27: 313 - 7.

65. Jaruja H.S. Mudga M.R. and Ganguly J. (1964)
Studies Of Metabolism Of Vitamin A. The Effect Of Hormones On Gestation In Retinoate Fed Female Rats.
Biochem. J. 111, 97 - 100.

66. Kramer and Zucker (1950)
Carotenoids
Amsterdam. Elsevier Publ. Co. pp. 126. 150. 161.

67. Kaun J.J. Hallenfelter K.O. Chaikin C.W. (1984)
Preclinical and Clinical Toxicology Of Selected Retinoids. In: Spern M.B., Roberts A.B., Goodman D.S. Eds. The Retinoids Vol. 2 New York Academic Press 287 - 326.

68. Karrer and Eugster (1950)
Synthesis Of Cartenoids II. Toal Synthesis Of Carotenes I.
Helv. Chim. Acta 33. 1172.

69. Kaiser M. and Stekol J.A. (1943)
Critical Study Of Carr-Price Reaction For The Determination Of B-Carotene and Vitamin A In Biological Materials.
J. Lab and Clin. Med. 28: 904 - 909.

70. Kimble M.S. (1939)
The Photocolorimetic Determination Of Vitamin A and Carotene In Human Plasma.
J. Lab. and Clin. Med. 24 : 1055.

71. Knapp K.H. Montes A. and Wurh M.K. (1970)
Int Laboratory Indices Of Nutritional Status During Pregnancy (Committee On Nutrition Of The Mother and Pre School Child Editor) Washington DC.: National Research Council. National Academy Of Sciences.

72. Kon S.K. and Hawson E.H. (1950)
Human Milk Vitamin Studies: Of Certain Vitamins and Other Constituents.
Med. Res. Council. Spec. Ser. No. 269 London HMSO.

73. Le Francois P. Chevassus-Agnes, Benejfice E. Dick J.C. Marie B. Parent G. Seyaat G. and Ndiaye A.N. (1980)
Vitamin A Status Of Populations In Three West African Countries.
Int. J. Vitam. Nutr. Res. 50. 352.

74. Le Page E.A. and Nilsson S. (1941)
Absorption Experiments With Vitamin A
J. Biol. Chem. 14. 747.

75. Le Page G.A. and Mott L.W. (1941)
Absorption Experiments With Vitamin A
J. Biol. Chem. 14. 747.

76. Lehninger A.L. (1974)
Vitamin A
Biochemistry 2nd Ed. Worth Publishers 352 - 355.

77. Lesher M. Brody J.K. Williams H.H. and Macy I.G. (1945)
Human Milk Studies Vitamin A and Carotenoids Content Of Colostrum and Mature Human Milk.
Am. J. Dis. Child 70, 182 - 192.

78. Lewis J.M. Bodansky O. and Haig C. (1941)
Level Of Vitamin A Deficiency In Infats and In Children.
Am. J. Dis. Child 62. 1129-3

79. Lewis J.M. Bodansky O. Falk K.G. and McGuire (1942)
Vitamin A Requirements In the Rat. The Relation Of Vitamin A Intake To Growth and To Concentration Of Vitamin A In the Blood Plasma, Liver and Retina. J. Nutr. 23, 351-6

80. Lewis J.M. Bodansky O. Lillienfeld M.C.C. and Schneider H. (1947)
Supplements Of Vitamin A and Carotene During Pregnancy. Their Effect On The Level Of Vitamin A and Carotene In the Blood Plasma Of Mothers and Newborn Infats.
Am. J. Dis. Child. 73, 143 - 156.

81. Loerch J.D. Underwood B.A. and Lewis K.C. (1979)
Response Of Plasma Levels Of Vitamin A to A Dose Of Vitamin A As An Indicator Of Hepatic Reserves In Rats.
J. Nutr. 109. 778 - 786.

82. Lund C.J. and Kimble M.S. (1943)
Vitamin A During Pregnancy. Labour and Puerperium.
Am. J. Obstet. Gynaecol. 46. 406 - 501.

83. Lund C.J. and Donova J.C. (1967)
Blood Volume During Pregnancy. Significance Of Plasma and Red Cell Volumes.
Am. J. Obstet. Gynaecol. 98 : 393-7

84. Lung C.J. and Kimble M.S. (1943)
Vitamin A and Carotene Of the Newborn Infants With Consideration Of Foetal Maternal Relationship.
Am. J. Obstet. Gynaecol. 46 : 207- 11

85. Mancini G. Carbonara A.O. Heremans J.F. (1965) Immunological Quantitation On Antigen by Single Radial Immunodi Fussion Immunochemistry 2, 234 - 254.

86. Martin D.W. (1980) Fat Soluble Vitamins: Vitamin A (Retinoids) In - Harper's Review Of Biochemistry. 18th ed. Lange Medical Publications. 14 - 16.

87. Marton R.A.F (1944) Chemical Aspects Of The Visual Process Nature 153, 69-72

88. McCollum E.V. and Davies M. (1913) The Necessity Of Certain Lipids In The Diet During Growth. J. Biol. Chem. 15: 167 - 175.

89. McCormick A.M. Napol J.L.. Schnoes H.K. Deluca H.F (1978) Isolation and Identification Of 5, 6 - Epoxyretinoic Acid : A Biological Active Metabolite Of Retinoic Acid. Biochemistry 17 : 4085 - 4090.

90. McGanity W.J. Little H.M. Coelho A. Jennings L. Calhoun E. and Dawson N.E.B. (1969). Am. J. Obstet. Gynecol. 103, 773 - 788.

91. McLaren D.S. and Ward P.G. (1962) Malaria Infection Of The Placenta and Foetal Nutrition E. Afr. Med. J. 39. 182 - 195.

92. Mejia L.A. and Chew F. (1988) Haematological Effects Of Supplementation On Anaemic Children With Vitamin A Alone and In Combination With Iron. Am. J. Clin. Nutr. 48 : 595 - 600.

93. Morrow B. (1952). Relationship between Intake and Serum Levels Of Absorbic Acid, Vitamin A and Carotene Of Selected Groups Of Children with Physical Signs Of Vitamin A Deficiencies. J. Nutr. 46, 445 - 458.

94. Muntrawaauwat N. and Olson J. (1979) Serum and Liver Concentrations Of Vitamin A In That Foetuses As A function Of Gestational Age. Am. J. Clin. Nutr. 32(3) 601 - 6.

96. Moore T. (1930)
Vitamin A and Carotene
Biochem. J. 24, 692.

97. Moore T. (1957)
Vitamin A
Elsevier Publishing Co. Amsterdam. 305 - 374.

98. Moore T. (1971)
Vitamin A Transfer From Mother To Offspring In Mice and Rats.
Int. J. Vitam. Nutr. Res. 41. 301.

99. Mourad R.L. Klein D. Meier F. (1975)
A Case Of Cridamar Syndrome: Acute Vitamin A Intoxication In Mother During Pregnancy.
J. Genet. Hum. 23: 135 - 154.

100. Mourey M. Siegenthaler G. Amedee-Manesme O. (1990)
Regulation Of Metabolism Of Retinol Binding Protein By Vitamin A Status.
Am. J. Clin. Nutr. 51: 643.

101. Nenid J.R. and Pearson W.N. (1962)
Macro and Micro Methods For The Determination Of Serum Vitamin A Using Trifluoroacetic Acid
J. Nutr. 74: 454 - 462.

102. O'Toole B.A. Frodsin R. Morisey J. Wilson J.G. and Mann G.C. (1974)
Vitamin A Deficiency and Reproduction in Rhesus Monkeys.
J. Nutr. 104. 1513 - 1524.

103. Olson J.A. (1961)
The Conversion Of Radioactive β-Carotene Into Vitamin A by Rat Intestine In Vivo.
J. Biol. Chem. 236 : 349.

104. Olson J.A. (1987)
Recommended Daily Intake (RDI) Of Vitamin A in Humans.
Am. J. Clin. Nutr. 45. 704 - 716.

105. Oluwin O. (1970)
Etiology Of Blindness In Nigerian Children.
Am. J. Ophthalmol. October. 533 - 539.

106. Oluwanya E.O. Oyemade O. and Ijoi A.E. (1987)
Household Food Security, Nutrition and Health

In Oyo Local Government Area. In Cassava: Life Line For The Rural Household.
Ikpi A.E. and Hahn N.D. Ed. 72 - 116.

106. Oomen J.M.V. (1971)
Xerophthalmia In Nigeria. Trop. and Geog. Med. 23. 246 - 249.

107. Palludan B. (1966)
Avitaminosis In Swine: A Study On The Importance Of Vitamin A For Reproduction. Copenhagen: Munksgaard. From Vitamin A In Pregnancy and Lactation. Reviewed By Bates (1983). Proc. Nutr. Soc. 42. 65 - 79.

108. Pulliam R.P. Dannenburg W.N. Burt R.L. Leslie H. (1962)
Carotene and Vitamin A In Pregnancy and The Early Puerperium.
Proc. Soc. Exptl. Bio. Med. 109. 913 - 916.

109. Rallis E.P. Papper E. Parley F. and Wauman E. (1941)
Vitamin A and Carotene Content Of Human Liver In Normal and In Diseased Subjects : Analysis Of 116 Human Livers.
Arch. Int. Med. 68. 102.

110. Robeson Cawley Weisler Stern Eddinger and Chechak (1955)
The Synthesis Of Geometric Isomers Of Vitamin A Via Methyl E-Methyl Gluconate.
J. Am. Chem. Soc. 77. 4111 - 4119.

111. Rodriguez M.S. and Irwin M.I. (1972)
A Conspectus Of Research On Vitamin A Requirements Of Man.
J. Nutr. 102. 909 - 968.

112. Roels O.A. Trout M. Dujacquier R. (1958)
Carotene Balances On Boys In Ruanda Where Vitamin A Deficiency Is Prevalent.
J. Nutr. 65. 115 - 127.

113. Sauberlich H.E. and Hodges H.E. Canham J.E. Wallace D.L. Rucker R.B. Mejia L.A. and Mohanram M. (1974)
Haematopoietic Studies In Vitamin A Deficiency.
Am. J. Clin. Nutr. 31(5) 876 - 85.

114. Shenai J.P. Chytil F. Jhaveri M. and Stahlman M.T. (1981)
Plasma Vitamin A and Retinol-Binding Protein In Premature and Term Neonates.
J. Paediatr. 99(2) : 302 - 5.

115. Silverman L.M. Christenson R.H. Grant G.H. (1986)
Amino Acid and Proteins In : Textbook Of Clinical Chemistry.
Norbert W. Tiez Edition. 559 - 562.

116. Smith J.E. Bore K.C. and Goodman D.S. (1978)
Regulation Of Retinol-Binding Protein Metabolism In Cultured Rat Liver Cell Lines.
Cell 15: 855 - 873.

117. Sobotka H. Khann S. and Loewenstutein E. (1943)
The Fluorescence Of Vitamin A.
J. Amer. Chem. Soc. 65. 1959 - 1961.

118. Standford-Smith J.H. and Whittle H.C. (1979)
Cornea Ulceration Following Measles In Nigerian Children.
Brit. J. Ophthamol 63 : 720 - 724.

119. Sommer A. Tarwotjo I. Hussaini G. and Susanto D. (1983)
Increased Mortality In Children With Mild Vitamin A Deficiency.
Lancet 2 : 350 - 8.

120. Stuenbock H. (1919)
White Corn Vs Yellow and A Probable Relation Between The Fat Soluble Vitamin and Yellow Plant Pigments.
Science N.Y. and Lancaster P.A. N.S.L. 352.

121. Steunbock H. and Boutwell P.W. (1920)
Fat Soluble Vitamin III. The Comparative Nutritive Value Of White and Yellow Maize.
J. Biol. Chem. 41 : 81 - 90.

122. Sweeny J.P. and Marsh A.C. (1970)
Separation Of Carotene Stereoisomers In Vegetables.
J.A.O.A.C. 53. 937 - 940.

123. Takahashi Y.I. Smith J.E. and Goodman D.S. (1977)
Vitamin A and Retinol-Binding Protein Metabolism During Foetal Development in The Rat.

Am. J. Physiol. 233, 263-7

124. Takahashi Y.I. Smith J.E. Minick M. and Goodman D.S. (1979)
Vitamin A Deficiency and Foetal Growth and Development In The Rat.
J. Nutr. 105, 299 - 1310.

125. Taylor G.O. and Akande E.O. (1975)
Socio Economic Factors and Serum Lipids in Pregnant Women.
Brit. J. Obstet. Gynaecol. 82 (4) 297 - 302.

126. Thompson D.L. Ruiz E. and Baker M. (1964)
Vitamin A and Reproduction in Rats.
Proc. Roy. Soc. London Ser. B. 159 : 510 - 535.

127. Thompson J.Y. Brande R. Coates M.E. Cowie A.T. and Ganguly J.A.N. (1950)
Further studies Of The Conversion Of β-Carotene To Vitamin A In The Intestine.
Br. J. Nutr. 4, 398.

128. Underwood B.A. (1990)
Methods of Assessment Of Vitamin A Status.
J. Nutr. Nov. Suppl. 12 : 1459 - 1463.

129. Urback G. Hickman K. and Harris P.L. (1952)
Effects Of Individual Vitamin A, C, E, and Carotene Administered At High Levels On Their Concentration In The Blood.
Exp. Med Surg. 10 : 3 - 20.

130. Vahlquist A. and Nilsson S. (1979)
Mechanic Of Vitamin A Transfer From Blood To Milk In Rhesus Monkeys.
J. Nutr. 109 (8) : 1456 - 63.

131. Vahlquist A. Rask L. Patterson P.M. and Berg T. (1975)
The Concentration Of Retinol-binding Proteins Prealbumin and Transferring In the Sera Of Newly Delivered Mothers and Children Of Various Ages.
Scand J. Clin. Lab. Invest 35. 569.

132. Van Bruggen J.T. and Straunfjord J.V. (1948)
High Vitamin A Intake and Blood Levels Of Cholesterol, Phospholipids, Carotene and Vitamins C, A, and E.
J. Lab. Clin. Med. 33. 67 - 74.

133. Venkatachalam P.S. Belvady B. and Gopalan C. (1962)
Studies on Vitamin A Nutritional Status Of Mothers and Infants In Poor Communities Of India.
J. Paediat. 61, 262 - 269.

134. Villard C.J and Bates L. (1986)
Effects Of Vitamin A Supplementation On Plasma and Breast Milk Vitamin A Levels In Poorly Nourished Gambian Women.
Hum. Nutr. Clin. Nutr. 41B, 47 - 58.

135. Vollbracht R. and Gilroy J. (1978)
Vitamin A Induced Benign Intracranial Hypertension.
Can. J. Neurol. Sci. 3(1) : 59 - 61.

136. Vonlennep E. El Knazen M. De Pierreux G. Amy J.J. Rodesch F. and Van Regemorter N. (1985)
A Case Of Partial Sirenomelia and Possible Vitamin A Teratogenesis.
J Prenat Diagn. 5 : 35 - 40.

137. Voorhoeve H.W.A. (1966)
Xerophthalmia and Kwashiorkor In 6 Children In Ibadan.
Trop. Geog. Med. 18. 15 - 19.

138. Wald G. (1954)
Enzymatic Reduction Of The Retinene To The Vitamin A Chemical. Abstract 4560B.

139. Wald G., Brown P.K., Hubbard R. and Drosdnik W. (1953)
Hindered Cis Isomers Of Vitamin A and Retinene: The Structure Of The Neo-B Isomer.
Natl. Acad. Sci. US 41 438.

140. Wald G. (1960)
The Visual Function Of The Vitamin A
Vitamin and Hormone 10 : 417.

141. Wald N. Idle M. Boreham J. and Bailey A. (1980)
Low Serum-Vitamin A and Subsequent Risk Of Cancer: Preliminary Results Of A Prospective Study.
Cancer. 2, 813 - 816.

142. Wald N.J. Cuttle N.S. and Dalton R.D. (1985)
The Effect Of Vitamin A Supplementation On Serum

Retinol and Retinol-Binding Protein Levels.
Cancer. Lett. 29: 203 - 213.

143. Willet W.C. Stamper M.J. Underwood B.A. Sampson L.A., Hennekens C.H., Wallingford J.C., Cooper L., Sieh C.C. and Speizer F.E. (1984) Vitamin A Supplementation and Plasma Retinol Levels : A Randomised Trial Among Women. J. Natl. Cancer. Inst. 73 : 1445 - 1448.

144. Willet W.C. Stamper M.J. Underwood B.A., Sampson L.A. and Hennekens C.H. (1983) Vitamin A. E and Carotene : Effects Of Supplementation On Their Plasma Levels. Am. J. Clin. Nutr. 38 : 559 - 566.

145. Wilson E.D. Fisher K.H. Garcia P.A. (1979) Vitamin A : In Principles Of Nutrition Ach ed. John Willey and Sons Inc. 171 - 179. 422.

146. Wilson J.G. Roth C.B. and Warkany J. (1983) An Analysis Of The Syndrome Of Malformations Induced By Material Vitamin A Deficiency. Effects Restoration Of Vitamin A At Various Times During Gestation. Am. J. Anat. 92 189 - 217.

147. Zechmeister L. and Polgar A. (1949) CIS-Trans Isomerization and Spectral Characteristics Of Carotenoids and Some Related Compounds. J. Am. Chem. Soc. 65. 1522 - 1528.

148. Zechmeister L. (1948) CIS-Trans Isomeric Carotenoids. Vitamins A and Arylpolyenes Academic Press. New York 251 pp.

149. Zechmeister Pinckard Greenberg Straus Fukui and Deuel Jr. (1949) Sterochemical Configuration and Provitamin A Activity III. Procarotene (A Poly CIS-Compound) and Its All-Trans Isomer In Rat. Arch. Biochem. 23, 242 - 245.

APPENDIX I

CONSENT FORM FOR VITAMIN A STUDIES IN PREGNANT AND NON PREGNANT NON LACTATING NIGERIAN WOMEN.

I ---------- GIVE MY CONSENT TO PARTICIPATE IN THE ABOVE NAMED PROJECT.

I HEREBY DECLARE THAT I WAS THOROUGHLY INFORMED OF THE PROTOCOL OF THE STUDY PRIOR TO MY CONSENT.

---------- ----------

SIGNATURE OF PARTICIPANT WITNESS

DATE---------- DATE----------

APPENDIX II

QUESTIONAIRE FOR VITAMIN A STUDIES IN PREGNANT AND NON PREGNANT NON LACTATING NIGERIAN WOMEN

PLEASE SPECIFY OR TICK THE APPROPRIATE ANSWERS TO THE QUESTIONS BELOW:

1. CODE NO----------
2. HOME ADDRESS----------
3. AGE/DATE OF BIRTH----------
4. EDUCATION 0 1 2 3 4 5

   HUSBAND

   WIFE

5. OCCUPATION:

   HUSBND

   WIFE

6. INCOME: <1000 1-3000 3-6000 6-9000 >9000

   HUSBAND

   WIFE

7. ARE YOU PREGNANT ? YES NO

8. IF YES TO 07 PLEASE STATE AGE OF PREGNANCY/ EXPECTED DATE OF DELIVERY:

9. PARITY----------

10. 24 HR DIETARY RECALL

    BREAST LUNCH SUPPER SNACK

11. DIETARY HABBIT

HOW OFTEN DO YOU EAT DAILY ?----------

IF YOU MISS ANY MEAL WHICH ONE DOY OFTEN MISS?

WHAT IS/ARE YOUR REASON/S FOR MISSING THE MEALS

WHAT FOODS DO YOU EAT DAILY----------

WHAT FOODS DO YOU EAT OCCASSIONALLY----------

WHAT FOODS DONT YOU EAT AT ALL----------

GIVE REASONS PLEASE----------

12. ASSESSMENT OF VITAMIN A INTAKE

HOW OFTEN DO YOU EAT THE FOLLOWING FOOD ITEMS WEEKLY?

FOOD ITEMS	FREQUENCY/WK
LIVER	
EGGS	
MILK	
CARROTS	
MANGOES	
SWEET POTATOES	
PALM OIL	

13. KNOWLEDGE ABOUT VITAMIN A

DO YOU KNOW WHAT VITAMIN A IS? YES NO

IF YES TO Q13 DO YOU KNOW WHAT DISEASE ITS LACK CAN CAUSES? YES NO

14. ARE YOU CURRENTLY ON ANY MULTIVITAMIN TABLETS

YES NO

15. IF YES WHY?----------

16. DO YOU SEE PROPERLY IN THE EVENINGS IN A DIMMLY LIT ENVIRONMENT

YES NO

17. IF HOW LONG DOES IT TAKE YOU TO ADJUST YOUR SITE WHEN YOU ENTER A DIMMLY LIT ENVIRONMENT?

1MIN <5MIN >5MIN 10MIN

18. HEALTH ASSESSMENT

WEIGHT (KG)----------

HEIGHT (CM)----------

EYE EXAMINATION

SCAR ULCER DRYNESS BITOT SPOT

CONJUCTIVA

CORNEA

PCV----------

SGOT----------

SGPT----------

URINALYSIS----------

PLASMA VITAMIN A----------

PLASMA B-CAROTNENE----------

PLASMA ALBUMIN----------

PLASMA TRANSTYRETTIN----------

PLASMA RETINOL BINDING PROTEIN----------.

B-CAROTENE AND VITAMIN A CONTENTS OF VARIOUS FOOD ITEMS COMMONLY CONSUMED (ug/100g)

FOOD ITEMS	B-CAROTENE	VITAMIN A
Rice	-	-
Bean portage	460	-
Bread	-	-
Amala	-	-
Eba (gari)	-	-
Plantain (fried)	45	-
Pap (ogi)	-	-
Yam	-	-
Egg (raw)	240	260
fried	180	320
boiled	200	320
Akara	465	-
Moinmoin	165	-
Beef		83
Fish	13	-
	-	
Ewedu	-	-
Gbure	-	-
Vegetable	15	-
	360	
Stew (g.oil)	-	-
(p.oil)	320	-
Lafun		-
Sugar	-	-
Milk	-	
		320
Soft drink	380	-
Margarine	-	00
	-	

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29 August 1989

Miss Iyabode Adeyefa
Department of Human Nutrition
College of Medicine
University of Ibadan

Dear Miss Adeyefa

Re: Viruses in [illegible]

The text of the study protocol of the above project has been reviewed by the Committee. The methodology is in conformity with the appropriate rules and guidelines laid down for conducting research in the University.

I therefore approve on behalf of the Joint University of Ibadan/University College Hospital Ethical Committee that you may proceed with the study.

Best regards.

Yours sincerely

Oyewale Tomori
Director - Chairman
Ethical Committee