EFFECT OF VITAMIN A THERAPY ON THE RETINOL AND RETIMOL-BINDING PROTEIN IN NIGERIAN CHILDREN WITH MEASLES, MALNUTRITION AND OTHER PATHOLOGICAL CONDITIONS

BY

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D E C L A R A T I O N

I declare that this work is wholly the author's and has not been part of any presentation for any other qualification.

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ABSTRACT

Vitamin A therapy was administered to Children with various pathological conditions other than measles and malnutrition (B), children suffering from malnutrition without measles infection (C), children with proven cases of measles infection without malnutrition (D), and children with measles and malnutrition (E). The changes in the plasma retinol and retinol-binding protein (RBP) were evaluated in these subjects.

Children between the ages of 6 weeks to 36 months were treated with 50,000 - 100,000 i.u of retinyl palmitate depending on the age of the child and severity of the disease condition. RBP and prealbumin wore determined using radial immunodifuasion technique, while retinol and retinyl ester were determined using the trifluoroacetic acid method.

The control population (A) had higher retinol and RBP than all other groups of subjects studied. These differences were significant ($p<O.Ol$) when A was compared with C, D and E. The mean plasma retinol differed significantly between groups B and C, D and E ($p<.0.01$, $p<.0.001$, respectively). Similarly, the mean plasma RBP between groups B and C, D and E were significant ($p<.0.02$, p c.0.001, respectively). In all categories of patients studied, vitamin A therapy significantly raised the mean plasma retinol (B: $p<0_o05$, C: $p<O.Ol$, D: $p<.0.005$, E: $p<O.005$) and RBP levels (B: $p<0.05$, C: $p<.0.05$, D: $p<.0.005$, E: $p<.0.005$). The raised retinol value was still significant ($p<0.05$) 4-7 days after therapy in D while the increase in RBP was not significant.

The reasons for the positive correlations existing between retinol and RBP, retinol and prealbumin, and between prealbumin and RBP in some of the categories of children studied were discussed.

This study has indicated that measles infection, and measles in association with other diseases such as malnutrition, significantly impair the metabolism of retinol, probably due to the insufficiency of the carrier protein, RBP as a consequence of inadequate protein intake or utilization- However, further study would be necessary to confirm this.

In view of the high incidence of eye complication associated with measles and malnutrition including blindness, the results of this investigation would form a necessary aspect of future public health education programme that might require the administration of vitamin A to children for the prevention of hypovitaminosis A and consequent associated pathological conditions. However, it is suggested that such a public health programme should include some form of protein supplementation.

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DEDICATION

To those scientists who dedicated their lives searching for the prevention and cure of blinding malnutrition disease in children.

1 INTRODUCTION

1.1 Definition

The term vitamin A, as commonly used refers to a group of compounds having biological activity of retinol, including retinal, retinoic acid and retinyl ester. These are also referred to as retinoids. The carotenoids which are found in green plants are the natural precursors of the retinoids. The retinoids and carotenoids exist predominantly as all-trans isomers (Figure 1.1).

1.2 History of the Research Problem

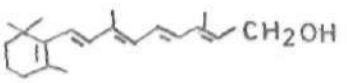
Vitamin A deficiency often occurring in association with protein energy malnutrition (PEM), parasitic infestation and gastroentritis, is one of the major nutritional problems of the world (WHO, 1976).

Vitamin A deficiency, although affecting many tissues of the body, is clinically most severe in its ability to damage the cornea of the eye. The surface of the cornea becomes dry and rough (a condition called xerophthalmia) and ultimately breaks down partially or completely, leading to permanent scarring and irreversible partial or total loss of sight.

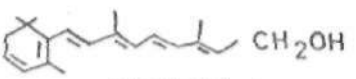
It is estimated that up to 100,000 children go blind every year, mostly in South East Asia, the Middle East, North Africa and some parts of Latin America (WHO, 1976; Pirie, 1978; McLaren, 1979). Despite the increasing knowledge of and concern over its control, blindness is still on the increase.

FIG. 1-1 : STRUCTURES OF VITAMIN A AND RELATED COMPOUNDS

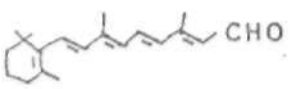
RETINOIDS



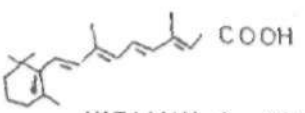
VITAMIN A_1 (RETINOL)



VITAMIN A_2
(DEHYDRORETINOL)

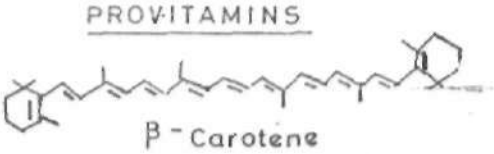


VITAMIN A ALDEHYDE (RETINAL)



VITAMIN A ACID
(RETINOIC ACID)

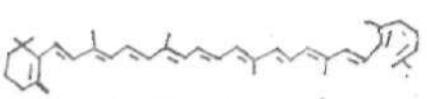
PROVITAMINS



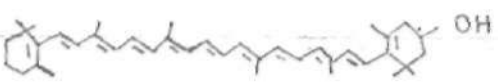
β - Carotene



α - Carotene



γ - Carotene



CRYPTOXANTHIN

ADAPTED FROM THE VITAMINS VOL. 2, 1967

In the developing countries, the blind child has a sad existence because he is unable to fend for himself in the absence of any organised social service programme. He is often abandoned by members of his family who themselves may be in abject poverty. A child in this situation has little to look forward to in life and the saddest aspect is that this condition can easily and cheaply be prevented by adequate intake of foods containing vitamin A.

In clinical reports from African countries, xerophthalmia is sometimes reported to accompany measles. The latter is a well-known important cause of death especially in young children throughout tropical Africa, Asia and Central America. Serious complications of this disease include brocho-pneumonia, diarrhoea, loss of appetite and vomiting. Unlike small-pox which has almost been eradicated through immunization sponsored by the World Health Organisation, measles still remains a major cause of death in many parts of the world.

In 1974 in Lagos, Nigeria, blindness was attributed to measles in 24 out of a total of 73 children attending the Pacelli school for the blind and 11 out of 14 new entrants to the same school in October 1975 had become blind following measles (Animashaun, 1977). In the same year all cases of measles were reviewed in about 114 in-patients admitted to Massey Street Children's Hospital, Lagos.

Ocular complications occurred in 47 children, a figure which was exceeded only by broncho-pnuemonia and malnutrition. Eighteen out of 47 cases of ocular complications were mild to moderate, while 29 were severe and resulted in blindness (Animashaun, 1977). In 1970, five cases of xerophthalmia from a single compound of Kambari tribe, during a local measles epidemic were brought to Catholic Hospital Yerwa, Northern Nigeria (Oomen, 1970).

The significance of the problems of xerophthalmia is under-estimated since vitamin A deficiency is linked to other pathological disturbances that lead to death. Most of the deaths associated with vitamin A deficiency occur in infants and children in the second year of life. According to McLaren (1979), about 25% of the survivors of severe ocular damage xerophthalmia, cornea ulceration and keratomalacia) remain totally blind, about 50-60% are partially blind, and only 15-25% escape with unimpaired sight.

In Zaria, research carried out by Inua (1979) showed lower level of plasma vitamin A in children with measles infection and malnutrition than in normal children. She suggested that blindness in children following measles infection is due more to hypovitaminosis A than to the disease itself. It is thought that adequate dietary intake of vitamin A prior to infection would help minimize the precipitation of hypovitaminosis A and occurrence of eye lesions following an attack of measles.

The objectives of the present investigation were:

i) to establish the retinol and RBP concentrations in apparently healthy Nigerian children, those with measles and malnutrition.

ii) to study the effect of vitamin A therapy on the plasma retinol and RBP concentrations in Nigerian children suffering from measles and malnutrition.

iii) to show the relationship between the plasma retinol levels and the plasma prealbumin and RBP concentrations in normal Nigerian children, those with measles and malnutrition.

1.3 Review of the Literature

1.3.1 Vitamin A

1.3.1.1 Historical Introduction

The history of the discovery and development of vitamin A started with investigations relating to carotenoids. Carotene was subsequently isolated from carrots by Wackenroder in 1831 (cited by Maddy, 1978).

The biological activity of vitamin A as fat soluble vitamin was first described in two separate laboratory experiments (McCollumn and Davis, 1913; Osborne and Mendel, 1913) when it was found that the growth of rats ceased prematurely when lard was used as the source of fat in experimental diets, whereas adequate growth was achieved when the dietary fat was either butter or fat extracted from egg yolk. This growth promoting substance present in butter or egg yolk was called vitamin A. However, it was not until six years later (Steenbock, 1919) that another characteristic of vitamin A was recognised, the association with yellow colour in plants.

The biochemical and physiological role of vitamin A was first recognised in association with visual cycles by Boll in 1876 (Wald, 1968) when he described how a freshly excised frog retina was pink in colour but became "bleached" on exposure to light. This change in colour was designated visual purple.

Deficiency of vitamin A has been shown to result in cessation of growth, keratinization of the epithelium in various parts of the body such as the respiratory, alimentary, reproductive and genito-urinary tracts (Wilson, et al., 1965).

1.3.1.2 Dietary sources

Dietary sources of vitamin A are of two kinds: (1) preformed vitamin A from animal sources mainly the liver and especially oils from fish liver, kidney, egg yolk, butter, cheese and milk. (2) Provitamin mainly B-carotene from many plants. β-carotene can be obtained from dark green leaves, pawpaw, carrot and red palm oil. Vitamin A intake comes from these sources in many parts of the world where animal products are seldom eaten. Although there are over 100 carotenoids, only about 10 have provitamin A activity. These include α-, β-, γ-carotene and cryptoxanthin (Fig 1.1). Beta carotene is by far the most widespread and most potent provitamin A (Sebrell and Harris, 1967).

1.3.1.3 Recommended intake

The recommended daily dietary allowances of vitamin A in terms of retinol for various age groups in the U.S.A. are as follows: infants 420μg; children 0.5 to 4 years old, 400μg; adult females, 800μg; adult males and pregnant women 1000μg, and lactating women, 1200μg (IVACG, 1976b). Other countries have made similar recommendations.

1.3.1.4 Metabolism

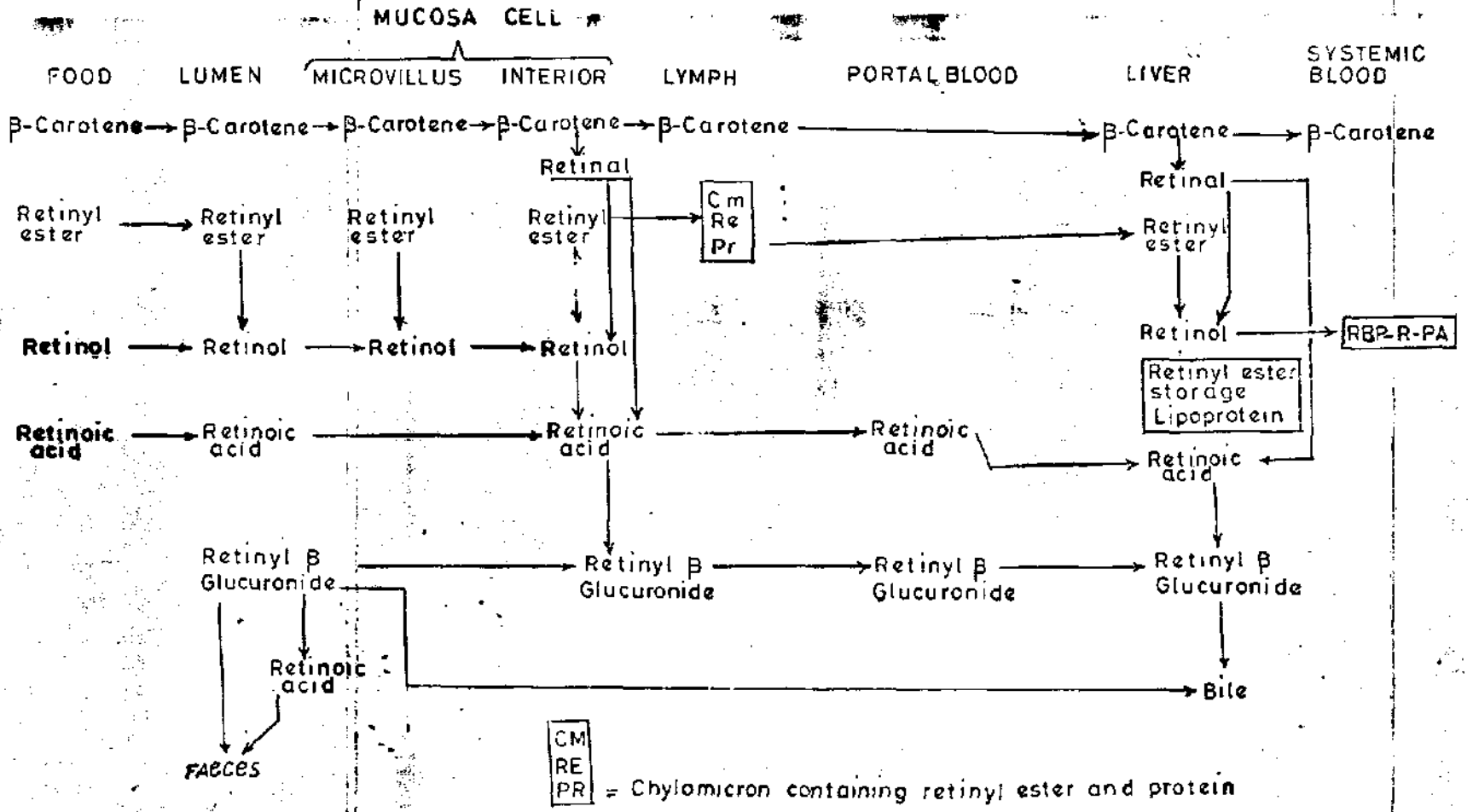
1.3.1.4.1 Absorption and Biosynthesis: Before absorption by the intestinal mucosa, dietary retinol and retinol precursors must be emulsified in the presence of lipids and bile salts (Sebrell and Harris 1967). The process requires an ester hydrolase, from pancreatic juice (Fig 1.2).

The provitamin A compounds contain one or two unsubstituted B-ionone rings. The formation of vitamin A from B-carotene entails first, the cleavage of the carotene into 2 molecules of retinal in the intestinal mucosa by a carotene 15', 15' dioxygenase (Olson, 1961); central fission theory); then the retinal is reduced to retinol. Though most of the carotenes are converted to retinol, some are absorbed unchanged. Retinol is esterified in the intestinal mucosa to produce esters predominantly of palmitic and stearic acids. The esters and the carotenoids are then transported to the liver by way of the lymphatic system as component of chylomicrons (Goodman et.al, 1966).

1.3.1.4.2 Uptake and Storage

Retinol is stored in the liver primarily as retinyl, ester predominantly as the palmitate (Sebrell and Harris, 1967). Intracellularly unesterified retinol liberated by esterase action on retinyl ester is bound by a specific cellular binding protein (cRBP) in the cytosol. Retinol is then esterified and transfered into a soluble lipoglycoprotein aggregate which is the storage form.

FIG. 1·2 : METABOLISM OF VITAMIN A IN MAN



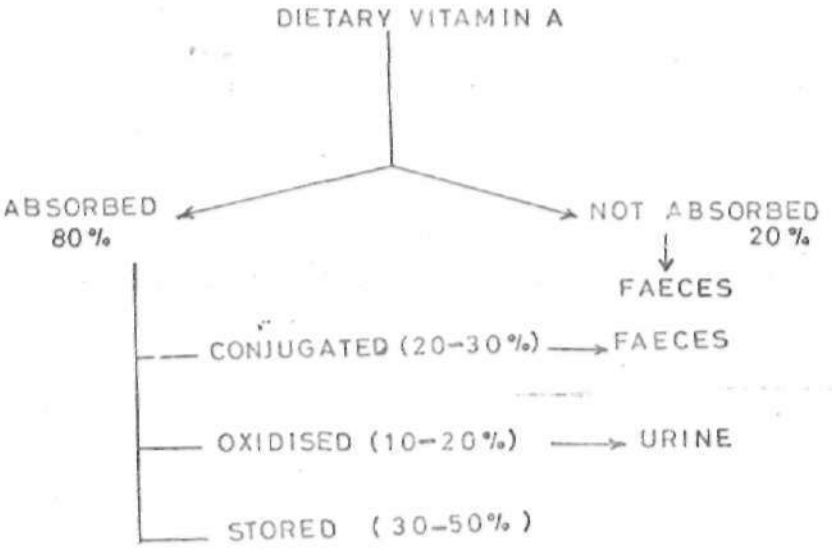
Retinyl ester hydrolase, which is tightly bound to the lipoglycoprotein aggregate, hydrolyses retinyl ester to retinol, which then forms a complex with a specific retinol binding protein (apo RBP) during its mobilization from the liver. (Plasma RBP and cellular RBP are quite different proteins). The retinol - RBP complex (holo-RBP) enters vesicles derived from golgi body and is then released to the plasma.

1.3.1.4.3 Excretion

Thirty to fifty percent of absorbed dietary vitamin A may be stored in the liver while the remaining portion is rapidly metabolised and excreted in faeces and urine (Figure 1.3, WHO, 1976). There are two major reactions in the excretion (1) conjugation of retinol and of retinoic acid with glucuronic acid in the liver followed by excretion of the conjugate into the bile and then into faeces (2) Metabolism of retinol and retinoic acid mainly in the liver and kidney to biologically inactive oxidation products (e.g. 4-keto derivative of vitamin A) followed by excretion of the degradation products through the urine (Fig 1.4).

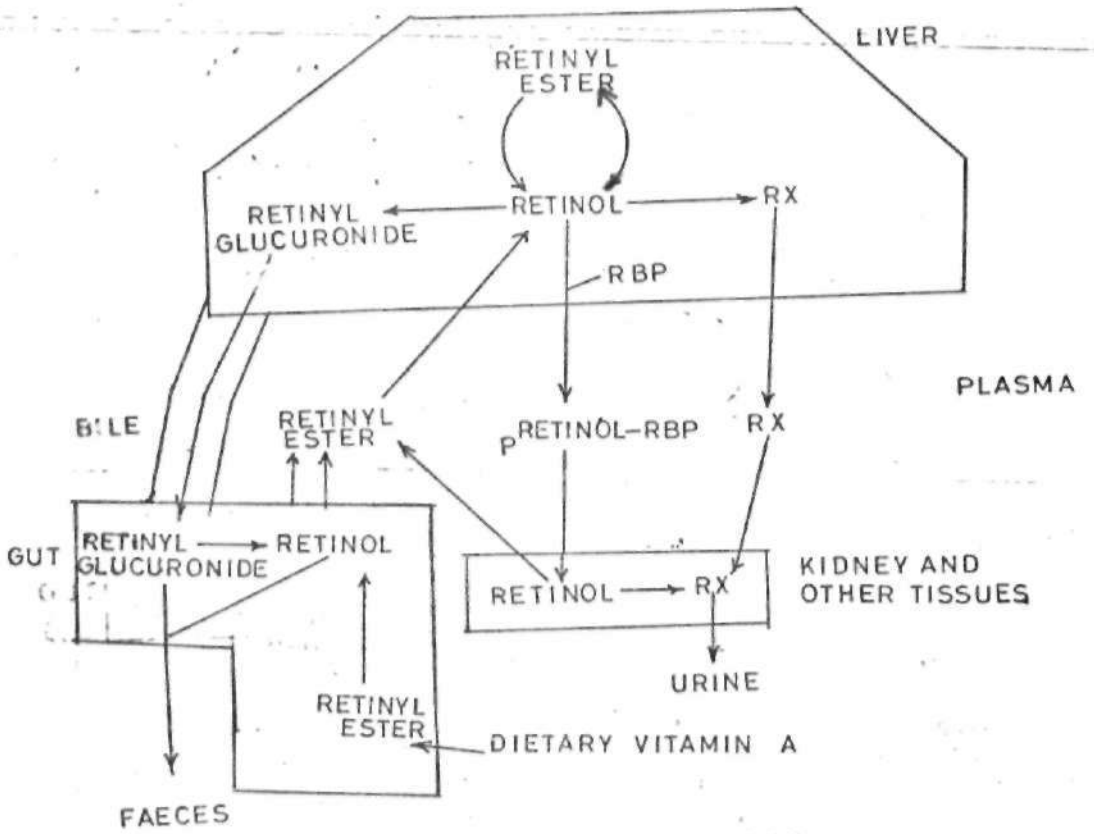
Some of the conjugated forms of vitamin A released into the bile are reabsorbed from gut and transported back to the liver, thereby allowing for the reutilization of metabolised retinol. Likewise, retinol released as an RBP complex is also transported in part back to liver from tissues.

FIG. 1·3 : UTILIZATION OF DIETARY VITAMIN A



ADAPTED FROM WHO (1976)

FIG.1.4: DYNAMICS OF VITAMIN A RECYCLING AND EXCRETION



ABBREVIATIONS

RX = BIOLOGICALLY INACTIVE OXIDATION PRODUCTS

RBP = RETINOL-BINDING PROTEIN

PREVIX P = PLASMA

PRETINOL-RBP = holo-RBP

ADAPTED FROM IVACG (1977).

The recycled retinol is esterified and transported to the liver in association with low density lipoproteins. This recycling process may be of critical importance when the vitamin A status is poor (IVACG, 1976b).

1.3.1.4.4 Transport in Plasma

A specific, small (MW 21,000) binding protein for retinol (apo-RBP) is synthesized in the liver (Smith et al, 1970). This apo-RBP binds retinol in a 1:1 molar complex in the cytoplasm (Raz et al, 1970). The resulting holo-RBP is taken up by the Golgi fraction and secreted into the blood. Holo-RBP forms a 1:1 molar complex with prealbumin (Smith and Goodman, 1971). These two proteins are secreted separately by the liver. Thus retinol-RBP - Prealbumin occurs as a 1:1:1 complex in which the binding is co-operative (Peterson, 1971). The binding of all-trans-retinol to RBP is highly, but not absolutely specific. A number of isomers of retinol and of retinal, as well as retinoic acid and retinyl acetate, can bind to apo-RBP with varying degrees of effectiveness (Harris et al, 1974). After transfer of retinol to cells of target tissues, apo-RBP is degraded in the kidney. The turnover of RBP is very high. Studies on experimental rats showed that the biological half-life of RBP is approximately 6.5 hours, corresponding to a synthesis rate of about 1.5µg per day (Paterson et al, 1973).

1.3.1.4.5 Plasma Concentration

The concentration of retinol in plasma is 30µg/100ml in normal subjects (IVACG, 1976b). Below this, there may be symptoms of deficiency though the normal level may vary depending on age and sex. Hypervitaminosis A is usually a result of excess intake, hypovitaminosis A may not necessarily be the result of low intake. It may also be due to the impairment of any of the following, absorption, lymphatic transport, storage in liver, mobilisation, transport associated with retinol-binding protein or utilization. Impaired absorption may be associated with gastroentritis (Harris et. al., 1974) while impaired transport associated with retinol-binding protein may be due to protein energy malnutrition (Smith et. al., 1975). Hypovitaminosis has been found associated with infections such as hepatitis, measles and respiratory infections (Inua et. al. 1979; Harold, 1976).

Plasma concentrations of retinol-binding protein and prealbumin in normal subjects are 40-50µg/ml and 200-300µg/ml respectively (Smith et. al., 1970; Smith and Goodman, 1971). The younger the child the lower the RBP. In Indian children (2-10 years old), the normal level is 30-40µg/ml and prealbumin is 160µg/ml (Pirie, 1979).

1.3.1.5 Functions

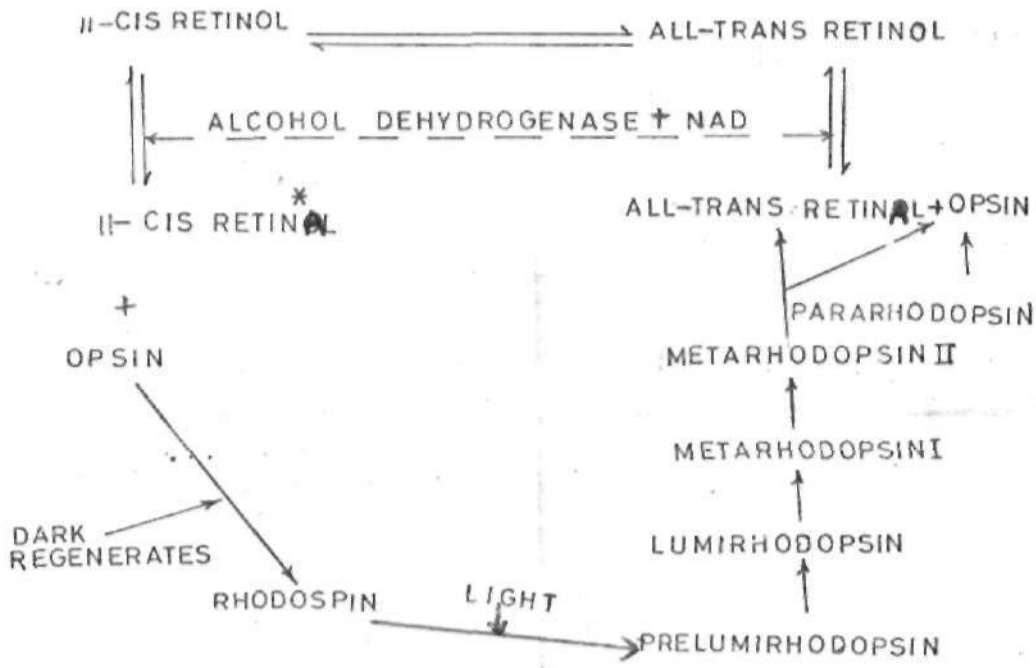
Retinol analogues have a series of functions in the body. They have two roles in the maintenance of vision. The first is the visual process and the second is in the maintenance of the structure of the eye epithelia (Wald, 1968).

In the visual process, all-trans retinol in the retina of the eye isomerizes to 11-cis retinol and then oxidized to 11-cis-retinal. This combines with the protein of the rod cells, opsin. Rhodopsin is formed and it undergoes a series of chromophoric changes which affect ion transport and membrane potentials resulting in a signal to the brain registered as vision. This is followed by the dissociation of rhodopsin releasing opsin and all-trans retinal (Fig. 1.5).

Vitamin A is important in the synthesis of mucopoly-saccharide e.g. chondriotin 4-and-6-sulphate; it is therefore necessary in the maintenance of differentiated epithelia cells and mucus secretion (De Luca and Wolf, 1968; Wong and Buck, 1971). It is also essential in the formation and development of bones and cartilage (Sebrell and Harris, 1967).

Vitamin A is also necessary for reproduction, and deficiency may lead to infertility, abnormal semen production, still births and neonatal deaths (Thompson et al, 1964).

FIG. 1·5 : ROLE OF VITAMIN A IN THE VISUAL CYCLE



* INDICATES THE ACTIVE FORM
(RUTH AND MYRTLE, 1975)

The synthesis and function of some hormones is affected by vitamin A deficiency. In deficiency state the adrenal cortex tissue is degraded, thus reducing the secretion of sex hormones (Harris et al, 1974). Vitamin A is also involved in the biosynthesis of glucocorticoid hormones (Wolf, 1962).

Retinoids and synthetic analogues - retinyl acetate, retinylidone dimedone and retinyl methyl ether are known to block the action of exogenous and endogenous oncogenic promoters in vivo, preventing carcinogens from producing new tumours but do not reverse the growth of established tumours (Harris et al., 1974).

Vitamin A also stimulates the synthesis of RNA and protein (IVACG, 1976b).

1.3.1.6 Effect of Vitamin A deficiency on the Cornea

Vitamin A deficiency which often accompanies protein calorie malnutrition, gives rise to severe changes in the cornea.

The first ocular sign of vitamin A deficiency is night blindness due to lack of rhodopsin in the retina rod cells. Changes in the conjuctiva follows, it becomes dry and wrinkled, the surface cells become keratinized, and goblet cells disappear. A Bitot spot, which is a white foamy plague of cells appear on the temporal conjuctiva.

Corneal xerosis follows the conjuctiva changes. Keratinization of the superficial layers of epithelium occurs and spreads over the whole cornea. The changes are reversible at this stage with vitamin A therapy. Without treatment cornea xerosis worsens and ulcer may develop on any part of the cornea. This leads to softening and dissolution of the cornea which is called keratomalacia (Pirie, 1978; Fig 1.6.).

1.3.1.7 Nutritional relationships of vitamin A

1.3.1.7.1 Effect of protein

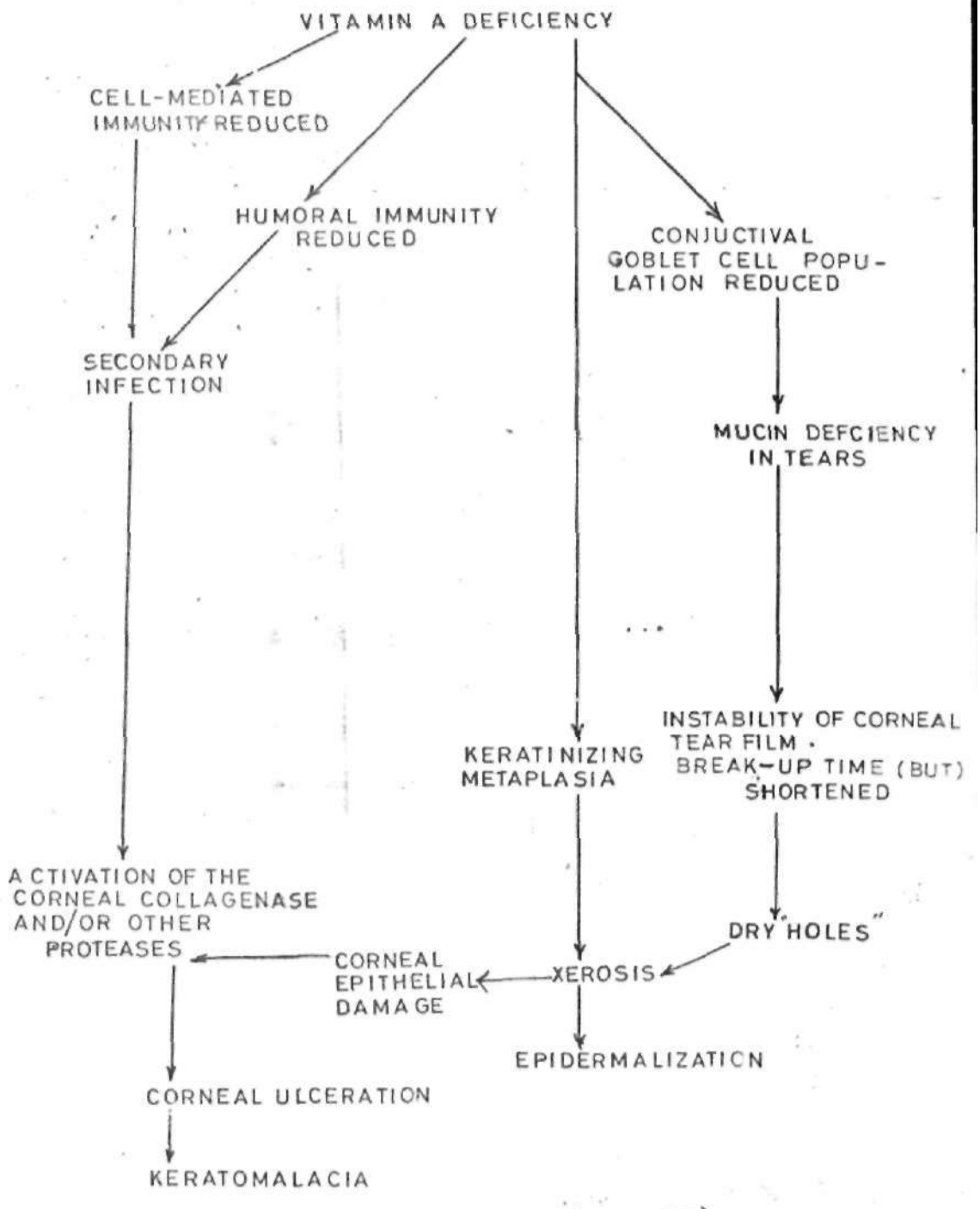
Severe protein deficiency interferes with vitamin A metabolism both indirectly by generally depressing the syntheis and release of proteolytic and lipolytic enzymes of the gut and pancreas and directly by limiting the synthesis of plasma transport proteins by the liver (IVACG, 1976a).

Retinol binding protein and prealbumin contain a high proportion of aromatic and other essential amino acids and are therefore more severely depressed than albumin in protein energy malnutrition (Smith et al, 1975; Ingenbleek et al, 1975). The depression of retinol binding protein in protein energy malnutrition also depresses the transport of retinol to the tissues.

1.3.1.7.2 Effect of Zinc

A linkage between zinc and vitamin A metabolism has also been suggested. By using animals deficient in both zinc and vitamin A, it was demonstrated that zinc is necessary for normal hepatic mobilization of vitamin A (Smith et. al., 1973).

FIG.1·6: A POSTULATED MECHANISM FOR THE PATHOGENESIS OF KERATOMALACIA IN VITAMIN A DEFICIENCY



(BASED ON BROWN et al 1970 :(W.H.O., 1976)

1.3.2 MEASLES

Measles is an acute communicable disease characterized by three stages (1) an incubation stage of approximately 10-12 days; (2) a prodomal stage with koplik's spots on the buccal and pharyngeal mucosa, mild to moderate fever, slight conjuctivitis and an increasingly severe cough; and (3) a final stage with a maculo-papular rash erupting successively over the neck and face, body, arms and legs, and accompanied by high fever. Conjuctival redness, photophobia and increased lackrymation sometimes accompanied by punctuate keratitis are part of the symptoms of measles infection (Dossetor, et. al., 1977; Ngwa, 1980; Plates 1.1 and 1.2).



Plate 1.1 - Nigerian child with measles rash



Plate 1.2- Post measles cornea ulceration in a Nigerian child

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Blood Specimens

Blood samples of about 3.0ml each were collected by venous puncture from children admitted at the Paediatric ward, Wusasa Hospital, near Zaria, or at the Ahmadu Bello University Teaching Hospital, Zaria, both in Kaduna State, Northern Nigeria.

2.1.2 Laboratory Chemicals and Reagents

All chemicals were of analytical grade, except that the chloroform, trifluoroacetic acid and hexane used were of spectroscopic grade. All chemicals were obtained from BDH, Poole, England and Hopkins and Williams, England.

2.1.2.1 Vitamin A Reference Standard

This was obtained from USPC, Rockvile, MD. U.S.A. Each capsule contains 8.475mg of retinyl acetate in cotton seed oil solution which is equivalent to 7.27mg of retinol.

2.1.2.2 Vitamin A (Injection)

This was obtained from Heyl & Co. Chem. Pharm. Fabrik, Berlin 37. Each ampoule contains 60µg retinyl palmitate in 2mls of a water miscible solvent.

2.1.2.3 Protein Reference Standard

Bovine serum Albumin was purchased from Biotest - serum Institute GMbH. Frankfurt/W. Germany.

2.1.2.4 Reference Standard for Plasma Proteins

Control Human Plasma for "Partigen" for immunological determinations was obtained from Behringwerke, AG, Marburg, W. Germany.

2.2 METHODS

2.2.1 Classification of subjects

Forty four children aged between 6 weeks and 36 months were medically examined by a consulting paediatrician and grouped into the following five groups -

1 This group consisted of children who were apparently healthy i.e. control group (A).

2 This group consisted of children with other pathological conditions other than measles and malnutrition but requiring hospitalisation (B).

3 This group was composed of children with malnutrition without signs of measles (C) (Plates 2.1 and 2.2).

4 This consisted of children with proven cases of measles without malnutrition (D) (Plate 1.1).

5 This was composed of children with measles and malnutrition (E).

2.2.2 Nutritional Assessment

Nutritional status was assessed using the Havard Standard curve (Fig 2.1). On arrival at Paediatric wards, weights of children were measured by means of a weighing pan scale. Age assessment was based on information supplied by the child's parent.



Plate 2.1 - A two year old Nigerian male child with kwashiokor



Plate 2.2 - A two year old Nigerian female child with marasmus

On the basis of these results the children were then classified as well-nourished if their weights were about 80% that of the Havard standard or mal-nourished if their weights were below 60% that of the Havard standard.

The biochemical assessments were based on the levels of total protein and prealbumin.

2.2.3 Diagnosis

Diagnosis was done by a qualified Paediatrician. Patients included children with many measles infection in which the rash had appeared. Other diseases included pneumonia, gastroentritis, kwashiokor, marasmus and minor illnesses such as fever. The controls were patients with minor illnesses but who showed no apparent signs of measles infection. or malnutrition.

2.2.4. Vitamin A dosage

Vitamin A palmitate dissolved in oil was used. Each ampoule consisted of 2mls of oil which contained the equivalent of 100,000 I.U of vitamin A. The administration of vitamin A was done by intramuscular injection by a Paediatrician at the following dosage rates. These values were subject to modification by the Paediatrician when he considered it appropriate

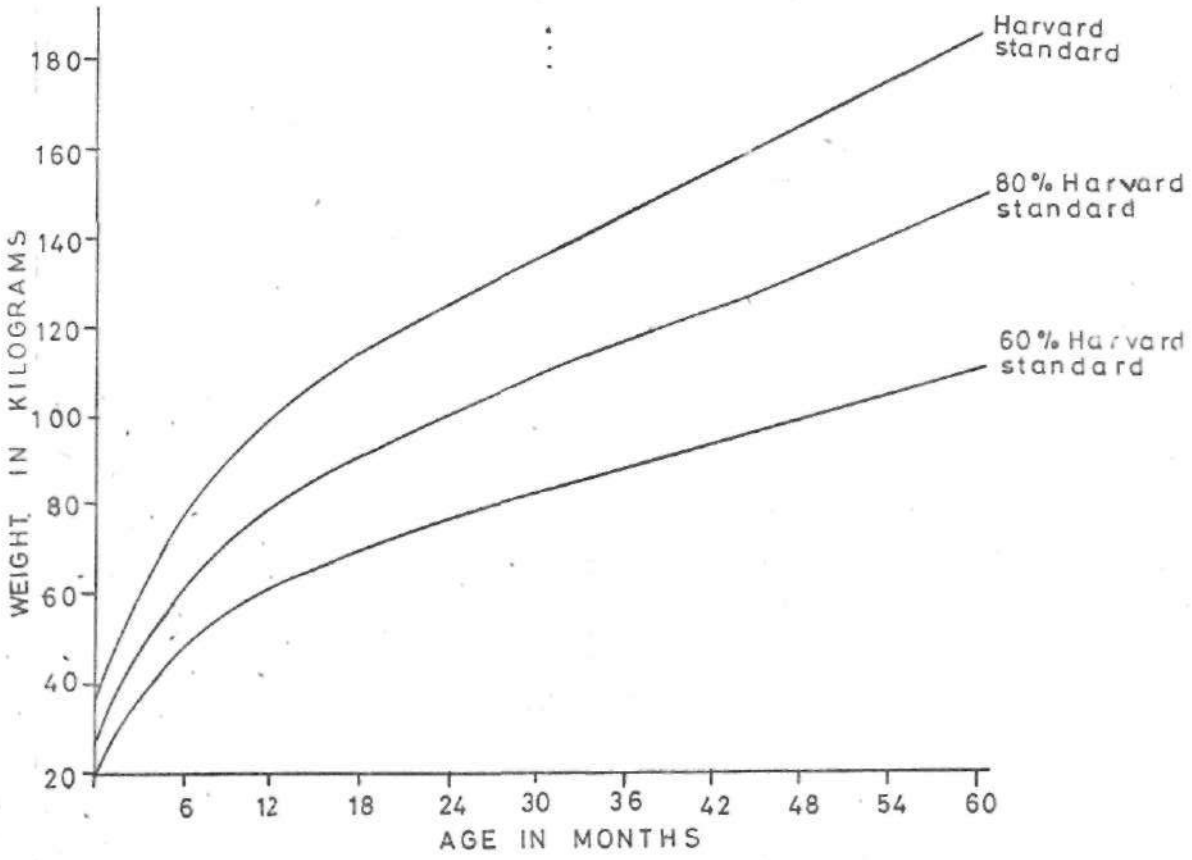
Children 1 year = 50,000 I.U

2 years = 100,000 I.U

3 years = 100,000 I.U

The dosage followed this pattern in all the groups.

FIG. 2·1 : WEIGHT CHART FOR CHILDREN (0-60 MONTHS) TO SHOW HARVARD STANDARD AND 80 % OF THIS (BELOW 60 % CLASSIFIED AS MARASMUS)



2.2.5 Collection and Processing of samples

Blood samples were collected before the administration of vitamin A (intramuscular injection) and thereafter, 24 hours or 4-7 days. Blood samples were collected using disposable syringes with stainless steel needles into heparinized glass tubes. They were centrifuged using an MSE bench centrifuge at a speed of 3000 r.p.m. for 10 minutes. The plasma was then frozen and stored when analysis could not be done on the same day.

2.2.6 Estimation of retinyl ester and retinol

The method adopted was a modification of that described by Pirie (Personal Communication, 1979). It was necessary to modify the method further so as to achieve complete separation of these components.

The whole procedure was carried out in dim light, as light can cause oxidation of vitamin A. An improvised 'shade' was therefore made by putting two drawers together such that their cavities are facing each other. All tubes at all times were wrapped with aluminium foil, up to the neck and securedly stoppered.

All glassware were washed thoroughly with detergent, rinsed with distilled water and dried at 120^{o}C in a conventional oven.

2.2.6 Extraction

0.5ml of plasma was pipetted into 10ml stoppered tube. 0.5ml of ethanol was added dropwise with shaking to precipitate protein. After adding 2.0ml of hexane (containing 100ug butylated hydroxyltoluene/ml as antioxidant) the mixture was agitated on a whirl mixer for 2 minutes.

The suspension was centrifuged for 10 minutes at medium speed using an MSE bench centrifuge. The hexane supernatant was then transferred with a pasteur pipette into a 10ml stoppered tube. An additional 2ml of hexane was added to the residue and the suspensic was whirlmixed for 1 minute. The mixture was centrifuged as above and the hexane supernatant removed and added to the first one. The supernatants were then evaporated under nitrogen at 40°C in a water bath. The residue was then taken up in 0.5ml of hexane and chromatographed on alumina.

2.2.6.2 Column Chromatography

10g Woelm Alumina (activity IV) was prepared each time by adding 10% water to neutral alumina of activity super 1. The globular powder was shaken by hand in an airtight container, until it became smooth running again (about 10 minutes) and then allowed to stand for 2 hours to attain equilibrium. A column consisting of funnel 0.5mm (diameter of stem) was plugged with glasswool and fitted with a 3cm length of polyethylene tubing, which was clamped with a screw clip to regulate the flow of eluent.

The column was filled with 100% hexane and allowed to run through three times to remove air bubbles. It was then left about three quarters full, and with the aid of spatula 1.0g of alumina activity grade IV was added. When alumina had settled, hexane was allowed to drain through the column to about 1cm above the level of alumina.

This was repeated 2 times. Columns prepared in this way were free of air bubbles.

The solution of extract in hexane (0.5ml) was applied to the column after the hexane had been allowed to drain to just above the surface of the column. Fractions were collected. The column was then eluted as follows:

1 3mls of hexane which eluted B-carotene. This fraction was not quantitated.

2 10ml 1% diethyl ether in hexane eluted retinyl ester.

3 10ml 20% diethyl ether in hexane eluted retinol.

The efluents were evaporated under nitrogen at 40°C in a water bath. The fractions collected were then each redissolved in 1.2ml of chloroform for colorimetric estimations.

2.2.6.3 Colorimetric estimation of retinyl ester and retinol

Fractions 2 and 3 were separately taken up in 1.2mls of chloroform and 0.8mls of TFA (trifluoroacetic acid) to make 2mls (Neeld and Pearson, 1963). The absorbance of the resulting transient blue colour was read in a Corning colorimeter Model 253 at 620nm after zeroing of the colorimeter with the blank consisting of chloroform and TFA mixed in the same proportion as in test sample. The absorbance given by fraction 2 represents that of retinyl ester in 'retinol equivalent'.

The absorbance given by fraction 3 represents that of retinol. The concentrations in ug retinol/2ml of reaction mixture was obtained from the standard calibration curves (Appendix, Figure 5.1) and converted to concentration in µg/100ml of plasma.

Absorbance measurements were taken within 30 seconds of adding the TFA reagent. Maximum colouration is obtainable if the proportion of TFA in the total reaction mixture is 40% (Pirie 1979, personal communication)

2.2.7 Estimation of total protein

Total protein in the specimens were estimated accoring to the biuret technique.

5.9ml of biuret solution was added to 0.1ml of plasma. The mixture was incubated at 37°C for 10 minutes. The absorbance at 540nm were measured against a blank prepared by adding 5.9 of biuret solution to 0.1ml of distilled water.

Bovine serum Albumin was used to prepare standards for calibration curve from which the concentration of total protein as albumin in the plasma samples were estimated.

2.2.8 Estimation of retinol-binding protein and prealbumin

These two plasma proteins were determined using the single radial immunodiffusion technique (Hoechst Pharmaceuticals (1975). In this technique a plasma protein antigen is applied to a cylindrical well of an agar gel plate into which a specific precipitating antiserum was present in uniform concentration.

The concentration of antigen in the plasma applied is determined by the amount of specific antibody with which it combined at equivalent proportions. This is proportional to the volume of the precipitin cylinder formed when diffusion ceased, i.e. when all the applied antigen combined with antibody at equivalence. As the thickness of the gel was constant, this corresponded to the area of the precipitin ring and this area varies directly with the amount of antigen applied, and inversely with the concentration of antibody in the gel (plate 2.3).

M-Partigen immunodiffusion plates were used and each plasma protein has its own specific plate. 5ul plasma (protein antigen) was pipetted into the cylindrical application well, and then allowed to diffuse into a thin film of agar gel in which a specific precipitating antiserum was already present.

Various dilutions of the Standard Human plasma protein were made and equal volume added to the wells as in the test samples. Each plate was allowed to develop for 48 hours. The diameters of the precipitin rings were measured using a measuring manifier with graticle (Polaron 10x).



Plate 2.3 - Radial Immunodiffusion plate for retinol-binding protein determination

2.2.9 EVALUATION OF METHODS

2.2.9.1 Separation on column

Separation of retinol from retinyl ester and B-carotene can be achieved by using a column of neutral alumina (activity IV) eluting with hexane containing increasing amount of diethyl ether. It has proved too difficult to estimate B-carotene in the samples collected since it is present in negligible amounts in plasma and to quantitate this, a large amount of plasma must be used. The specimens were collected from children 3 years of age who still depend more on preformed vitamin A than the carotenoids for their vitamin A requirements. The B-carotene washed off the column with 3mls of 100% hexane was therefore not estimated.

Retinyl ester is completely eluted with 10mls of 1% diethyl ether in hexane. Retinol is absent from this fraction (Appendix, Table 5.1).

Retinol is eluted with 10mls of 20% diethyl ether in hexane.

2.2.9.2 Evidence of separation on column

0.5µg of retinol was applied to column and eluted with hexane containing increasing amount of diethyl ether. Ninety percent of the retinol was found in the fraction eluted with 10mls 20% diethyl ether in hexane. There was no retinol detected in the fraction eluted with 100% hexane nor in that eluted with 1% diethyl ether in hexane.

With retinyl ester, there was 100% recovery in the fraction eluted with 10ml 1% diethyl ether in hexane.

β-carotene is washed off the column completely with 3mls of 100% hexane (Appendix, Table 5.1).

2.2.9.3 Recovery of retinoids from alumina column

The recovery of materials from a column of neutral alumina was investigated by applying known amounts of retinyl ester and retinol separately to the standard column and eluting according to the procedure described. Appendix, Table 5.2 shows results obtained. Results from subsequent analysis were corrected using the % recovery to offset the loss incurred.

2.2.9.4 Reproducibility of retinol and retinyl ester determination

As the reaction of TFA with retinoids produces transient blue colour, the reproducibility of estimation was determined. 1ug of retinyl palmitate was taken each time and reacted with the same amount of TFA. The optical density readings were obtained within 30 seconds after the addition of TFA. The results obtained were consistent and therefore reproducible (Appendix, Table 5.3).

2.2.10 STANDARD CALIBRATION CURVES

2.2.10.1 Retinol and Retinyl Ester

For retinol and retinyl esters, standards were prepared from pure vitamin A reference standard in the form of all-trans retinyl acetate in oil. Each capsule contained 29.1mg of retinol in 1gm of oil.

A sample of oil was weighed and dissolved in chloroform to give a concentration of 0.5mg of retinol/ml of chloroform. This was diluted 1/100 in ethanol to give 5µg of retinol/ml of chloroform. and the absorbance at 325nm was determined using CE 505 Double beam ultraviolet spectrophotometer. This is to test the purity of the retinyl acetate. The $E^{1\%}_{1cm}$ was then calculated to be 1850. This value is comparable to the literature value of 1835 (Herbert, 1970). Different amounts of the stock standard in chloroform were taken and made up to 2.0ml with chloroform and TFA. Absorbance readings were measured at 620nm using Corning colorimeter model 253. The standard curve was then plotted as shown (Appendix, Fig 5.1).

2.2.10.2 Total Protein

Appropriate dilutions of the stock albumin standard was made to obtain working standard of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0g/dl. The standards were reacted with biuret working solution as described for the test samples and calibration curve plotted as shown (Appendix, Fig 5.2).

2.2.10.3 Retinol Binding protein and prealbumin

Control plasma for partigen was used as standard for both plasma proteins. Dilutions of this control were made using phosphate saline buffer (PSB) to obtain dilutions of 1:1, 1:2, 1:4, 1:8 and 1:10. These were treated as the test samples and calibration curves were plotted as shown in Figures 5.3 and 5.4 for both retinol-binding protein and prealbumin, respectively.

3 RESULTS

The results of individual analysis are shown in the Appendix (Tables 5.4 - 5.7). The mean values of retinol and retinol-binding protein for each category of subjects studied before and after treatment are shown in Table 3.1.

3.1 Pre-Therapy analysis

3.1.1 Total Protein

The mean plasma total protein values in children with manifest malnutrition without measles infection (C) and those children with measles infection coupled with malnutrition (E) were significantly lower ($p \leq 0.005$, $p < 0.01$, respectively) than in the control group (A) (Appendix, Table 5.4). Other inter-group comparisons showed no significant differences.

3.1.2 Retinol, Retinol-binding protein and Prealbumin

From Table 3.2, the control population (A) had higher retinol and retinol-binding protein than all other groups of subjects studied before vitamin A therapy. These differences were significant ($p < 0.001$) when group A was compared with children with manifest malnutrition without measles infection, children with proven cases of measles infection without malnutrition or children with measles infection coupled with malnutrition. The mean plasma retinol and retinol-binding protein differed significantly between some of the categories of patients studied (Table 3.3.).

The mean prealbumin levels in all categories of various pathological conditions studied were significantly lower (B: $p < 0.05$, C: $p < 0.01$, D and E: $p < 0.001$) than in the control group (A) (Appendix, Table 5.1).

3.2 Vitamin A Therapy

Vitamin A therapy significantly raised the mean plasma retinol and retinol-binding protein within 24 hours after therapy in all categories of patients studied (Table 3.4). While the mean plasma retinol levels in patients with proven cases of measles infection without malnutrition was still significantly raised 4-7 days after vitamin A therapy, the mean plasma retinol-binding protein showed no significant change (Table 3.4).

Total vitamin A values were computed by adding the values of retinyl ester and retinol (Appendix, Tables 5.4-5.8). Retinyl ester levels showed considerable increases in all individuals irrespective of disease category (Appendix, Tables 5.5-5.8). However, 4-7 days after vitamin A therapy, retinyl ester values tended to return to pretherapy values.

The correlation coefficients for retinol-binding protein, prealbumin and retinol were determined in each category of children studied, and are shown in Figures 3.1-3.9.

Table 3.1 – PLASMA RETINOL AND RETINOL-BINDING PROTEIN IN NIGERIAN *CHILDREN WITH OR WITHOUT MEASLES AND MALNUTRION* (MEAN+S.E).

Groups of Children		Retinol n	Retinol (µg/100ml)	Retinol-binding Protein n	Retinol-binding Protein (µg/ml)
Apparently healthy children who did not require admission (A) (Receiving no vitamin A therapy)		5	21.40±0.84	5	24.94±1.33
Children with other pathological conditions without measles or malnutrition but requiring admission (B)	a	5	17.22±2.48	5	21.00±2.33
	b	5	24.20±1.65	5	28.70±2.63
Children suffering from malnutrition without measles infection (C)	a	8	8.49±0.90	6	13.67±1.92
	b	6	16.35±1.83	6	19.17±1.13
Children with proven cases of measles infection without malnutrition (D)	a	11	14.13±1.10	9	19.26±1.16
	b	9	23.60±1.90	9	28.42±1.63
	c	8	24.46±4.99	8	27.38±5.42
Children with measles infection and with malnutrition (E)	a	15	9.27±0.79	12	13.46±0.84
	b	11	18.22±1.06	8	19.5±0.97

S.E = standard error

n = number of children studied

a = before vitamin A therapy

b = twenty four hours after therapy

c = Four to seven days after therapy.

Table 3.2 - STATISTICS OF THE COMPARISON OF MEAN PLASMA RETINOL AND RETINOL-BINDING PROTEIN LEVELS BETWEEN CONTROL GROUP AND OTHER STUDIED GROUPS

Two population groups compared [a]		df	t statistics	Results [b]	Probability
			Retinol		
A and B	Before vitamin A therapy	8	1.60	NS	$p < 0.1$
	Twenty four hours after therapy	8	-1.55	NS	$p < 0.1$
A and C	Before vitamin A therapy	11	9.72	S	$p < 0.001$
	Twentyfour hours after therapy	9	2.33	S	$p < 0.05$
A and D	Before vitamin A therapy	14	4.14	S	$p < 0.001$
	Twentyfour hours after therapy	12	-0.85	NS	-
	Four to seven days after therapy	11	-0.50	NS	-
A and E	Before vitamin A therapy	18	8.51	S	$p < 0.001$
	Twentyfour hours after therapy	14	1.88	S	$p < 0.01$
			Retinol-binding protein		
A and B	Before vitamin A therapy	8	.47	NS	-
	Twentyfour hours after therapy	8	1.27	NS	-
A and C	Before vitamin A therapy	10	5.06	S	$p < 0.001$
	Twentyfour hours after therapy	9	3.32	S	$p < 0.01$
A and D	Before vitamin A therapy	12	3.070	S	$p < 0.01$
	Twentyfour hours after therapy	12	-1.43	NS	-
	Four to seven days after therapy	11	-0.38	NS	-
A and E	Before vitamin A therapy	15	8.89	S	$p < 0.001$
	Twentyfour hours after therapy	11	3.37	S	$p < 0.01$

[a] The population groups are as defined in Table 3.1

[b] Level of significance was set at $\alpha = 0.05$

df = degrees of freedom; S = Significant difference

NS = No significant difference.

Table 3.3 - STATISTICS OF COMPARISON OF MEAN PLASMA RETINOL AND RETINOL-BINDING PROTEIN LEVELS BETWEEN GROUPS OF CHILDREN WITH MEASLES, MALNUTRITION AND OTHER PATHOLOGICAL CONDITIONS

Two population groups compared		df	tstatisfics	Results	Probability
			Retinol		
B and C	Before vitamin A therapy	11	3.91	S	$p < 0.01$
	Twenty four hours after therapy	9	2.49	S	$p < 0.05$
B and D	Before vitamin A therapy	14	1.34	NS	-
	Twenty four hours after therapy	12	0.22	NS	-
C and E	Before vitamin A therapy	21	-0.63	NS	-
	Twenty four hours after therapy	15	-0.95	NS	-
D and E	Before vitamin A therapy	24	3.75	S	$p < 0.001$
	Twentyfour hours after therapy	18	2.64	S	$p < 0.02$
			Retinol-binding protein		
B and C	Before vitamin A therapy	10	2.80	S	$p < 0.02$
	Twenty four hours after therapy	9	2.64	S	$p < 0.05$
B and D	Before vitamin A therapy	12	0.75	NS	-
	Twenty four hours after therapy	12	0.09	NS	-
C and E	Before vitamin A therapy	17	-0.19	NS	-
	Twenty four hours after therapy	12	-0.22	NS	-
D and E	Before vitamin A therapy	19	4.12	S	$p < 0.001$
	Twenty four hours after therapy	15	4.54	S	$p < 0.001$

Table 3.4 - STATISTICS OF THE COMPARISON OF MEAN PLASMA RETINOL AND RETINOL-BINDING PROTEIN LEVELS IN CHILDREN WITH MEASLES, MALNUTRITION AND OTHER PATHOLOGICAL CONDITIONS BEFORE AND AFTER VITAMIN A THERAPY

Groups of children [a]	df	t statistics	Results [b]	Probability
		Retinol		
B	4	5.20	S	$p < 0.005$
C	5	3.50	S	$p < 0.01$
D	8	4.36	S	$p < 0.005$
	*7	2.46	S	$p < 0.05$
E	10	7.29	S	$p < 0.005$
		Retinol-binding protein		
B	4	3.34	S	$p < 0.05$
C	5	3.24	S	$p < 0.05$
D	7	4.73	S	$p < 0.005$
	*5	1.25	NS	-
E	7	5.27	S	$p < 0.005$

* Comparison 4-7 days after therapy. All other comparisons are based on 24 hours after therapy.

a The population groups of children are as defined in Table 3.1.

b Level of significance was set at $\alpha = 0.05$

df = degrees of freedom; S = Significant difference

NS = No significant difference.

FIGURE 3·1 : RELATION BETWEEN LEVELS OF RBP AND RETINOL IN APPARENTLY HEALTHY NIGERIAN CHILDREN.

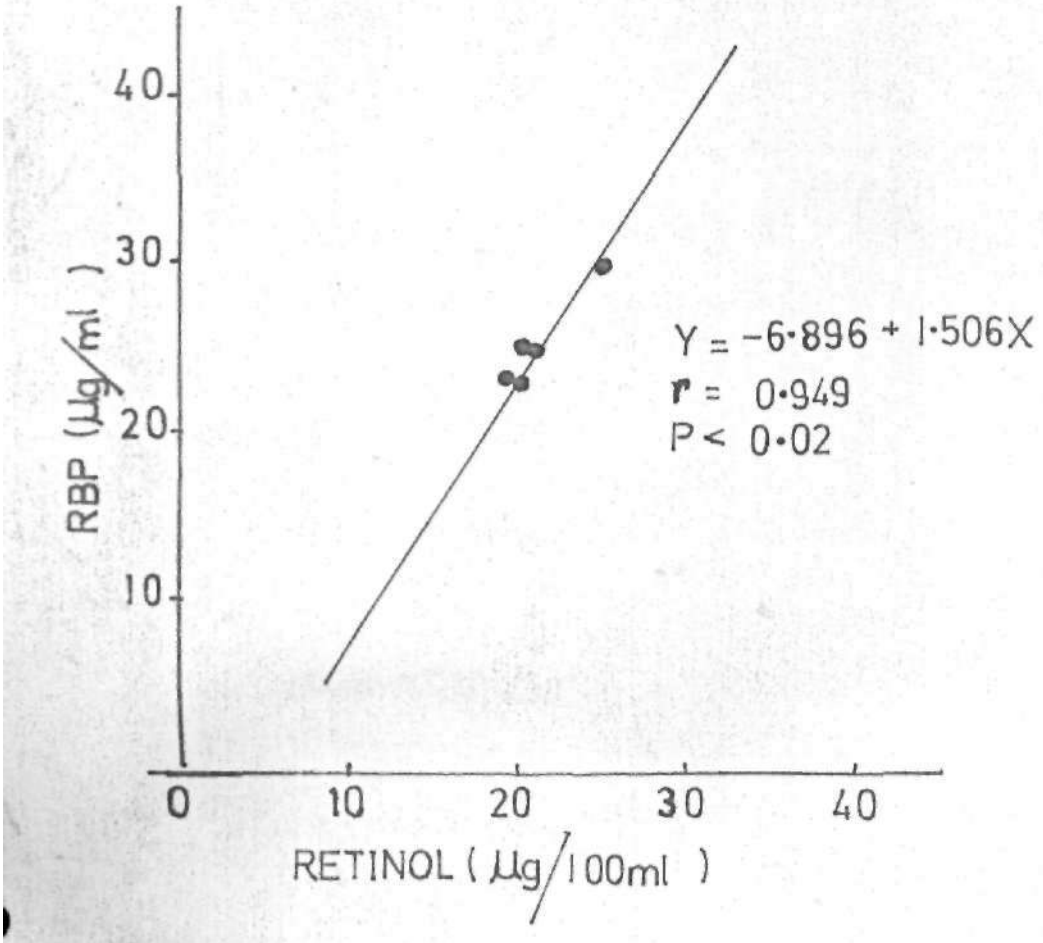


FIGURE 3.2: RELATION BETWEEN LEVELS OF RBP AND PREALBUMIN IN APPARENTLY HEALTHY NIGERIAN CHILDREN

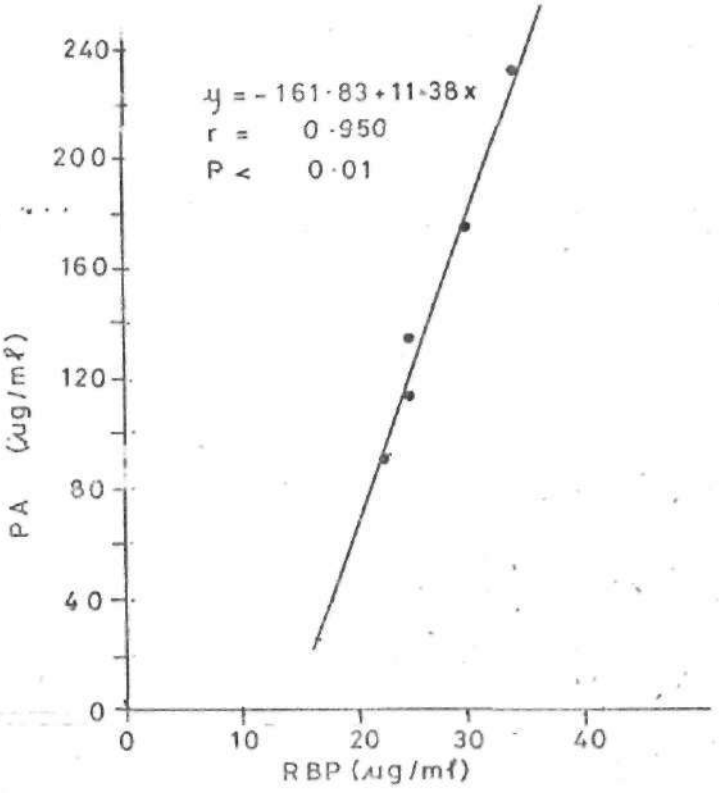


FIG.3·3: RELATION BETWEEN LEVELS OF RETINOL AND PREALBUMIN IN APPERENTLY HEALTHY NIGERIAN CHILDREN

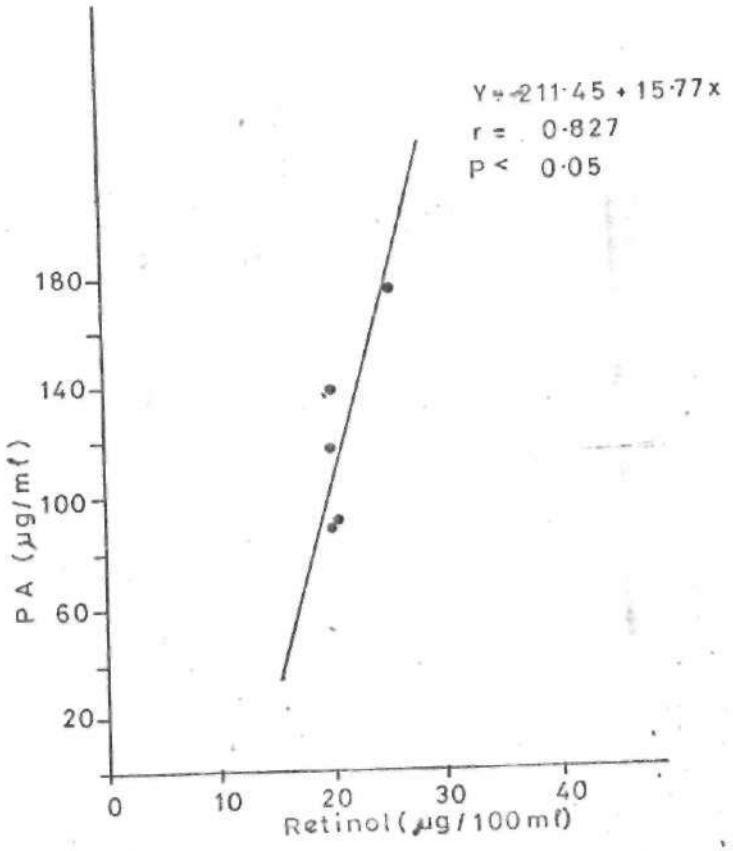


FIG. 3·4: RELATION BETWEEN LEVELS OF RBP AND RETINOL 24 HOURS AFTER VITAMIN A TREATMENT IN NIGERIAN CHILDREN WITH VARIOUS PATHOLOGICAL CONDITIONS OTHER THAN MEASLES AND MALNUTRITION

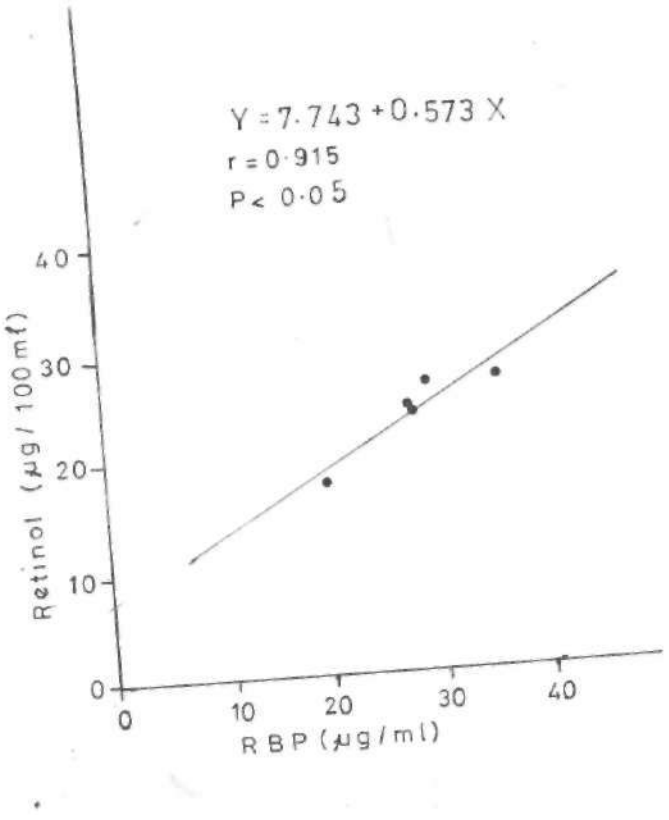


FIG.3-5 : RELATION BETWEEN LEVELS OF RBP AND RETINOL 24 HOURS AFTER VITAMIN A TREATMENT IN NIGERIAN CHILDREN SUFFERING FROM MALNUTRITION WITHOUT MEASLES INFECTION

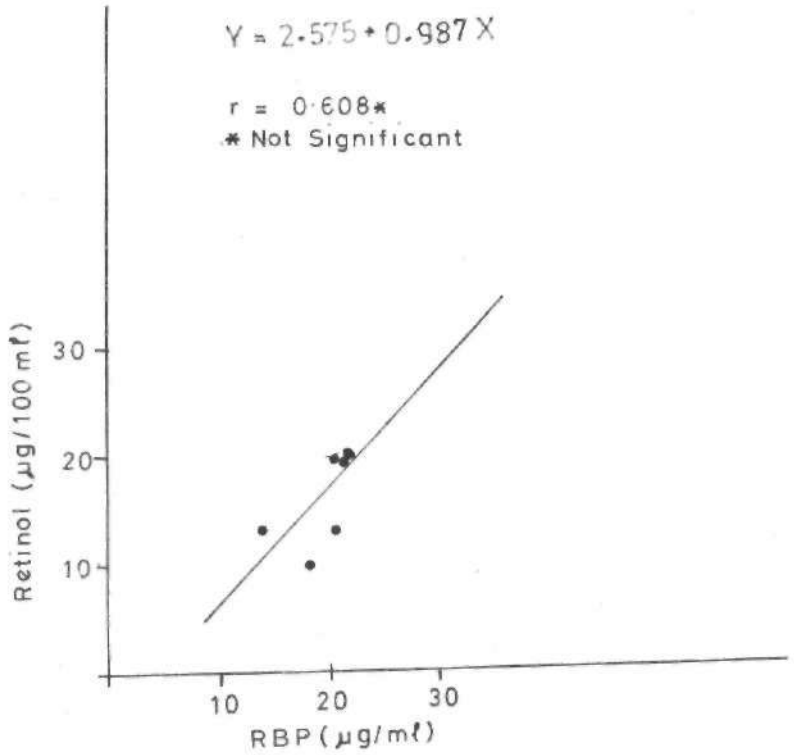


FIG.3·6 RELATION BETWEEN RBP AND RETINOL 24 HOURS AFTER VITAMIN A TREATMENT IN NIGERIAN CHILDREN WITH PROVEN CASES OF MEASLES INFECTION WITHOUT MALNUTRITION

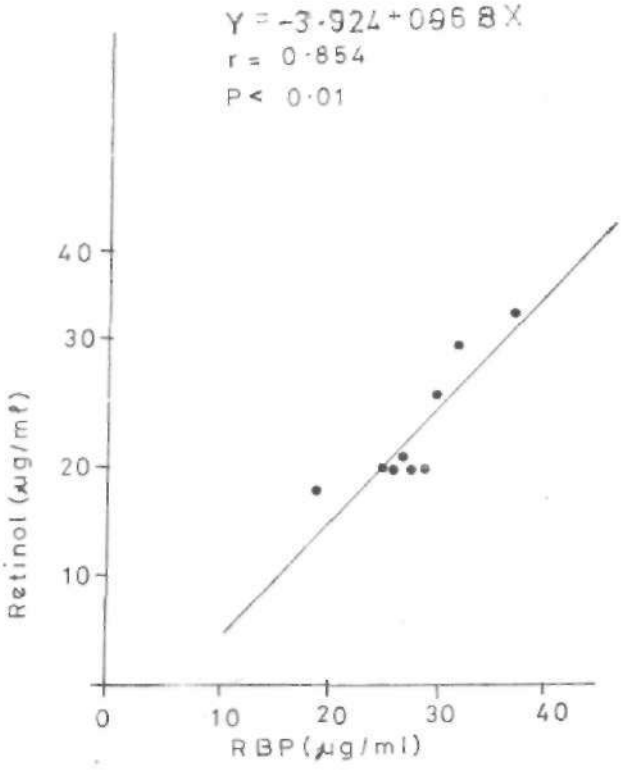


FIG. 3·7: RELATION BETWEEN RBP AND RETINOL 4 – 7 DAYS AFTER VITAMIN A TREATMENT IN NIGERIAN CHILDREN WITH PROVEN CASES OF MEASLES INFECTION WITHOUT MALNUTRITION

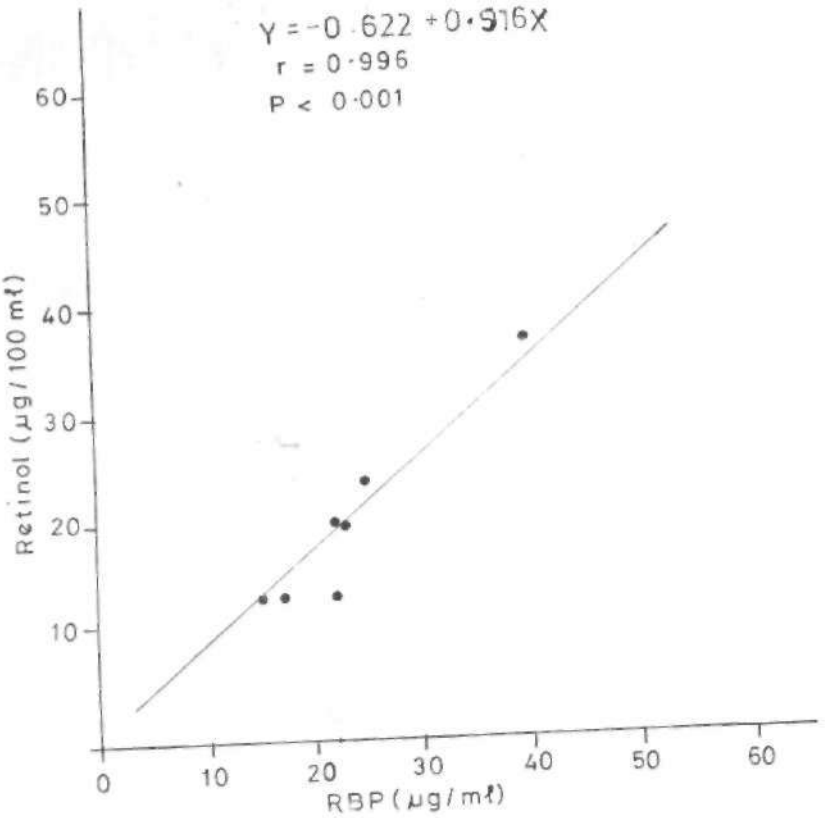


FIG.3.8: RELATION BETWEEN LEVELS OF RBP AND RETINOL 24 HOURS AFTER VITAMIN A TREATMENT IN NIGERIAN CHILDREN WITH MEASLES INFECTION AND MALNUTRITION

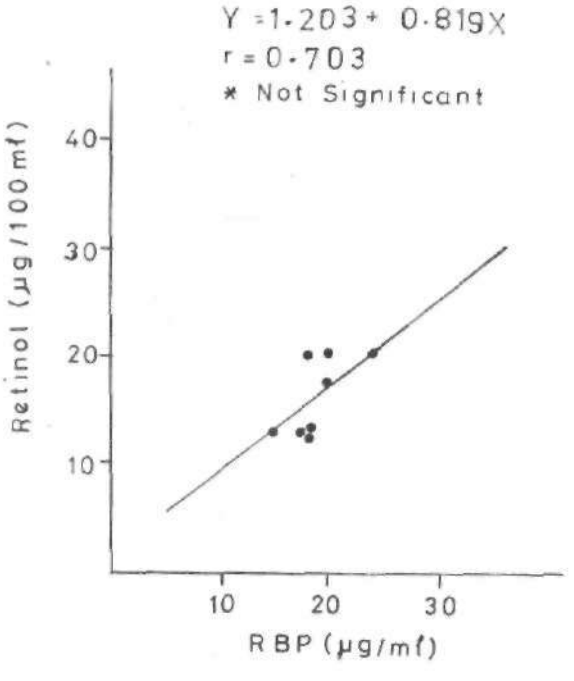
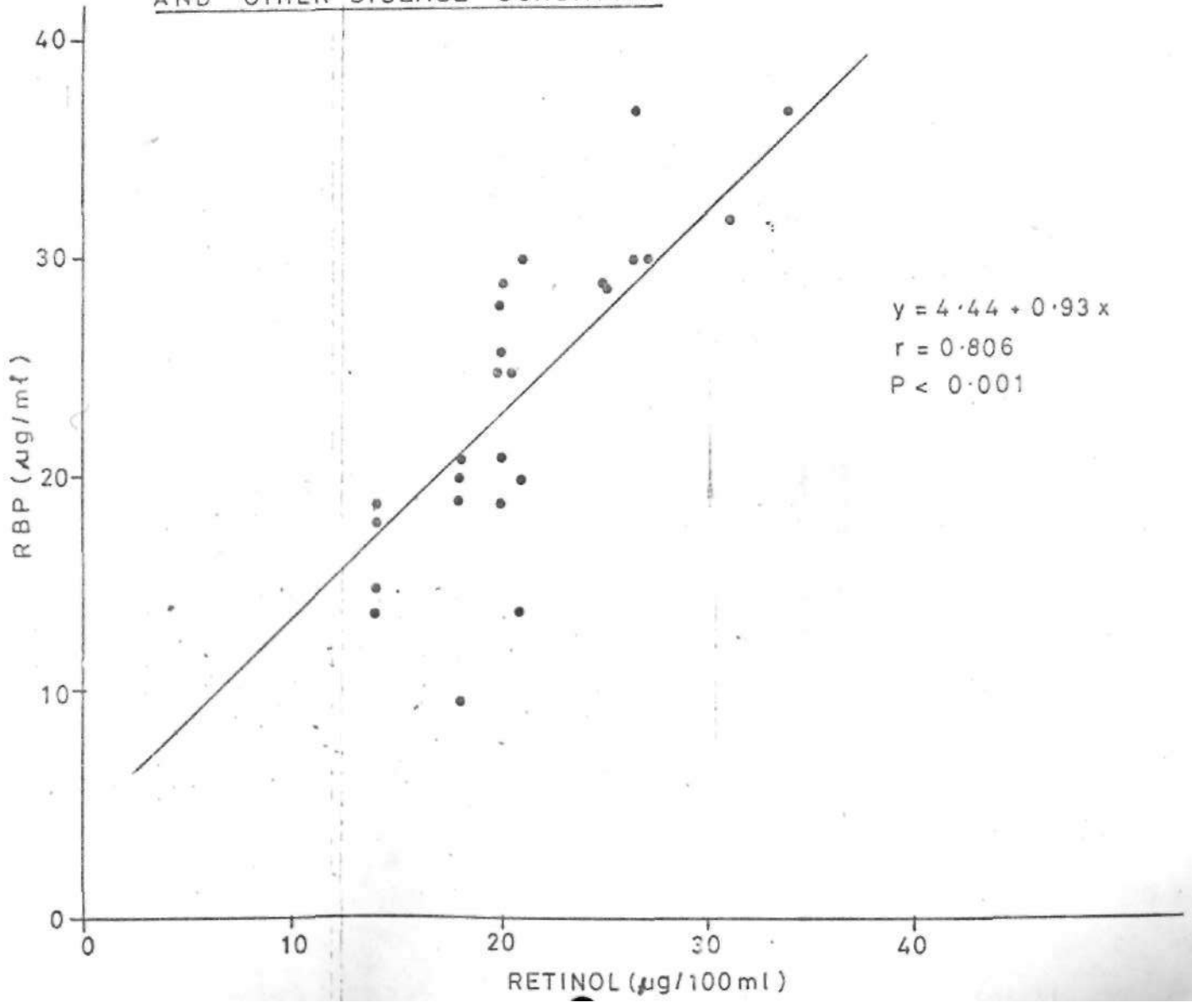


FIG. 3·9: RELATION BETWEEN LEVELS OF RBP AND RETINOL 24 HOURS AFTER VITAMIN A TREATMENT IN NIGERIAN CHILDREN WITH MEASLES, MALNUTRITION AND OTHER DISEASE CONDITIONS



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4. DISCUSSION

4.1 Control Group

The mean retinol, RBP, prealbumin and total protein values obtained in the control group were within the range of values reported for normal control groups in similar studies done elsewhere (Smith et al, 1975; WHO, 1976; Harold, 1976; IVACG, 1976). These workers suggested that adequate protein intake as well as good nutrition practice were factors maintaining the physiological levels of these parameters in their subjects.

The significantly positive correlations that exists between RBP and retinol (Figure 3.1), RBP and prealbumin (Figure 3.2), retinol and prealbumin (Figure 3.3) in the present study were expected, because the availability of retinol in plasma is dependent on the availability of RBP to form holo-RBP (Peterson et al, 1974). Also, under physiological conditions more than 90% of the plasma RBP is complexed to prealbumin in a 1:1 molar RBP-prealbumin complex (Kanai et al, 1968; Peterson, 1971; Raz et al, 1970).

4.2 Diseases other than measles or malnutrition

The lower but non-significant values of mean plasma retinol and retinol-binding protein (RBP) in those with diseases other than measles or malnutrition in comparison with the control group (Table 3.2) may have been due to intercurrent infections.

Generally, it has been shown that lower than normal levels of retinol are found in a wide variety of infections in experimental animals which might also adversely affect the transport and utilization of vitamin A in the body (IVACG, 1976b). The significantly reduced prealbumin levels were probably due to the same reason.

The significant increases in the levels of both RBP and retinol 24 hours after vitamin A treatment suggest that the release of holo-RBP (retinol-RBP complex) from the liver depends on the availability of vitamin A.

The significantly positive correlation existing between retinol and RBP 24 hours after therapy (Figure 3.4) suggests that the 1:1 relationship between RBP and retinol is not affected by these diseases.

4.3 Measles and Malnutrition

The reduced plasma retinol values in the present study confirms ealier studies in Zaria (Inua, 1979). In the present study, the mean plasma RBP and prealbumin levels were also lower than normal (Table 3.1; Appendix, Table 5.5). The reason for these differences may be due to inadequate protein intake and/or utilization. However, the reduced plasma retinol, RBP and prealbumin in children with measles and malnutrtion did not differ significantly from those in children with malnutrition, indicating that malnutrition has a more marked effect in reducing the plasma retinol, RBP and prealbumin values than measles.

The response to therapy after 24 hours in these patients agrees with the studies of other workers in malnourished children from other parts of the world (Reddy et al, 1978). The results of the combined effect of measles and malnutrition, and the subsequent response to therapy were similar in malnourished children. However, it will require further study to demonstrate the beneficial role of vitamin A therapy in the prevention or cure of hypovitaminosis A in children with measles and malnutrition.

4.4 Malnutrition

The reduced values of retinol, RBP and prealbumin in malnoursihed children in this study are in agreement with values reported by Smith et al (1975) for patients with protein calorie malnutrition (PCM). In Kwashiokor (Smith et al, 1973), reduced vitamin A levels have been found to be associated with decreased concentrations of RBP and prealbumin. Clinical signs of vitamin A deficiency may or may not be present (IVACG, 1976b).

Although Peterson et al (1974) reported a 1:1 RBP: retinol ratio under normal physiological conditions, the RBP: retinol ratio in children with malnutrition in the present study was higher than normal, indicating that the reduction in vitamin A levels was probably due to factors other than as a consequence of reduced plasma retinol carrier proteins.

The significantly raised RBP and retinol levels in malnourished children 24 hours after therapy may have been due to increased metabolism of injected vitamin A. This agrees with the findings of Venkataswamy, et al (1977). The significantly lower retinol and RBP values in malnourished children even 24 hours after therapy in comparison with the control group indicates that the malnourished children who are also clinically vitamin A deficient do not seem to have a sufficient store of RBP in their livers to bring the serum RBP up to normal within 24 hours after vitamin A injection (Ventakaswamy et al, 1977). However, the malnourished children in the present study did not have clinical signs of vitamin A deficiency, although the malnutrition may have been complicated by other diseases which have been shown to exacerbate the reduction in plasma RBP values in such children (IVACG, 1976b).

4.5 Measles Infection

Measles infection have been found to be associated with a fall in retinol concentration and this fall was seen to be greater than that associated with malnutrition (Inua, 1979). However, the results of the present study indicate that measles infection though associated with reduced levels of retinol, RBP and prealbumin was more marked in malnutrition than measles infection, when these groups were compared with the control groups.

In measles infection, it has been shown that the decrease in vitamin A concentration may be due to an increased vitamin A metabolism to repair damaged tissues, or due to a decrease in vitamin A absorption due to a damaged epithelium, or to an increased urinary vitamin A excretion, or to an inadequate dietary intake of vitamin A due to a loss of appetite or a sore mouth following an attack of measles (Inua, 1979). In malnutrition however, the reduced values of retinol may be a result of low levels of plasma RBP and prealbumin (Smith et al 1973).

The positive effect of vitamin A therapy in raising the plasma retinol and RBP values in measles infection might suggest that after vitamin A therapy, there seems to be a gradual but concomitant increase in the metabolism of the injected vitamin A and its carrier proteins. This could certainly have been the case since there was a significantly positive correlation between plasma retinol and RBP values 24 hours and 4-7 days after therapy (Figures 3.6 and 3.7).

The results of the present investigation have indicated that measles infection markedly reduce plasma retinol, RBP and prealbumin levels. It is apparent therefore that children with measles infection are likely to benefit from vitamin A therapy. Because of the need for protein in vitamin A metabolism in the body, it is suggested that future studies requiring vitamin A therapy should be done in association with varying regimens of protein.

5 - APPENDIX

Table 5.1 - THE SEPARATION OF B-CAROTENE, RETINYL ESTER AND RETINOL ON COLUMN OF ALUMINA (GRADE IV)

Fractions	Recovery of added material (%)		
	β-carotene	Retinyl Ester	Retinol
3mls 100% hexane	100	-	-
10mls 1% diethyl ether in hexane	-	99	-
10mls 20% diethyl ether in hexane	-	-	90

Table 5.2 - THE RECOVERY OF RETINYL PALMITATE AND RETINOL FROM A COLUMN OF NEUTRAL ALUMINA ACTIVITY GRADE IV

Number of elutions	Recovery (%)	
	Retinyl Palmitate	Retinol
1	100	90
2	100	92
3	100	90
4	98	90
5	100	90
Mean ± SE	99.6±0.39	90.4±0.39

Results from subsequent analysis corrected by 1.005 for retinyl ester and 1.11 for retinol offset the loss incurred.

SE = STANDARD ERROR

Table 5.3 - THE REPRODUCIBILITY OF THE ABSORBANCE OF RETINYL PALMITATE (1µg/ml) WITH TFA AT 620nm

No of reading	Absorbances
1	0.130
2	0.130
3	0.120
4	0.130
5	0.130
6	0.130
7	0.120
8	0.130
9	0.130
10	0.130

Mean ± SE = 0.128±0.002.

Table 5.4 PLASMA VITAMIN A, RBP, PREALBUMIN AND TOTAL PROTEIN CONCENTRATIONS IN APPARENTLY HEALTHY NIGERIAN CHILDREN WHO DID NOT REQUIRE ADMISSION

Specimen No.	Sex	Age (months)	Total Protein g/100ml	Prealbumin µg/ml	Total vitamin A µg/100ml	Retinyl ester µg/100ml	Retinol µg/100ml	RBP µg/100ml	Diagnosis
1	M	24	7.9	90	32.5	12.2	20.3	22.5	Anorexia
2	M	6	7.8	175	36.7	12.2	24.5	30.0	Fever
3	M	1½	-	138	26.4	6.1	20.3	24.6	Diarrhoea
4	M	6	6.6	117	26.4	6.1	20.3	24.6	Sore throat
5	F	6	6.2	90	32.5	12.2	20.3	23.0	Pneumonia
Mean ± SE		8.7± 3.92	7.13± 0.38	122.00± 16	30.9± 1.99	9.76± 1.49	21.14± 0.84	24.94± 1.33	

SE = STANDARD ERROR

Table 5.5 PLASMA VITAMIN A, RBP, PREALBUMIN BEFORE AND AFTER THERAPY AND TOTAL PROTEIN CONCENTRATIONS BEFORE AND AFTER THERAPY IN NIGERIAN CHILDREN WITH OTHER PATHOLOGICAL CONDITIONS WITHOUT MEASLES OR MALNUTRITION BUT REQUIRING ADMISSION

Specimen No.	Sex	Age (months)	Total Protein g/100ml	Prealbumin µg/ml	Total vitamin A ug/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml	24 hours after therapy				Diagnosis
									Total vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/100ml	
1	M	18	6.3	76	24.1	6.1	18.0	22.5	85.6	61.1	24.5	28.5	Diarrhoea and vomitting
2	M	24	6.0	69	32.5	12.2	20.0	20.5	88.1	61.1	27.0	36.5	Burns on lower extremities
3	M	12	6.9	67	19.5	6.1	13.5	20.5	128.5	104.0	24.5	28.5	Burns in the leg + discharging eyes
4	F	15	7.3	117	30.5	6.1	24.4	28.0	100.2	73.2	27.0	30.0	Pneumonia
[a]5	F	24	6.1	39	16.3	6.1	10.2	13.5	73.0	55.0	18.0	20.0	Postmeasles Pneumonia
Mean± SE		18.6± 2.39	6.52± 0.25	73.6± 12.53	24.58± 3.10	7.32± 1.21	17.22± 2.48	21.0± 2.32	95.08± 9.26	70.88± 8.78	24.2± 1.64	28.7± 2.63	

a Patient had a history of measle but not recent.

SE = STANDARD ERROR

Table 5.6 PLASMA VITAMIN A, RBP, PREALBUMIN AND TOTAL PROTEIN CONCENTRATIONS BEFORE AND AFTER THERAPY IN NIGERIAN CHILDREN WITH MALNUTRITION WITHOUT MEASLES INFECTION

Specimen No.	Sex	Age (months)	Total Protein g/100ml	Prealbumin µg/ml	Total Vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml	24 hours after therapy				Diagnosis
									Total Vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml	
1	F	30	3.8	53	10.2	NEG	10.2	18.5	81.4	61.1	20.3	21.0	Kwashiokor Pneumonia
2	M	24	3.6	29	12.9	6.1	6.8	10.0	105.3	91.8	13.5	14.0	Gastroentritis Malnutrition anorexia
3	M	15	5.1	59	12.9	6.1	6.8	10.0	129.2	122.4	10.2	16.0	Gastroentritis Marasmus
4	M	24	5.2	32	19.0	12.2	6.8	10.0	56.8	36.5	20.3	20.5	Marasmic Kwashiorkor
5	M	24	-	-	13.5	NEG	13.5	20.5	31.8	18.3	13.5	20.5	Marasmus
6	M	24	-	39	12.9	6.1	6.8	10.0	69.1	48.8	20.3	21.0	Malnutrition
7	M	24	-	-	19.0	12.2	6.8	-	-	-	-	-	Marasmus
8	F	18	5.2	-	10.2	NEG	10.2	13.0	-	-	-	-	Malnutrition discharging eyes
Mean ± SE			22.87± 1.60	4.58± 0.36	42.4± 5.85	13.83± 1.21	6.71± 1.42	8.49± 0.89	13.07± 1.67	78.93± 14.18	63.15± 15.56	16.35± 1.84	19.17± 1.13	

SE = STANDARD ERROR

Table 5.7 PLASMA VITAMIN A, RBP, PREALBUMIN AND TOTAL PROTEIN CONCENTRATIONS BEFORE AND AFTER THERAPY IN NIGERIAN CHILDREN WITH PROVEN CASES OF MEASLES INFECTION WITHOUT MALNUTRITION

Specimen No.	Sex	Age (months)	Total Protein g/100ml	Prealbumin µg/ml	Total vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml	24 hours after therapy				4-7 days after therapy				Duration of 3rd specimen collection (days)
									Total Vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml	Total Vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml	
1	M	12	6.4	53	19.6	6.1	13.5	17.0	61.1	42.8	18.0	19.9	-	-	-	-	-
2	M	24	6.1	36	19.6	6.1	13.5	15.0	91.8	61.2	30.6	32.0	44.1	30.6	13.5	15.0	7
3	M	36	6.9	67	26.4	6.1	20.3	26.0	118.2	97.9	20.3	28.0	-	-	-	-	-
4	M	24	6.9	42	19.6	6.1	13.5	17.0	112.1	91.8	20.3	26.4	13.5	NEG	13.5	17.0	7
5	M	24	6.9	62	22.7	9.2	13.5	21.8	131.7	97.9	33.8	37.0	65.8	12.2	53.6	60.0	7
6	F	6	6.9	72	19.6	6.1	13.5	18.5	64.2	42.9	21.4	30.0	26.4	6.1	20.3	22.5	7
7	F	24	7.9	60	26.4	6.1	20.3	22.5	76.3	48.9	27.4	30.0	-	-	-	-	-
8	M	24	-	-	19.6	6.1	13.5	-	-	-	-	-	42.7	18.3	24.4	25.0	4
9	F	12	6.2	53	19.6	6.1	13.5	18.5	-	-	-	-	26.4	6.1	20.3	22.5	4
10	M	12	6.5	-	19.6	6.1	13.5	-	63.0	42.7	20.3	28.5	48.8	12.2	36.6	40.0	5
11	M	10	7.3	62	12.9	6.1	6.8	17.0	81.4	61.1	20.3	24.5	19.6	6.1	13.5	17.0	7
Mean± SE		17.82± 2.73	6.8± 0.17	56.3± 3.87	20.51± 1.12	6.38± 0.28	14.13± 1.10	19.24± 1.6	88.87± 8.74	65.23± 8.04	23.6± 1.85	28.42± 1.63	35.91± 6.15	11.58± 3.30	24.46± 4.98	27.38± 5.42	6.0± 0.50

SE = STANDARD ERROR

Table 5.8 PLASMA VITAMIN A, RBP, PREALBUMIN AND TOTAL PROTEIN CONCENTRATIONS BEFORE AND AFTER THERAPY IN NIGERIAN CHILDREN WITH MEASLES AND MALNUTRITION

Specimen No.	Sex	Age (months)	Total Protein g/100ml	Prealbumin µg/ml	Total vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml	24 hours after therapy			
									Total vitamin A µg/100ml	Retinyl Ester µg/100ml	Retinol µg/100ml	RBP µg/ml
1	F	15	5.3	40	31.3	24.5	6.8	10.0	135.9	122.4	13.5	18.0
2	M	18	6.4	-	19.0	12.2	6.8	-	38.6	18.3	20.3	-
3	M	36	6.2	-	6.8	NEG	6.8	10.0	54.7	36.7	18.0	20.5
4	M	12	4.7	-	19.6	6.1	13.5	-	97.7	73.2	24.5	-
5	F	9	5.6	45	16.3	6.1	10.2	18.0	79.1	61.1	18.0	20.5
6	M	24	-	-	12.9	6.1	6.8	-	-	-	-	-
7	M	18	6.0	48	19.6	6.1	13.5	15.0	69.2	48.9	20.3	18.5
8	M	15	5.2	21	6.8	NEG	6.8	10.0	-	-	-	-
9	M	12	5.6	-	13.5	NEG	13.5	14.0	31.8	18.3	13.5	18.5
10	M	9	4.5	40	19.0	12.2	6.8	13.0	56.1	42.6	13.5	15.0
11	F	12	6.1	40	16.3	6.1	10.2	13.5	-	-	-	-
12	M	14	6.8	65	16.3	6.1	10.2	15.0	-	-	-	-
13	F	18	-	65	31.8	18.3	13.5	18.0	136.6	116.3	20.3	24.5
14	M	24	-	39	12.9	6.1	6.8	10.0	66.1	48.1	18.0	-
15	M	11	6.0	60	19.0	12.2	6.8	15.0	68.4	48.1	20.3	20.5
Mean ± SE		16.47± 1.84	5.7± 0.20	46.37± 4.35	17.41± 1.83	9.27± 1.50	9.27± 0.76	13.45± 0.86	75.84± 10.40	57.64± 10.40	18.22± 1.06	19.5± 0.97

SE = STANDARD ERROR

FIG. 5-1: CALIBRATION CURVE FOR RETINOL AND RETINYL ESTER (AS RETINOL EQUIVALENT)

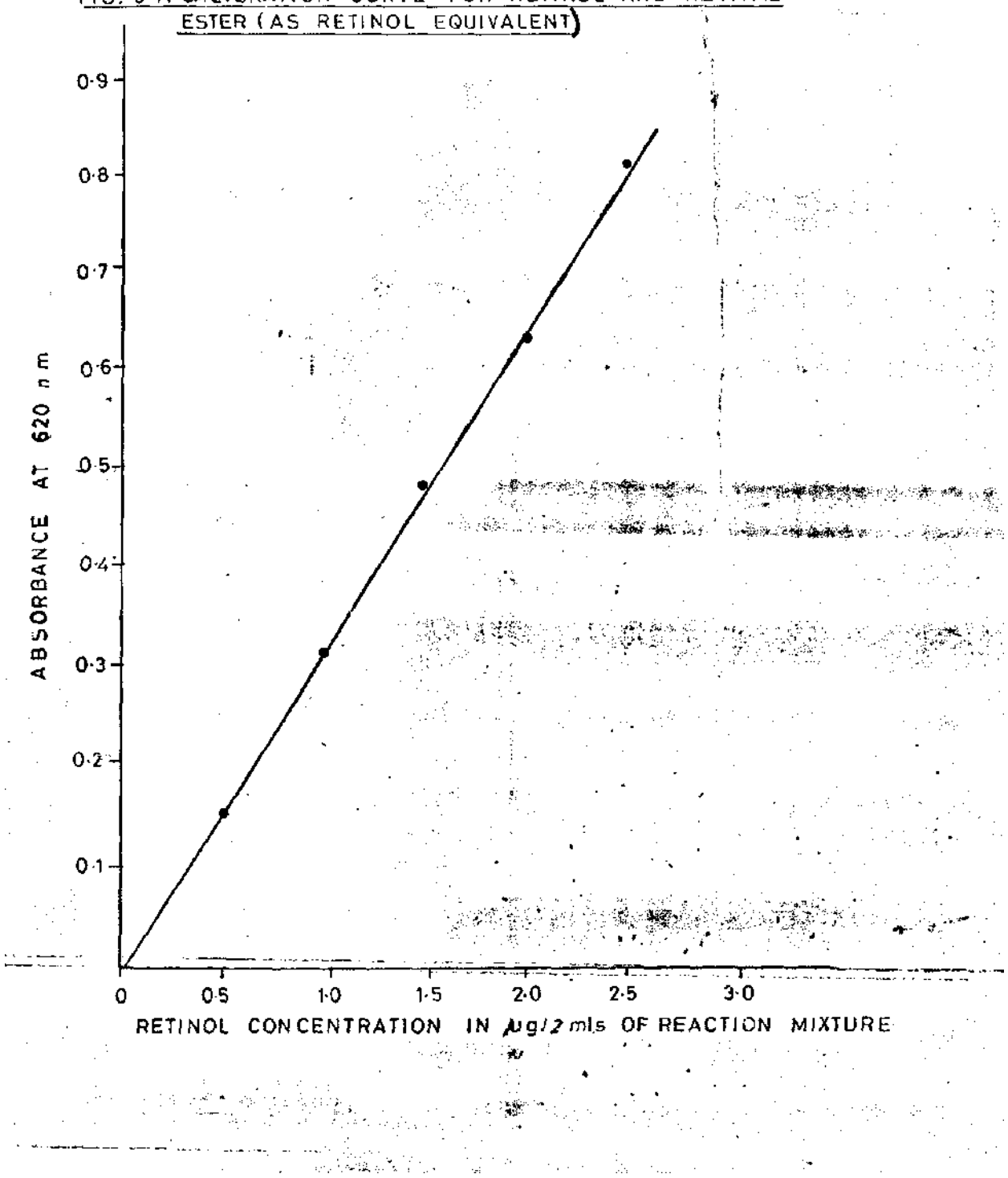


FIG. 5·2 : CALIBRATION CURVE FOR PROTEIN ESTIMATION

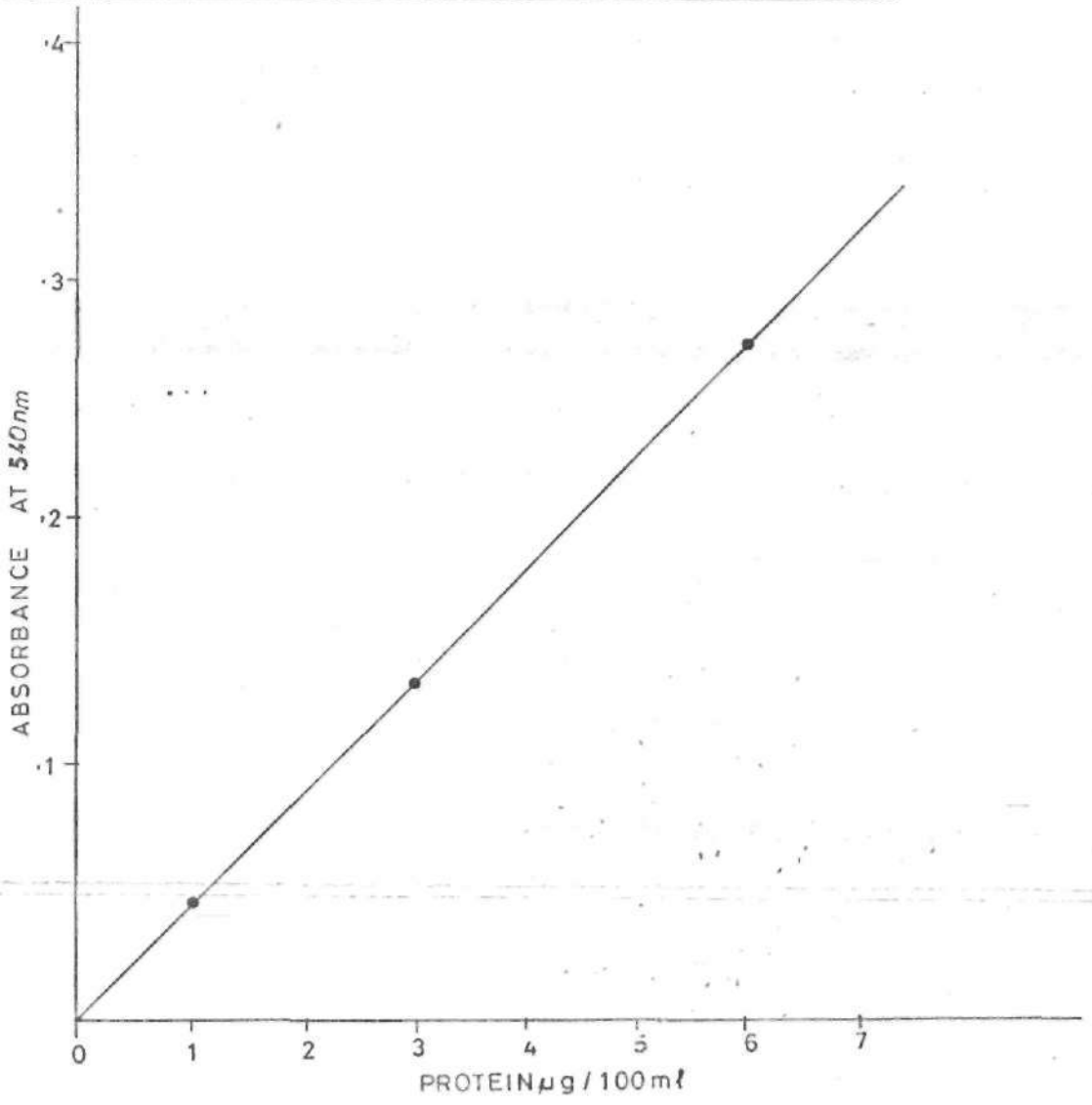


FIG. 5·3: CALIBRATION CURVE FOR RETINOL-BINDING PROTEIN ESTIMATION

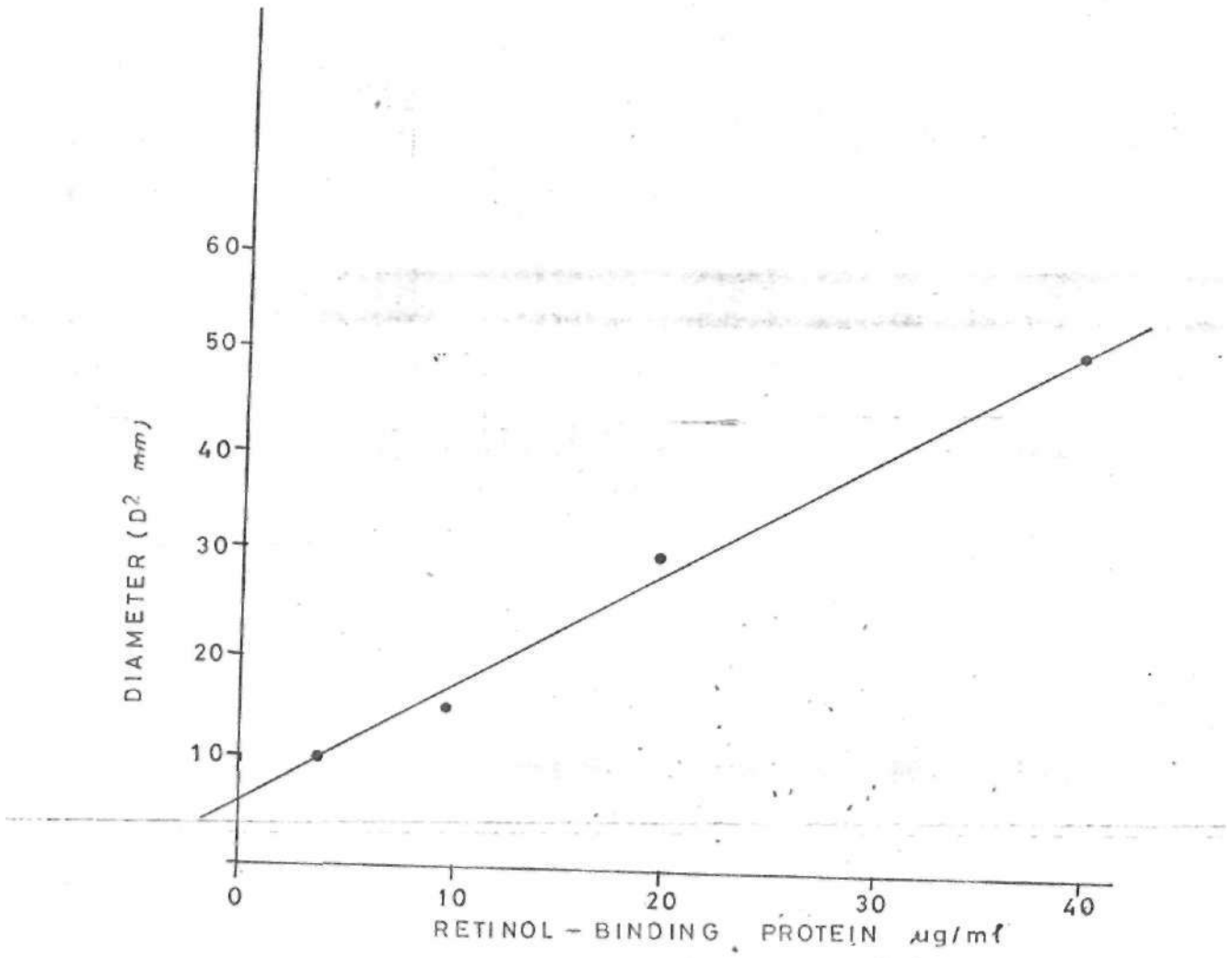
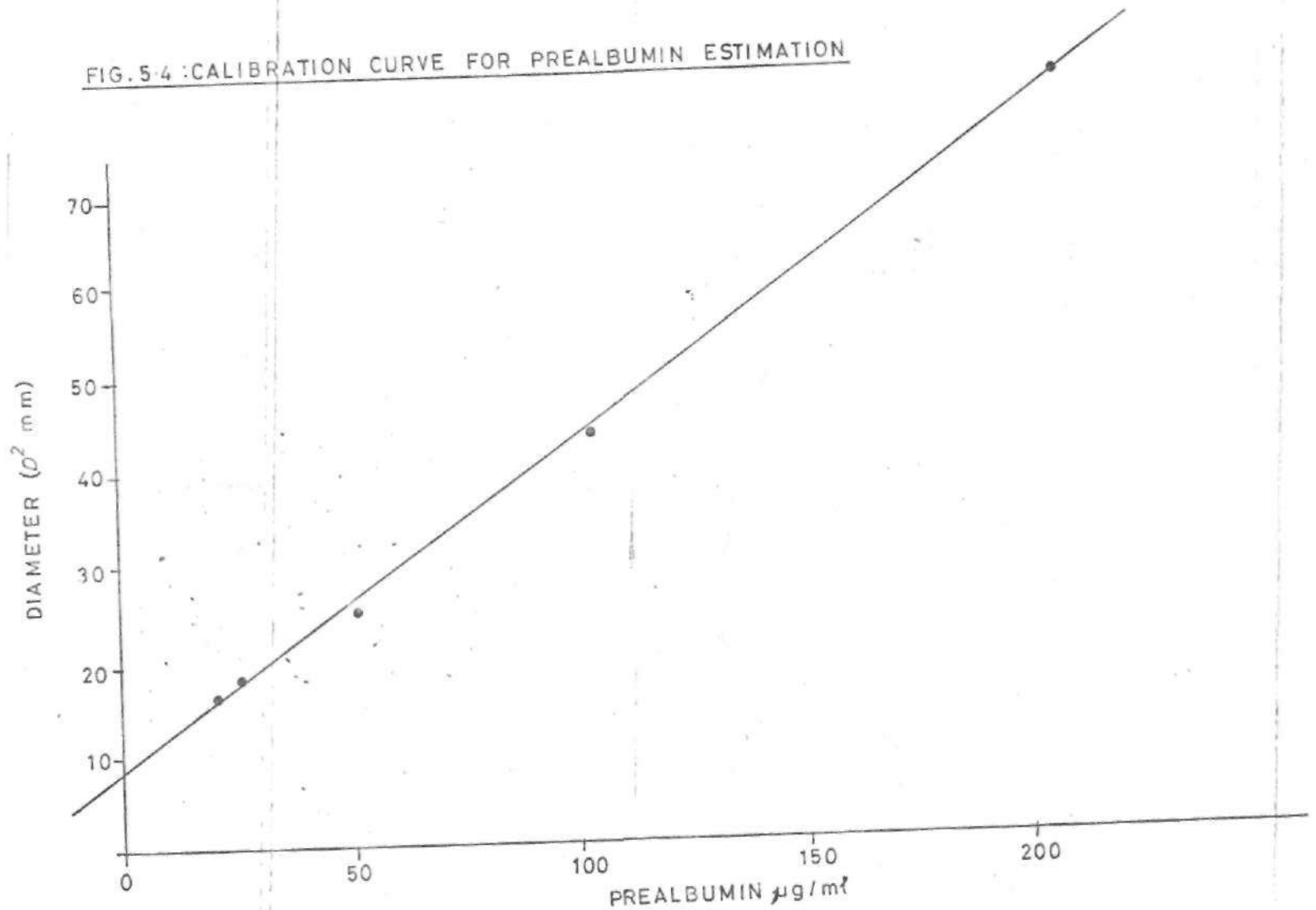


FIG. 5·4 :CALIBRATION CURVE FOR PREALBUMIN ESTIMATION



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