VITAMIN A STUDIES IN CHILDHOOD

By

WILLIAM MORTON FYFE

M.B., CH.B., F.R.F.P.S.(Glasg.),
M.R.C.P.(Ed.),M.R.C.P.(Lond.),D.C.H.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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PREFACE

The investigations on which this thesis is based were carried out in the wards and biochemical laboratory of the Royal Hospital for Sick Children, Glasgow. Part of this work has already been published in the Lancet, 1956, I, 610 under the title "Vitamin A Levels in Idiopathic Hypercalcaemia".

I am grateful to Professor Stanley Graham for suggesting this investigation. Most of the patients studied were in Dr. J. H. Hutchison's wards and it is a pleasure to express my indebtedness to him for this access to his patients and much encouragement and advice; the remaining patients were under the care of Professor Graham, Mr. W. M. Dennison and Dr. R.A. Shanks and to them also I would express my thanks. I am grateful also to Dr. H. E. C. Wilson for making available the resources of his department and for unstinted technical advice.

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PART I. HISTORICAL INTRODUCTION.

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--- HISTORICAL INTRODUCTION ---

THE BIRTH OF DIETETICS

Since the dawn of medical history man has speculated on the part played by the diet in maintaining health and producing disease. Until the eighteenth century A.D. when the application of the experimental method to the study of human nutrition placed knowledge on a scientific basis, medical opinion was founded on the theories of the ancient philosophers and the observations of physicians.

In ancient Egypt, more than one thousand years B.C., disease was generally attributed either to the influences of magic or to the actions of a vengeful deity. However, the historian Herodotus suggests that food was held in some part responsible when he states that the ancient Egyptians "Purge themselves every month, three days successively seeking to preserve health by emetics and clysters, for they suppose that all diseases to which men are subject proceed from the food they use." This does perhaps scant justice to the part played by rational medicine as revealed in the Smith papyrus. Here we are told that the surgical patient is frequently "moored to his mooring stakes." This meant that he was kept on his ordinary diet and given no drugs (Sigerist, 1951).

The concept that diet is an important factor in the causation of disease formed an integral part of Greek medicine in the

pre-Christian era. Germane to this concept was the humoral theory of pathology which was destined to dominate therapeutics for many centuries. Its origins lay in the theories of the Greek philosophers of the first six centuries B.C. Thales (ca. 652-548 B.C.), the founder of the earliest school of the Greek philosophers, believed that water was the ultimate principle of all things while Anaximenes (ca.570-500 B.C.) adopted air and Heraclitus of Ephesus (ca. 535-475 B.C.) fire. To Empedocles (ca. 495-435 B.C.) however is given the credit, if such it be, of postulating the theory of the four elements. According to this view everything on earth, including the human body, was composed of four simple factors, namely, fire, air, earth and water. Alcmaeon of Croton, who lived about 500 B.C., had already advanced the concept of Isonomia that health consisted in a state of perfect harmony while disease was due to a disturbance of this harmony. Empedocles combining this concept with the theory of the four elements believed that so long as the four elements were in harmony the human body remained healthy but whenever they were in disharmony disease resulted. In the ensuing years, qualities were attributed to these elements and fire, earth, air and water were considered to have the qualities, hot, cold, dry and moist respectively. It was accepted that all foodstuffs were composed of the four elements, and it was believed that their digestibility and nutritive value were

determined by the related qualities of which four degrees were postulated. Moreover, man was thought to contain four complexions, each formed by the blending of the qualities of two elements. The complexions were sanguine, phlegmatic, choleric and melancholic and the predominant complexion determined the individual's appearance and character. The Greek physicians contemporary with these philosophers believed that the body fluids stood between the food ingested and the matter which constituted the body. These physicians in their search for the underlying cause of disease were impressed by the changes in body fluids which accompanied disease processes, such as haemorrhage, discharges and the alterations in the urine and faeces. At first, the blood was credited with being the cause of all pathological disorders, while later, physicians attributed this role to the bile. Slowly the theory evolved that there were four body fluids, or humours, namely blood, phlegm, yellow bile and black bile. In health these fluids were in a balanced state, but disease resulted if this balance was upset either by the body receiving insufficient food, or the wrong type of food, or by some other means. The final stage in the development of the theory of the four humours was the association of each of the complexions with one of the humours as follows:-

Complexion	Qualities	Humour
Sanguine	Hot and moist	Blood
Phlegmatic	Cold and moist	Phlegm
Choleric	Hot and dry	Yellow bile
Melancholic	Cold and dry	Black bile

Children were considered to be phlegmatic at first becoming sanguine and choleric with growth. As phlegm has the qualities moist and cold, young children were thought to be best nourished by foods which were hot and moist. Galen (ca.A.D. 130-200) credited Hippocrates with being the originator of the theory of the four humours, although it is probable that it was not fully elaborated until mediaeval times. However, this theory in some form was responsible for the prominent position of dietetics in the therapeutics of the Hippocratic era.

The theory of the four humours dominated medical opinion until the dawn of the eighteenth century. The continued belief in this unfounded philosophy throughout twenty-four centuries is to be attributed to its inclusion in the teachings of Hippocrates and Galen. These teachings together with the work of all the Greek scholars, which were to influence so greatly the pattern of the later middle ages and the renaissance, were preserved in the cloisters of Europe and in the Arabian schools of medicine, where Rhazes and Avicenna were later to become names almost as often quoted in English medical literature as Hippocrates and

Galen. From the dietetic point of view the well known medical poem "Regimen Sanitatis Salernitanum" is worthy of mention. It emanated from the school of medicine at Salerno about the twelfth century A.D. Because dedications to Anglorum regi appear in some of the early manuscripts it has been suggested that this poem was compiled for a king of England. It is more probable, as Singer (1928) suggests, that this reference was included in the hope that readers would consider that medicine good enough for the great was good enough for those of humbler estate. Whatever may have been the reason, this poem was widely read throughout Europe and was largely responsible for the establish-:ment of Hippocratic and Galenic teaching in England during the renaissance.

In 1539, Sir Thomas Elyot (A.D. 1490-1546), a diplomatist in the court of Henry VIII, published a book entitled "The Castell of Helth." A copy of this book "in some places augmented by the first author thereof" published in "the yere of our lorde 1541" is included in the Euing collection of Glasgow University Library. This book written in the English language probably reflects contemporary opinion when foods are classified as those engendering choler, fleume and melancholy. As is so characteristic of his time Sir Thomas Elyot quotes as his authority on "remedies against the distemperaunce of every humour" Soranus of Ephesus who had lived fourteen hundred years previously in

the second century A. D.

Even in the seventeenth century dietetics was still dominated by the humoral theory. This is shown by the following extract from Thomas Muffets' "Healths Improvement" (1655):-

> "Yea, my self have known a young Maide, of an exceeding moist and cold complexion, whose meat for two years was chiefly pepper, wherewith another would have been consumed, though she was nourished: for it is hot in the third, and dry in the fourth degree."

Throughout all these centuries of belief in this speculative philosophy dietary regimens formed an important part of therapeutics. The classification of foodstuffs according to the degree of the quality each article was presumed to possess must have presented many problems. However, the choice of foods for various ages and different diseases appears to have been determined more by observation and experience than by inductive reasoning.

In the closing years of the seventeenth century the classification of salts into 'acid' and 'alcaline' led to a simple chemical conception of disease which was applied to children by Walter Harris (1647-1732). In his book "De Morbis Acutis Infantum", which was published in 1689 and translated into English by John Martyn in 1742, he advanced the hypothesis that "As the Acidity of the Humours is the primary cause of all

the Disorders with which the tender age is wont to be tormented, the whole Art of Cure turns entirely on subduing the acid". He suggested that testaceous powders made from oyster shells, crab's eyes and claws etc. and various other alkaline remedies should be used in the treatment of children's diseases. This concept of acidity and alkalinity became widely accepted and foods were classified as to whether their influence was acid or alkaline.

In his classic treatise on rickets entitled "De Rachitide", which was published in 1650, Francis Glisson (A.D. 1597-1677) still adhered to the humoral theory as he attributed rickets to a cold and moist distemper. Among the dietary predisposing causes he included "most kinds of fish, and crude meats which are not well prepared by Coition", over-eating, "flesh hardened by smoke and seasoned with much salt", "salt fish, and cheese almost of any kind plentifully fed on", "bread newly taken out of the oven and not yet cold", "almost all sweet things condited with sugar and old and strong wines, especially being drunk on an empty stomach". In contrast, Lind's treatise on scurvy published in 1753 shows that medical thought at that time had advanced beyond the humoral pathology. Lind also discounts the theory of acidity and alkalinity. Lind realised that lack of fruit and vegetables was an "occasional cause" of scurvy and that the provision of these foods in adequate amount prevented

the disease or cured it once it had developed. He attributed the curative properties of fruit and vegetables to their ability to correct a digestive upset caused by the excess of "sweet farinaceous substances unfermented", and "salted or dried flesh and fish" in the "sea diet".

In the eighteenth century, the discovery that air and water could be resolved into simpler substances led to the final downfall of the elemental and consequently the humoral theory, and heralded the birth of the modern science of dietetics. Antoine Laurent Lavoisier (1743-1794) was the first man to apply these new chemical discoveries to the study of human metabolism. He appreciated that respiration was really a process of oxidation and demonstrated that the quantity of oxygen absorbed and carbon dioxide excreted depends primarily on food, work and temperature. Tragically this great Frenchman was guillotined in 1794 by the French Revolutionists.

Another famous Frenchman, Francois Magendie (1783-1855), an exponent of the experimental method at a stage in history when this was not yet an accredited method of investigation, was the first to use animal experiments in the investigation of dietetic problems. He demonstrated that animals fed on fresh meat as their sole nutriment remained in good health, while those fed on sugar or starch or fat became ill. He concluded from this experiment that foods containing nitrogen are essential for

health. This conclusion has been amply confirmed by all subsequent work. It is interesting that Magendie noticed that dogs fed exclusively on bread developed sore eyes and opacities of the cornea. (Drummond and Wilbraham, 1939).

The next major contribution to dietetics was the accurate analysis of the chemical composition of food by the German chemist Professor Justus Von Liebig (1803-1873). Liebig's major work entitled "Organic Chemistry in its Application to Physiology and Pathology" was published in 1842. This work was fully reviewed in the same year in the Lancet by Henry Ancell, Lecturer on Medical Jurisprudence at the School of Anatomy and Medicine, Grosvenor-place, St. George's Hospital, and Surgeon to the Western General Dispensary. Ancell states that Liebig

> "proves that the diet of Man consists of two essential parts subserving totally different purposes in his economy. The one part being composed of materials, capable of assimilation to his various organs and tissues, and containing a peculiar principle essential to his existence, now well known to chemists as proteine; these are the nitrogenised principles of his food and are called 'plastic elements of nutrition'. The other part being composed of elements employed continually for purposes other than the nutrition of the tissues, but equally essential to the continuance of life, of which the support of respiration and the production of heat are among

"the most obvious and important; these are the non-nitrogenised elements of his food and are called 'elements of respiration'." Liebig believed that the nitrogenous constituents of food were directly converted into blood after digestion and then later became transformed into flesh, a view which still reflects the influence of the humoral theory.

In 1863, Liebig devised a formula for the "perfect" infant food. This food consisted of cow's milk, flour, potassium bicarbonate and malt, and sold for sixpence a quart. It was the forerunner of numerous farinaceous infant foods which were increasingly used for infant feeding during the latter half of the nineteenth century. Evidence of the imperfections of these foods was provided by the malnutrition which resulted from their use and the rise in the incidence of rickets and scurvy.

During the second half of the nineteenth century a great deal of research, especially in Germany, was devoted to determining the amount of protein, carbohydrate and fat utilised by the body under a variety of conditions. Karl von Voit (1831-1908) and Max von Pettenkofer (1818-1901), working in Munich, constructed a special respiratory apparatus, the cost of which was defrayed by King Maximilian II of Bavaria. This apparatus was designed to measure the total carbon excretion, including that of respiration, and also the total nitrogen excretion.

From these two observations, calculations were made as to the substances utilised by the body. This work led Voit to believe that the adequacy of the diet was to be measured in terms of protein, carbohydrate and fat consumed in the correct proportions. Voit's pupil, Max Rubner (1854-1932), who became director of the Hygienic Institute of Berlin, established the importance of the caloric value of the diet. He calculated the caloric value of protein carbohydrate and fat as follows:-

1 G. of protein	4·1 Calories
1 G. of carbohydrate	4·1 Calories
1 G. of fat	9·3 Calories

These"Standard Values" have since been uniformly accepted for calculating the caloric value of a diet. Using an animal calorimeter, Rubner also showed that the amount of heat given off by a dog to the calorimeter was the same as that calculated from an estimate of the metabolism using a respiration apparatus, according to the method of Pettenkofer and Voit. This demonstration that the laws of conservation of energy were applicable to animal metabolism provided a final verification of Voit and Pettenkofer's work.

Further metabolic experiments were carried out by W. O. Atwater and E.B. Rosa using a large calorimeter capable of measuring the heat given off by a man living in it. This work was extended by F. G. Benedict in the Nutrition Laboratory

in the Carnegie Institution in Boston and by E. F. Du Bois and Graham Lusk in Cornell University Medical College in New York City.

THE DISCOVERY OF THE VITAMINS

During the nineteenth century it had been shown that food contained protein, carbohydrate, fat and salts and it was assumed that, given in the correct proportions, these provided all the dietetic requirements for health. The first hint that food contained other substances essential for health came from animal feeding experiments carried out in 1881 by N. Lunin, a Russian assistant to Professor Bunge at the University of Basle. Lunin was investigating the effect on mice of selective additions of mineral salts to a diet consisting of protein, fat and carbohydrate mixed in the same proportions as in milk. He found that, while mice could live on milk, they only survived a short time on this synthetic mixture even when the whole of the mineral ash of milk was added. As a result of this experiment Lunin asked the following questions:-

> "Cannot cane sugar take the place of sugar of milk? Or are the inorganic constituents of milk chemically combined and only assimilable in this combination?---- Or does milk contain in addition to protein, fat, carbo-:hydrates other organic substances which are also indispensable to the maintenance of life?". (Bunge, 1890)

Lunin, however, did not follow up the exciting possibility suggested in his third question. Ten years later, Socin, also working in Professor Bunge's laboratory, carried out similar animal feeding experiments. The results confirmed the existence of these indispensable substances in milk and indicated that they were present also in egg yolk. Socin commented that these new substances should be discovered before new feeding experiments were attempted but he made no effort to do so. Hopkins (1929) suggests that this was possibly because Professor Bunge did not believe in the existence of these new dietary constituents and explained the failure of synthetic diets on the separation of inorganic constituents from organic combinations during the preparation of the diets.

It was not until the early years of the twentieth century that Professor Pekelharing in Holland, Dr. Stepp in Germany and Professor Hopkins in England, each working independently, confirmed the existence of these substances by further animal feeding experiments. Professor Hopkins deserves special mention as he, more than the others, appreciated the full significance of the new dietary essentials which he termed "accessory substances." In 1912,he published his most important work which described experiments showing that, while rats failed to grow on an artificial mixture of isolated casein, fat, carbo-:hydrate and salts, the addition of a small quantity of milk to

this diet induced normal and continued growth. Further Hopkins demonstrated that the effect on growth was not due to the small increase in food intake of the rats receiving the added milk. It is fortunate historically that as early as 1906 Hopkins, in the course of a lecture to the Society of Public Analysts devoted primarily to a discussion of the relationships between Analysts and Medical practitioners, expressed his conviction that unsuspected dietetic factors existed, and that the errors in the diet in rickets and scurvy comprised these "minimal qualitative factors". Professor Hopkins was awarded the Nobel prize in 1929 for his contributions to dietetics.

DEFICIENCY DISEASES

The idea that disease could result from an isolated deficiency in the diet was first suggested by Professor Grijns in 1901 to explain the facts then known about beri-beri. In 1882, Takaki, who later became Director General of the Medical Department of the Japanese Navy, demonstrated the importance of a dietetic factor when he showed that the addition of more protein foods to the diet of naval personnel eliminated beri-beri on long sea voyages. In 1897, Professor Eijkman showed that the continuous consumption of polished rice caused beri-beri; he concluded that rice contained a poison capable of producing this disease and that rice polishings contained the antidote. Professor Grijns, however, realised that an alternative explanation of Professor

Eijkman's observations was that the disease was due to a deficiency of an essential nutrient present in rice polishings. Knowledge of the new accessory substances led to the further development of this concept. It gradually became appreciated that there existed a group of diseases each one due to the lack of a particular accessory substance in the diet. Casimir Funk was one of the early proponents of this theory and in 1912 he suggested the name "Vitamines" for the deficient substances.

"The deficient substances, which are of the nature of organic bases, we will call vitamines".

As the ending 'amine' has chemical implications which are not applicable to this group of substances, Drummond suggested that the final 'e' be omitted and the word vitamin is now in general use.

VITAMIN A.

During the second and third decades of the twentieth century, Osborne and Mendel in Yale University and McCollum and his colleagues in the University of Wisconsin conducted many feeding experiments on rats which confirmed and supplemented Hopkins' work on the accessory dietary factors.

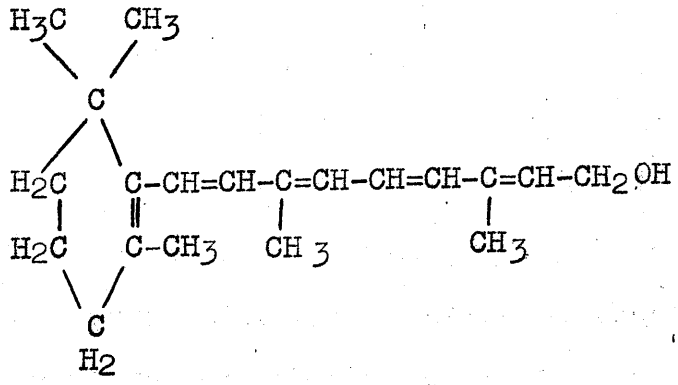
In 1913, Osborne and Mendel showed that the growth promoting substance in milk is present in the butter fat fraction. Independently, in the same year, McCollum and Davis demonstrated that ether extracts of eggs or butter also contain the growth

promoting factor. These experiments established that the growth promoting factor is fat soluble, and in 1917 McCollum and Simmonds suggested that it be called "fat soluble A". McCollum and Simmonds also drew attention to the similarity between the xerophthalmia produced in rats by a diet deficient in fat soluble A and the conjunctivitis which was seen in children fed on fat deficient diets, and which had been described by Mori and Bloch. They concluded that this type of conjunctivitis was not due to a 'fat starvation' but to a lack of fat soluble A "which occurs in just those foodstuffs which they (Mori and Bloch) observed to possess curative properties".

In 1918, Mellanby produced rickets in puppies by feeding them on a diet of separated milk, wheaten bread, linseed oil, yeast, orange or lemon juice and sodium chloride; the rickets could be cured by the addition of ten grams of butter or cod liver oil but not by ten grams of cottonseed oil or olive oil. Mellanby considered that these results were "for the most part in keeping with the idea that rickets is a disease primarily due to a deficiency of fat soluble A".

The realisation that the anti-rachitic substance was a separate entity distinct from the growth promoting anti-xerophthalmic factor was not long delayed. McCollum et al. (1922a) showed that the quantitative distribution of these two substances varied in different foods; they found that rats fed throughout

their period of growth and a considerable period of their adult life on a diet deficient in calcium and fat soluble A factor were much better nourished when supplied with 1% cod liver oil than with 10% butter fat. McCollum et al. (1922b) also showed that heat and oxidation destroyed the anti-xerophthalmic but not the anti-rachitic properties of cod liver oil. Hume (1922) and also Goldblatt and Soames (1922) found that irradiation with ultra-violet light failed to prevent rats fed on a diet deficient in fat soluble A factor from developing xerophthalmia. These and similar experiments showed that McCollum's fat soluble A factor comprised two separate substances, the growth promoting anti-xerophthalmic factor and the anti-rachitic factor, which became known as vitamin A and vitamin D respectively. The isolation of vitamin A and the discovery of its chemical formula was the work of Karrer, Heilbron, Holmes and Corbett between 1930 and 1937. The final synthesis was achieved in 1947. Vitamin A is an alcohol with the formula



THE CAROTENOIDS

In 1920, Rosenheim and Drummond showed that the distribution of fat soluble A in a number of fats runs parallel with the lipochrome pigments. In 1928, Euler et al. claimed that carotene could restore growth in vitamin A deficient rats. Moore (1930) confirmed this work of Euler et al., and also showed that there was a considerable quantity of vitamin A in the livers of vitamin A deficient rats restored to health by a carotene diet. This demonstrated the in vivo conversion of carotene to vitamin A. It is now known that vitamin A can be formed from at least four carotenoids. These vitamin A precursors or provitamins A are alpha -, beta - and gamma - carotene and cryptoxanthine. In human nutrition a considerable part of the vitamin A value is represented by the carotenoids in green and yellow vegetables. Eggs, milk and butter which are the usual sources of vitamin A also provide some carotenoids.

VITAMIN A DEFICIENCY.

Although vitamin A was unknown until the present century the therapeutic properties of vitamin A containing foods were known to the ancient Egyptians. Thus the Ebers papyrus, written about 1600 B.C., contains a reference to the use of liver in the treatment of eye disease. The nature of the eye disease benefited by liver is not stated but it is probable that it was night blindness (Drummond and Wilbraham, 1939). Certainly Hippocrates was familiar with night blindness as is shown by the following extract from the Second Book of Prorrhetics

(Adams translation):

"Persons having protracted defluxions of tears who are attacked by nyctalopia, are to be questioned whether they had any previous complaint in the head".

Celsus (ca A.D. 100) in "De Medicina" advises sufferers from night blindness to "be anointed with the blood of a liver (particularly the liver of a he goat; if that can't be had, of a she goat) that drops from it while roasting; and they ought to eat the liver itself". (Grieve's translation). It is generally believed that Celsus was not a physician but an encyclopaedist who recorded everything that was known in his time about several subjects, as well as medicine, which seemed to him worth saving for posterity.

The earliest description of xerophthalmia was probably given by Jacques Guillemeau in the sixteenth century (Drummond and Wilbraham, 1939) and, according to Mackay (1934), Joseph Brown is to be credited with the first clear clinical description of keratomalacia, which he published in the Edinburgh Journal of medical Science in 1827. Bitot in 1863 recognised the clinical relationship between xerosis of the conjunctiva and certain kinds of night blindness; his name has since been applied to characteristic spots present on the conjunctiva in xerophthalmia. According to Kohn (1903), the association of keratomalacia with malnutrition in infancy was first noted by Mackensie in 1857.

It was later emphasised by Czerny and Keller in 1906 when they described the nutritional disturbance which they termed mehlnärschaden (Ross, 1921)

There is general agreement that young children develop signs of vitamin A deficiency more readily than adults. In 1904, Mori described fifteen hundred and eleven cases of a disease in which excessive appetite, diarrhoea, dryness of the skin and hair, loss of weight and swelling of the abdomen were associated with an ocular disturbance whose severity varied from night blindness in mild cases, through xerophthalmia and keratomalacia to blindness in the most severe cases. The maximum age incidence of this disease was two to five years, and cod liver oil was an effective cure. Blegvad (Ross, 1921) found that about six hundred to seven hundred cases of xerophthalmia had occurred in Denmark between 1909 and 1920. Bloch (1921 and 1924) observed sixty-three cases of xerophthalmia in Denmark and noticed that before the eye lesions appeared, the affected children became quiet and apathetic and lost weight; also they became unusually susceptible to infections of the skin and of the respiratory and urinary tracts. The suggestion of McCollum and Simmonds that this eye disease is due to vitamin A deficiency is now generally accepted. In 1924, Parsons stated that xerophthalmia is rarely seen in the children's clinics in Great Britain and that during the previous thirteen years only thirteen cases of the mild form of xerophthalmia-

i.e. xerosis of the conunctiva - had been seen at the Birmingham and Midland Eye Hospital, and not one case of the severe form of the disease, keratomalacia. On the other hand, according to Mackay (1934), Dawson found approximately one hundred cases of xerosis of the conjunctiva during 1928 and 1929 among the children then attending nursery and elementary schools in the Darlington area. Also, in 1931, Spence described fourteen cases of xerophthalmia seen in the ophthalmological out-patient department of a Newcastle hospital in the previous year. However, xerophthalmia and keratomalacia are late manifestations of vitamin A deficiency, and, since their aetiology became widely known, have been rarely seen in European countries.

In recent years, research has been directed to determining the early manifestations of vitamin A deficiency. Different symptoms and signs have been described by various authors as preceding the eye changes but there has been no general agreement. Thus, while Mori (1904) and Bloch (1924) considered that loss of weight was an early symptom, Spence (1931) found that xerophthalmia occurred in children whose general health and rate of growth were well maintained, and that cure of the eye disease did not necessarily cause a rise in weight. Spence (1931) found that seven of eleven children with xerophthalmia had indolent skin sepsis and Mackay (1934) suggested that the earliest symptom of vitamin A deficiency in babies was an increased susceptibility

to minor infections of the skin, but Sweet and K'Ang (1935) found little evidence of skin sepsis in their two hundred and three cases of 'Avitaminosis A'. Again, while Spence (1931) and Blackfan and Wolbach (1933) described an increased number of epithelial cells in the urine persisting until treatment was given, Sweet and K'Ang (1935) found such an increase in only two of their two hundred and three cases. Hydrocephalus, facial paresis, anaemia, haematuria and gynaecomastia have also occurred in some but not in all cases of vitamin A deficiency in infants (Blackfan and Wolbach, 1933; Cornfeld and Cooke, 1952; Bass and Caplan, 1955). The inconstancy of these symptoms and signs suggests that they may not be due directly to vitamin A deficiency and renders them of little value for detecting mild degrees of hypovitaminosis A.

The degree of night blindness appears to provide a reliable index of vitamin A deficiency. Its measurement, however, requires the co-operation of the patient, and Harris and Abbasy (1939) consider that it is unreliable under eleven years of age. Moreover, these authors believe that repeated estimations must be made to ensure accuracy, and that this and the difficulties of the method make it of value chiefly to the specialised research worker. Friderichsen and Edmund (1937) have attempted to assess "latent avitaminosis A" in infants between the ages of two months and eighteen months by the magnitude of the faintest light irritant

that is able to elicit certain reflex movements via the infant's eye, when the infant is adapted to darkness by a stay of at least thirty minutes in a perfectly dark room. The difficulties inherent in such a method would appear to be very great.

The value of the vitamin A content of the blood as a measure of vitamin A deficiency will be discussed in Part II of this thesis.

HYPERVITAMINOSIS A.

Although the diet must contain a certain amount of vitamin A to maintain health, overdosage can cause toxic effects. This was shown in animals by Takahashi et al. in 1925. The unpleasant symptoms which follow a meal of polar bear liver have been known to Arctic explorers for many years. They are headache, drowsiness, irritability, vomiting and peeling of the skin of the face or even of the whole body. It was not until 1942, however, that Rodahl and Moore (1943) identified the toxic substance in polar bear liver as vitamin A.

In recent years concentrated preparations of vitamin A have been manufactured for use as medicines. Overdosage with these concentrates has produced toxic effects in children. The syndrome of chronic hypervitaminosis A in a child due to an excessive intake of vitamin A over a long period was first described by Josephs in 1944. Since then several cases have been reported in the American literature (Toomey and Morissette, 1947;

Caffey, 1950; Gribetz et al., 1951; Arena et al., 1951; Goldzier et al., 1952) and two cases in the British literature (Pickup, 1956). Caffey (1950) found that the minimal preclinical latent period of vitamin A poisoning was about six months, and the minimal toxic daily dose was about 75,000 units. The commonest initial manifestations of chronic hypervitaminosis A are anorexia, irritability, pruritus and a failure to gain weight. Tender soft tissue swellings appear and radiographs show underlying cortical hyperostoses of the bones. Caffey (1950) has stressed this cortical hyperostosis and states that it is always present on two or more of the long bones, and most frequently on the ulna and metatarsals. The hair becomes coarse and much hair may be shed. Skin rashes and fissuring at the corners of the mouth with cracking and bleeding of the mucous membranes of the lips occur. The liver and spleen may be enlarged and hydrocephalus has also been described (Gribetz et al., 1951; Arena et al., 1951). The blood vitamin A level is always high. Rapid recovery occurs when the vitamin A is stopped.

In 1951, Marie and Sée described an acute form of hypervitamin-:osis A in infants following the accidental ingestion of a massive dose of vitamin A in the form of a concentrated preparation of vitamins A and D. These infants suffered from vomiting, drowsiness and a marked bulging of the fontanelle. Complete

recovery occurred in one or two days.

It is interesting that infants may develop a reversible hydrocephalus both from hypovitaminosis A and acute and chronic hypervitaminosis A. Also that in rabbits, maternal vitamin A deficiency can produce in the progeny hydrocephalus due to stenosis of the cerebral aqueduct (Millen et al., 1953).

PART. II. AUTHOR'S INVESTIGATIONS.

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METHODS.

Estimation of the Vitamin A and Carotenoid Content of the Blood

As neither vitamin A nor carotenoids are present in appreciable amounts in the blood corpuscles (May et al., 1940), plasma or serum is used when estimating the vitamin A and carotenoid content of the blood.

Two methods are available for estimating the serum or plasma vitamin A. The first method is based on the antimony trichloride colour reaction described by Carr and Price (1926) for the quantitative estimation of the vitamin A content of cod liver oils. Carr and Price showed that the intensity of the blue colour produced by adding a 30% solution of antimony trichloride in chloroform to a solution in chloroform of the oil to be tested was a measure of the vitamin content of the oil; the intensity of the blue colour was measured in a Lovibond tintometer. Subsequent experience has confirmed the reliability of this method of assaying oils. It was adopted by S. W. Clausen (McCoord and Luce-Clausen, 1934) for estimating the vitamin A content of small quantities (2 ml.) of blood. In 1938, Dann and Evelyn increased the accuracy of the method as applied to oils by substituting a photoelectric colorimeter for the visual matching of colours. The following year Kimble (1939) used the photoelectric colorimeter for plasma

assay. May et al. (1940) used this instrument for assaying the vitamin A content of 1 ml. serum or plasma samples. They estimated the carotenoid content of the same samples, and were thereby able to correct the vitamin A reading for irrelevant absorption due to carotenoids. A disadvantage of methods employing the antimony trichloride reaction is the technical difficulty resulting from the rapidity with which the maximum blue colour, representing the vitamin A activity, fades.

The wavelength at which solutions of vitamin A show the maximum light absorption is in the ultra-violet portion of the spectrum at about 328 millimicrons. The exact wavelength varies within a narrow range with the solvent. Measurement by spectrophotometry of the light absorption at this wavelength is the basis of the alternative method of quantitative assay. With extracts of serum or plasma, however, the total absorption at 328 millimicrons is due not only to vitamin A but also to other substances principally the carotenoids. The true absorption due to vitamin A can be determined by measuring the light absorption at 328 millimicrons before and after irradiation with ultra-violet light to destroy the vitamin, when the difference in the two readings represents the vitamin A content (Chevallier and Dubouloz, 1936; Chevallier et al., 1938; Bessey et al., 1946). Caster and Mickelsen (1955) have pointed out that the validity of this method of correcting for irrelevant

absorption depends on the assumptions that ultra-violet light converts vitamin A to products having no absorption at 328 millimicrons, that interfering substances originally present do not undergo appreciable change in their absorption at 328 millimicrons and that ultra-violet light causes quantitative destruction of vitamin A. An alternative method of correcting for irrelevant absorption is that of Morton and Stubbs (1946). This depends on a mathematical procedure in which the irrelevant absorption is assumed to be linear over a small range near 328 millimicrons.

In the present investigation the carotenoid and vitamin A content of plasma was estimated by spectrophotometry by a modification (Appendix I) of the method of Bessey et al. (1946). The plasma was saponified and then extracted with benzene. The carotenoid content of the benzene extract was determined by measuring the optical density at 460 millimicrons. The reading was converted to 'micrograms carotenoids per 100 ml. plasma' by reference to a standard curve prepared from solutions of international standard beta-carotene in benzene. The vitamin A content of the benzene extract was determined by measuring the optical density at a wavelength of 328 millimicrons before and after irradiation with ultra-violet light to destroy the vitamin A; the difference in the two readings represents the vitamin A content. This final value was converted into

'International Units (I.U.) vitamin A per 100 ml. plasma' by reference to a standard curve prepared from solutions of pure vitamin A acetate in benzene.

Synthetic vitamin A palmitate in arachis oil (Avoleum, B.D.H.) was used in the present investigation as a rich source of vitamin A. Solutions in benzene of this vitamin A preparation gave a curve similar to that obtained with solutions of pure vitamin A acetate in benzene.

Many of the estimations of the carotenoid and vitamin A content of the plasma were done in duplicate. The results are detailed in Appendices 3 to 8a. In the tables, however, a single value is shown which is an average of duplicate estimations when these were done.

VITAMIN A STUDIES

in

HEALTHY CHILDREN

REVIEW OF THE LITERATURE.

(a) PLASMA VITAMIN A.

The significance of the plasma vitamin A level depends on how closely it reflects the nutritional status with regard to the vitamin. Grossly excessive or deficient intakes of the vitamin do alter the plasma level. Thus it is high in chronic hypervitaminosis A (Josephs, 1944; Toomey and Morissette, 1947; Caffey, 1950; Pickup, 1956), and it falls in vitamin A deficiency before the appearance of clinical manifestations (May et al., 1940) Persons from the more favourable socio-economic groups have higher values than those from the less favourable (Aron, 1949). But, although Shank et al. (1944) found a relationship between the plasma vitamin A level and dietary intake of the vitamin in children with inactive rheumatism, Szymanski and Longwell (1951) found no such correlation in healthy children from the upper middle class groups. Szymanski and Longwell (1951) also found that the season of the year had no effect on the plasma vitamin A level. Kimble (1939) showed that the blood vitamin A level was unaltered by ordinary meals.

The sensitivity of the blood vitamin A level to variations in vitamin A intake and the relation of the blood level to the body stores of the vitamin have been investigated by animal and human experiments. As the liver is the principal storehouse of vitamin A in the body the liver vitamin A content is taken to

represent the body stores of the vitamin. McCoord and Luce-Clausen (1934) showed that the level of vitamin A in the blood of rats was not significantly altered by oral supplements of the vitamin although the liver stores were greatly augmented. This has been confirmed by subsequent workers (Lewis et al.,1941a; Josephs,1942) though Lewis et al.,(1941a) found that at low levels of vitamin A intake the concentration of the vitamin in the blood was directly related to the intake. Lewis et al. (1941b) also showed that when rats were put on a vitamin A free diet the blood vitamin A was maintained at a high level for a variable interval before beginning to fall; the duration of the interval depended on the size of the liver stores before the diet was started. Glover et al. (1947a) confirmed that the plasma vitamin A level of rats is maintained near normal even when the liver stores approach exhaustion. They found, however, that the plasma vitamin A level was proportional to the concen-:tration of vitamin A alcohol in the liver, although not to the total liver stores which consist mainly of vitamin A ester.

The results of human studies correspond with those obtained by animal experiment. In humans a large oral dose of vitamin A produces a transient rise in the blood vitamin A level lasting about twenty-four hours. The effect of repeated large oral doses of vitamin A on the blood level cannot be assessed therefore until at least twenty-four hours after the last dose

of the vitamin. Lewis et al. (1941b) gave infants vitamin A in doses of 17,000 U.S.P. units daily for periods up to five months. Forty-eight hours after stopping the vitamin supple-:ments the plasma vitamin A levels of the infants over six months were similar to those of a comparable unsupplemented group. On the other hand the plasma vitamin A levels of the infants under six months were slightly higher than those of a comparable unsupplemented group. When a child of four years was given 120,000 U.S.P. units of vitamin A daily for one month there was no more than a minimal rise in the serum vitamin A estimated twenty-four hours after the last dose of the vitamin (Josephs, 1944). Daily supplements of 50,000 I.U. vitamin A given to healthy adults for as long as twenty-two months did not raise the plasma vitamin A level except for a few hours after intake. When the dose was increased to 250,000 I.U. daily the plasma vitamin A level of a healthy adult was doubled in twelve days; however ten days after stopping the vitamin the plasma level had returned to normal.

The effect of vitamin A deprivation has been investigated in infants and adults. May et al. (1940) found that when infants were given an abundant supply of carotenoids and vitamin A followed by an extremely restricted intake, the level of vitamin A in the blood became lower but did not drop below normal even after a period of weeks. Lewis et al. (1941b)

gave a vitamin A free diet to infants aged six weeks to four months. After periods on the diet ranging from two to six weeks, only one of four infants tested had an abnormally low blood vitamin A level. However after periods on the diet ranging from two to four-and-a-half months, six of eight infants tested had abnormally low blood levels. The two infants who showed no marked fall had been given vitamin A supplements for one month before starting the diet. An experimental study of vitamin A deprivation in adult volunteers was conducted by the Medical Research Council in 1942. On a diet containing negligible amounts of vitamin A and less than 70 I.U. of alpha- and beta-carotene, eight months elapsed before the plasma vitamin A levels of the volunteers slowly began to decline.

These experimental studies show that in health the body tries to maintain a constant blood vitamin A level despite variations in the intake of the vitamin. Aron (1949) considers that this is due to the existence of a mechanism for controlling the level of vitamin A in the blood and that the liver plays an important part in this regulatory process. Vitamin A deprivation or over-dosage must be severe and prolonged to cause this mechanism to break down. It is only when the liver reserves approach exhaustion or the liver storage mechanism is temporarily overwhelmed that alterations in the blood vitamin A level occur.

There are, however, factors other than the intake and body stores of vitamin A which influence the amount of the vitamin in the blood. Large scale studies show that age, sex, and fever cause consistent alterations. During the first forty-eight hours of life the concentration of vitamin A in the blood falls rapidly but on the fourth day a sudden rise restores the concentration to the newborn level (Lewis et al., 1943). Children under sixteen years of age have lower values than adults of the same families (Aron, 1949). The effect of sex on the blood vitamin A level varies with age. Szymanski and Longwell (1951) showed that the plasma vitamin A levels of female infants between the ages of six months and fifteen months were higher than those of male infants of the same age. Between the ages of fifteen months and sixteen years, however, they found no difference between the sexes. In contrast to the results in infancy, Kimble (1939) found that in adult life males have higher plasma vitamin A levels than females. It must be emphasised that after the first forty-eight hours of life age and sex cause only small alterations in the blood vitamin A level. Therefore the range of values for either sex at any particular age overlap almost completely those at any other age.

In contrast to the slight effect of age and sex, fever, whether due to disease or artificially produced, causes a pronounced fall in the blood vitamin A level (Lindquist, 1938;

Clausen and McCoord, 1938). It is important, therefore, to know that fever is absent before attaching another significance to a low blood vitamin A level.

Evidence that the blood vitamin A level of humans does not always reflect the body stores of the vitamin was provided by a comparison of the plasma level with the vitamin A content of biopsy specimens of the liver (Popper et al., 1943a). It was found that when the plasma level was high the hepatic concentration was also high but when the plasma level was low the hepatic level might be high or low.

In certain animals vitamin A deficiency in the mother determines the development of congenital abnormalities in her offspring (Wilson and Warkany, 1950; Millen et al., 1953). As fever greatly reduces the concentration of vitamin A in the blood of humans, Bicknell (1950) suggested that whenever infections occur during pregnancy the foetus must be exposed to a sudden severe deficiency of vitamin A. He further suggested that it may be this deficiency and not the toxin of rubella which interferes with foetal development. However, this hypothesis is unlikely to be correct as the blood vitamin level of the newborn infant is independent of the mother's blood vitamin A level (Byrn and Eastman, 1943; Lund and Kimble, 1943). Moreover the cord blood vitamin A level of one identical twin may be more than twice that of the other

twin (Lund and Kimble, 1943).

Recently it has been shown that 10% to 20% of the vitamin A in the blood of healthy persons is in the form of the ester while the remainder is present as the alcohol (Hoch and Hoch, 1946; Popper et al., 1948). It appears that vitamin A ester is the form in which the vitamin is transported from the gut to the storage depots regardless of the form in which it has been ingested. On the other hand vitamin A alcohol is the form in which the vitamin is supplied to the blood from the liver. A vitamin A deficient diet produces a rapid fall in the alcohol fraction while the ester fraction may fluctuate up or down. It appears therefore, that vitamin A alcohol is the significant fraction in relation to vitamin A activity in the body.

(b) Vitamin A Tolerance Test.

In 1934, Chesney and McCoord reported that the rise in the blood vitamin A level following a standardised oral dose of haliver oil was less in children with coeliac disease than in healthy children. In this study the serum vitamin A level was estimated before and two, four, six, nine, twelve, and twenty four hours after the test dose of the vitamin. This procedure has since been used to study diseases other than coeliac disease and has been called by some workers the vitamin A absorption test and by others the vitamin A tolerance test.

Pratt and Fahey (1944) suggested that it was clinically adequate to determine the blood vitamin A level at the time of the expected maximum rise after the oral test dose of the vitamin. They showed that using 0·1 ml. percomorph liver oil per pound of body weight, the maximum rise in the blood vitamin A level generally occurred four hours after the test dose in infants under 6 months and five hours after the test dose in older children.

The height to which the blood vitamin A level rises following an oral dose of the vitamin must depend on the rate of absorption from the intestine on the one hand and the rate of storage or urinary excretion on the other. Urinary execretion of vitamin A does not occur in health but has been reported in certain pathological conditions.

In healthy persons the rate of absorption is affected by the size of the dose and the preparation of vitamin A administered. Increasing the dose of vitamin A increases the amount of vitamin A absorbed. It is necessary, therefore, to use a standardised dose of vitamin A for the tolerance test. In healthy children aqueous preparations of vitamin A give higher blood levels than equivalent doses of oily preparations Lewis et al., 1947; Kramer et al., 1947; Sobel et al., 1949) regardless of whether the aqueous preparations contain vitamin A as the ester or the alcohol (Kagan et al., 1950a). It has been

shown that this is due to the better absorption of aqueous preparations. In animal experiments more vitamin A is deposited in the liver and less excreted in the faeces after an oral dose of an aqueous preparation than after an equivalent dose of an oily preparation. The smaller faecal excretion has also been shown in children (Lewis et al., 1947). Frazer (1947) believes that fat is absorbed by two routes. Some is hydrolysed to fatty acids which are then absorbed into the portal vein and so reach the liver. The rest is finely emulsified into minute particles which pass into the intestinal cells in particulate form and thence to the thoracic duct and systemic circulation. Aqueous preparations of vitamin A are fine colloidal suspensions of oil containing the vitamin in an aqueous medium. The small size of the vitamin A containing particles in such preparations may permit a more rapid and complete absorption by the lymphatic route than occurs with the larger particles of the oily preparations which presumably have to be hydrolysed or emulsified before absorption. The low interfacial tension which would exist between the small particles of aqueous preparations and the intestinal wall would ease their penetration (Sobel et al., 1948). It is difficult, however, to reconcile this theory with the observations of Gribetz and Kanof (1951) who found that in a case of chylous ascites in the neonatal period an oral dose of percomorph oil

did not raise the serum vitamin A level while an oral dose of an aqueous preparation produced a satisfactory rise. This suggests that, although oily preparations are absorbed by the lymphatic route, aqueous preparations are absorbed into the portal vein. The cause of the better absorption of aqueous preparations remains therefore undecided, but there is evidence which suggests that they are absorbed by a different method from oily preparations.

Mendeloff (1954) found that, when vitamin A tolerance tests were done on healthy persons using vitamin A palmitate in corn oil, the act or thought of eating caused a rapid and marked rise in the concentration of vitamin A in the serum. He attributed this to the lacteals being activated in some way by eating to expel their contained vitamin A into the blood stream.

The amount of vitamin A in the body stores might affect the rate of storage of the vitamin. One would expect the rate of storage to be increased when the body stores were low and decreased when they were high. If this were so a small rise in the blood vitamin A level in the vitamin A tolerance test would occur when the body stores were low, even although the absorption of the vitamin was normal. Because of this possibility some workers have administered large doses of vitamin A for several days before the test (Barnes et al.,1950).

In normal individuals, however, vitamin A tolerance tests before and after massive doses of vitamin A show a similar rise in the blood vitamin A level (Popper et al., 1943b;Paterson and Wiggins, 1954). This indicates that with normal or high body stores the rate of storage is constant. It is still possible, however, that when the body stores are very low the rate of storage may be increased.

Sobel et al. (1949) have shown that the rise in the serum vitamin A level following an oily preparation of vitamin A is less in children under four months than in older children.

These factors which alter the rates of absorption and storage of vitamin A in healthy children must be taken into account when using the vitamin A tolerance test in the study of disease.

(c) PLASMA CAROTENOIDS

After Moore (1930) demonstrated that carotene is converted *in vivo* into vitamin A which is then stored in the liver, it was assumed that the conversion took place in the liver. However Sexton et al.(1946) showed that beta-carotene administered parenterally to vitamin A depleted rats caused carotene but not vitamin A to accumulate in the liver and did not relieve the symptoms of vitamin A deficiency. Glover et al. (1947b) demonstrated the presence of vitamin A in the small intestine of vitamin A depleted rats six hours after the oral administration

of a large dose of beta-carotene in oil, that is at a time when there was little vitamin A in the liver. These results have been confirmed by other workers and the accumulated evidence, which has been ably reviewed recently by Kon and Thomson (1951), leaves little doubt that the principal site of conversion of the carotenoids to vitamin A is not the liver but the intestinal wall. The mechanism of the transformation of the carotenoids into vitamin A would appear to be that the carotenoid precursors are oxidised into vitamin A aldehyde which is then reduced to vitamin A (Glover and Redfearn, 1954). The intestinal wall is not the exclusive site of this transformation as animal experiments have shown that, even when the small intestine is removed, certain preparations of carotene when parenterally administered are converted into vitamin A (Bieriand Pollard, 1954; Kon et al., 1955).

Nicholls and Nimalasuria (1941) found that phrynoderma in children responded to treatment with cod liver oil orally, but not to treatment with carotene orally. They suggested that carotene is poorly converted into vitamin A in the child's body. Further observations on the effect of carotene orally in vitamin A deficient children are necessary before this suggestion is finally accepted.

The carotenoids in the blood are the overflow into the blood stream of carotenoid precursors, which have failed to be

converted into vitamin A in the intestinal wall, together with inactive carotenoid pigments. Although the fate of the blood carotenoids is obscure, it is believed that most of the carotenoid precursors remain unconverted into vitamin A and of no value to the body.

In health the plasma carotenoid level varies with the carotenoid content of the diet. Clausen and McCoord (1938) showed that the plasma carotenoid level of children fell markedly within one week when pigmented vegetables, butter, eggs and cream were excluded from their diet. Again in an invest-:igation carried out by the Medical Research Council in 1942 the plasma carotenoid level of adult volunteers given a diet containing less than seventy international units of alpha- and beta- carotene fell within three months from an average value of one hundred and fifty international units per 100 ml. to a value varying from twelve to forty international units, little if any of which was due to carotenoid precursors of vitamin A. Seasonal variations in the concentration of the carotenoids in the blood reflect the higher dietary content of these pigments in the summer months. In children over four years of age, Szymanski and Longwell (1951) found that between June and November the plasma carotenoid levels were higher than between December and May. Very high carotenoid intakes raise the blood carotenoids to a high level; yellow colouration of the skin may

develop but it is doubtful if there are any other toxic effects. It is interesting as pointed out by Josephs(1944) that the body makes no attempt to control the blood level of carotene, which is virtually non-toxic, but does try to maintain a constant blood level of vitamin A which is potentially toxic.

A considerable proportion of ingested carotenoids are excreted unchanged in the faeces but the proportion varies with the dietary source. Wilson et al. (1937) showed that the proportion was increased by exclusion of fat from the diet.

The carotenoid content of the blood, like the vitamin A content, is influenced not only by the dietary intake but also by age, sex and fever. The carotenoid content of the cord blood of newborn infants is less than that of older children but is related to the mother's blood level (Lund and Kimble,1943). Szymanski and Longwell (1951) studied a group of ninety-five children and made repeated estimations on each child from infancy throughout childhood. They found that the carotenoid level remained low for the first three months of life but was much higher at six months of age. This increase was presumably due to the introduction of mixed feeding. However the rise continued to a peak at one year after which it fell until three years of age. Thereafter it remained almost constant until nine years when a further drop occurred. These authors also found that in infancy girls had higher levels than boys, while

at nine years the boys had the higher levels. Fever causes a marked decrease in the concentration of the carotenoids in the blood (Clausen and McCoord, 1938; May et al., 1940). It is clear then that the concentration of the carotenoids in the blood depends primarily on the dietary content of the carotenoids. When the intake is adequate, however, the concentration is influenced by age, sex and fever.

PRESENT INVESTIGATION

The plasma carotenoid and vitamin A levels of thirty healthy children were estimated. There were seventeen males and thirteen females and their ages ranged from seven weeks to ten years. Some of these children were in hospital for minor surgical procedures, some for medical conditions unrelated to vitamin A metabolism and the remainder had recovered from the disease which had necessitated their admission to hospital. None of them had received vitamin A concentrates for at least forty-eight hours before the date on which the estimations were made. For this reason the results have been called 'fasting' and this term is used, where applicable, throughout the remainder of this thesis.

A vitamin A tolerance test was done on fifteen of these children. After withdrawal of the blood sample for estimation of the fasting levels they were given a standardised oral dose of vitamin A. The preparation of vitamin A used was synthetic vitamin A palmitate in arachis oil (Avoleum, B.D.H.) and this was given in a dose of 12,500 I.U. per kilogram of body weight up to a maximum dose of 250,000 I.U.). Four hours after the dose of vitamin A a further sample of blood was taken for estimation of the plasma carotenoid and vitamin A levels. The patients received their ordinary meals throughout the test.

This method of performing the vitamin A tolerance test

has been used throughout the present study except that on a few occasions a different dose of the vitamin A preparation was used. Such variations from the standardised dose are indicated in the text. For convenience of reference the description of the vitamin A tolerance test is repeated in Appendix 2.

RESULTS

The results are shown in Table 1 (page 52) and in Appendix 3.

Fasting Plasma Vitamin A

The fasting plasma vitamin A levels ranged from 83 I.U. per 100 ml. to 200 I.U. per 100 ml. with a mean value of 135·4 I.U. per 100 ml. The mean of the levels of the four infants under one year of age was lower than that of the older children but there was no other significant variation with age or sex (Table 2, page 53). The mean of the values obtained for the children over one year of age investigated in the winter months is slightly higher than that for the children over one year of age investigated in the summer months (Table 3, page 53); the difference is not, however, statistically significant.

Vitamin A Tolerance Test.

The plasma vitamin A level four hours after the test dose of the vitamin varied widely from case to case, ranging from 183 I.U. per 100 ml. to 2068 I.U. per 100 ml. In four (cases 5, 8, 9 and 16) of the fifteen cases it was less than

450 I.U. per 100 ml. The mean of the plasma vitamin A levels four hours after the test dose of the vitamin is 716·4 I.U. per 100 ml.

Plasma Carotenoids

The plasma carotenoid level was unaltered by the test dose of vitamin A. For this reason the values shown in Table I and in subsequent tables for the children on whom a vitamin A tolerance test was done, are the average of the fasting plasma carotenoid level and the plasma carotenoid level four hours after the test dose of vitamin A. The four infants under one year of age, the oldest of whom was seven months, had much lower plasma carotenoid levels than the older children. The plasma carotenoid levels of the children over one year of age ranged from 43 µgm. per 100 ml. to 153 µgm. per 100 ml. with a mean value of 78·5 µgm. per 100 ml. The mean of the values obtained for the girls over one year of age is slightly higher than that for the boys over one year of age (Table 2 page 53) but the difference is not statistically significant. The boys over five years of age had higher levels than the boys under that age (Table 2) but the number of boys on which this observation is based is small. The mean of the values for the children over one year of age investigated in the winter months is similar to that for the children over one year of age investigated in the summer months, (Table 3, page 53).

DISCUSSION

The range of the values and the mean value for the plasma vitamin A in the present investigation are similar to those obtained by other workers (Table 4, page 54). On the other hand, the range of values and the mean value for the plasma carotenoids in the present investigation are much lower than those reported by Szymanski and Longwell (1951) for children in the United States of America (Table 4). This compares with the finding of Moore and Leitner (1949) that the plasma carotenoid levels of adults living in London were lower than the plasma carotenoid levels reported for adults in the United States of America. The low plasma carotenoid levels of the four infants under one year of age were no doubt due to the low carotenoid content of their diet. The higher levels of the boys over five years of age compared with boys under that age are also probably best explained on the basis of the carotenoid content of their diet.

The slightly higher plasma vitamin A levels of the children investigated in the winter months may have been due to their receiving vitamin A concentrates during these months.

The rise in the plasma vitamin A level in the vitamin A tolerance tests bore no relation to the fasting plasma vitamin A level. Moreover, previous work has shown that in healthy persons increasing the body stores of vitamin A does not affect

the rise in the plasma vitamin A level in the tolerance test. It seems probable, therefore, that the variation in rise from case to case was due to differing rates of absorption rather than differing rates of storage. Mendeloff (1954) has shown that when vitamin A tolerance tests are done using synthetic vitamin A palmitate in corn oil, flat curves in healthy subjects can be avoided by giving a meal two hours after the test dose of the vitamin. The small rise in the plasma vitamin A level in four of the tolerance tests (cases 5, 8, 9 and 16) is noteworthy, therefore, as all the children had a meal during the test, although the timing of the meal was not controlled. As judged by the mean of the plasma vitamin A levels four hours after the test dose of the vitamin, a normal rise occurred in the plasma vitamin A level of the two infants aged three months and four months. This contrasts with the low rise observed by Sobel et al. (1949) in infants of this age.

SUMMARY

(1) The literature relating to the factors affecting the plasma carotenoid and vitamin A levels and the vitamin A tolerance test in healthy children is reviewed.

(2) The fasting plasma vitamin A levels of thirty healthy children were estimated. The levels ranged from 83 I.U. per 100 ml. to 200 I.U. per 100 ml. with a mean value of 135·4 I.U. per 100 ml. Of the children over one year of age, those

investigated in the winter months i.e. October to March had slightly higher levels than those investigated in the summer months, i.e. April to September. It is suggested that this difference, although not statistically significant, may have been due to the children receiving vitamin A concentrates in the winter months. The infants under one year of age had slightly lower levels than the older children but there was no other significant variation with age or sex.

(3) Vitamin A tolerance tests were done on fifteen healthy children using a standardised oral dose of an oily preparation of vitamin A palmitate. The rise in the plasma vitamin A level varied widely from case to case. It is suggested that this was due to varying rates of absorption of the vitamin preparation.

(4) The plasma carotenoid levels of twenty-six healthy children over one year of age were estimated. The levels ranged from 43 µgm per 100 ml. to 153 µgm. per 100 ml. with a mean value of 78·5 µgm. per 100 ml. The girls had slightly higher levels than the boys but the difference is not statistically significant. The boys over five years of age had slightly higher levels than the boys under that age. The plasma carotenoid levels of four healthy infants under one year of age were estimated. In each case the level was below 20 µgm per 100 ml.

TABLE 1.

PLASMA CAROTENOID AND VITAMIN A LEVELS OF HEALTHY CHILDREN.

Case No.	Age	Sex	Plasma Carotenoids µgm. per 100 ml.	Plasma Vitamin A I.U. per 100 ml.	
				Fasting.	4 hrs. after test dose of Vitamin A.
1	7 wks.	M.	17	104	-
2	3 mos.	M.	16	154	637
3	4 mos.	M.	20	125	808
4	7 mos.	F.	19	125	531
5	1yr. 3mos.	M.	53	117	183
6	1 yr. 6mos.	F.	58	164	487
7	1 yr. 7mos.	M.	67	112	737
8	1 yr. 7mos.	M.	72	110	316
9	2 yr.	M.	43	175	192
10	3 yr.	M.	64	196	514
11	3 yr.	F.	94	171	-
12	3 yr.	M.	59	160	-
13	4 yr.	M.	128	164	1367
14	4 yr.	M.	45	94	1256
15	5 yr.	F.	99	100	991
16	5 yr.	M.	53	121	192
17	6 yr.	M.	79	139	-
18	6 yr.	F.	55	104	-
19	7 yr.	F.	153	200	-
20	8 yr.	F.	133	162	-
21	8 yr.	F.	64	141	-
22	8 yr.	F.	59	83	-
23	8 yr.	M.	83	154	467
24	9 yr.	M.	143	129	-
25	9 yr.	F.	66	150	-
26	10 yr.	F.	92	102	-
27	10 yr.	M.	56	106	-
28	10 yr.	F.	102	139	-
29	10 yr.	F.	76	116	-
30	10 yr.	M.	45	144	2068

TABLE 2.

PLASMA CAROTENOID AND VITAMIN A LEVELS OF HEALTHY CHILDREN RELATED TO AGE AND SEX.

Age Group	No. of Cases		Mean Plasma Carotenoid Level µgm. per 100 ml.		Mean Plasma Vitamin A Level I.U. per 100 ml.	
	Male	Female	Male	Female	Male	Female
Under 1 yr.	3	1	18	19	128	125
1 - 5 yrs.	9	3	65	84	139	145
6 -10 yrs.	5	9	81	89	134	133

TABLE 3.

PLASMA CAROTENOID AND VITAMIN A LEVELS OF HEALTHY CHILDREN OVER ONE YEAR OF AGE RELATED TO THE SEASON OF THE YEAR IN WHICH THE ESTIMATIONS WERE DONE.

Month in which Estimations were made.	No. of Cases.	Mean Plasma Carotenoid Level µgm. per 100 ml.	Mean Plasma Vitamin A Level I.U. per 100 ml.
April to September	11	74·9	128·1
October to March	15	81·1	142·9

TABLE 4.

VITAMIN A AND CAROTENOID CONTENT OF THE SERUM OR PLASMA OF HEALTHY SUBJECTS.

Observers	Date	Age of Subjects	Vitamin A per 100 ml. serum or plasma		Carotenoids per 100 ml. serum or plasma.	
			Mean	Range	Mean	Range
Moore and Leitner	1949	Adults	121 I.U.	-	91·8 µgm.	-
Lewis et al.	1941	3 wks. to 18mos.	-	45-141 U.S.P.U.	-	-
		6 yrs. to 12 yrs.	-	70-197 U.S.P.U	-	-
Sobel et al.	1949	14mos. to 12 yrs.	132 U.S.P.U. (37µgm.)	-	-	-
Krause and Pierce	1947	School Children	38 µgm.	-	116 µgm.	-
Szymanski and Longwell	1951	2 mos. to 16 yrs.	-	36-56* µgm.	-	42-328* µgm.
Present Series	1957	7 wks. to 10yrs.	135·4 I.U. (46µgm.)	83-200 I.U. (282-68 µgm.)	78·5 + µgm.	16-153 µgm.

* These are 'Median' values, that is 50 per cent of the children had levels within or below this range.

+This mean value relates to the children over 1 year of age.

VITAMIN A STUDIES

in

FIBROCYSTIC DISEASE OF THE PANCREAS

REVIEW OF THE LITERATURE.

Children with fibrocystic disease of the pancreas suffer from chronic ill-health and are frequently confined to bed by the recurrent respiratory infections which characterise the disease. No cure is known and survival beyond puberty is unusual. In the Royal Hospital for Sick Children, Glasgow, fibrocystic disease of the pancreas was found in 5·1% of a thousand consecutive autopsies performed between 1950 and 1954. Because of its importance as a cause of invalidism and death in childhood this disease has been intensively investigated in recent years.

Widespread recognition of the pancreatic changes in this disease dates from a clinical and pathological study of thirteen vitamin A deficient children by Blackfan and Wolbach in 1933. Post-mortem examinations in eleven of these children showed that six of them had identical extensive pancreatic lesions. Blackfan and Wolbach appreciated that the pancreatic changes were not due to vitamin A deficiency as the pancreatic lesion and vitamin A deficiency frequently occurred independently. They suggested, on the contrary, that the pancreatic lesion, if extensive, might cause defective absorption of fat and hence of vitamin A. Deficiency of vitamin A would then occur despite an adequate intake of the vitamin.

The next major contribution to our knowledge of this disease

was made by Dorothy Andersen in 1938. She described the clinical features and pathological findings of forty-nine children who at post-mortem had pancreatic fibrosis. Clinically these cases were divisible into three groups. Five infants died before the age of one week due to intestinal obstruction from inspissated meconium. Nineteen infants died between the ages of one week and six months from respiratory infections and nutritional disturbance. The remaining twenty-five children presented with the coeliac syndrome and died between the ages of six months and fourteen years and six months of a respiratory infection. Two infants in the second group and one child in the third group had xerophthalmia, and at post-mortem ten of the forty-four patients in these two groups showed keratinising metaplasia of the epithelium of various organs which was interpreted as evidence of vitamin A deficiency. Dorothy Andersen suggested that vitamin A deficiency might be a factor in causing the pulmonary lesions.

Blackfan and May (1938) were the first to estimate the concentration of vitamin A in the blood in fibrocystic disease of the pancreas. They found low blood vitamin A levels in two children with this disease, and observed that a daily dose of oleum percomorphum gradually restored the levels to normal. When vitamin A absorption tests were done on these two children using oleum percomorphum the rise in the blood

vitamin A level was less than in healthy children. Blackfan and May concluded that although vitamin A could be absorbed the efficiency of absorption was probably diminished. Subsequent workers have confirmed that children with fibrocystic disease of the pancreas have low blood vitamin A levels and that following a standardised dose of an oily preparation of vitamin A ester a smaller rise occurs in the blood vitamin A level of these children than of healthy children (May et al., 1940; May and McCreary, 1941; McCoord et al., 1948; Danielson et al., 1949). May et al. (1940) also confirmed that adequate daily doses of percomorph liver oil restored the blood vitamin A level of a child with fibrocystic disease of the pancreas to normal. May and McCreary (1941) found low plasma carotenoid levels in this disease.

The poor absorption of oily preparations of vitamin A ester has led to the investigation of means of improving vitamin A absorption in fibrocystic disease of the pancreas. When pancreatin is given along with an oily preparation of vitamin A the rise in the blood vitamin A level is much greater than when the oily preparation of vitamin A is given alone (Clausen et al., 1946; McCoord et al., 1948; May and Lowe, 1948; Gibbs, 1950). Gibbs (1950) showed that pancreatin did not have this effect in patients with coeliac disease or in healthy individuals and suggested that its use might be of value

in differential diagnosis.

The absorption of preparations of vitamin A other than vitamin A ester has been investigated. An oral dose of vitamin A alcohol raises the blood vitamin A level of children with fibrocystic disease of the pancreas much more than an equivalent dose of vitamin A ester (Clausen et al., 1946; McCoord et al., 1948). Animal experiments have shown that during the absorption of vitamin A ester it is hydrolysed in the intestine to vitamin A alcohol (Gray et al., 1940; Eden and Sellers, 1950). The absence of pancreatic lipase from the intestine of children with fibrocystic disease of the pancreas will prevent or seriously retard such hydrolysis and this is believed to explain the preferential absorption of the alcohol over the ester form of vitamin A in this disease. Sobel (1952), however, considers that in fibrocystic disease of the pancreas vitamin A alcohol is better absorbed than vitamin A ester only when dissolved in the unsaponified fraction of fish liver oil which contains no neutral fat. He states that when vitamin A alcohol is dissolved in an oil which is an ester, like maize oil, the vitamin A alcohol is not preferentially absorbed. On the other hand Katsampes et al. (1953) found that in equivalent vitamin A dosage solutions of vitamin A alcohol and vitamin A aldehyde in Wesson oil, which is itself an ester, were much better absorbed by children with fibrocystic disease of the pancreas

than solutions of vitamin A acetate in Wesson oil. These workers also observed that in fibrocystic disease of the pancreas ingested vitamin A alcohol appears in the blood as the ester, which indicates that the enzyme that causes the esterification is not pancreatic lipase.

The absorption of emulsions of vitamin A ester and of aqueous preparations of vitamin A ester has also been studied. Emulsions of vitamin A ester in Tween 20 (May and Lowe, 1948) or on dextrimaltose (Danielson et al., 1949) produce a normal rise in the blood vitamin A level of patients with fibrocystic disease of the pancreas. The work of Lewis et al. (1950) suggests that this is due to a reduction in the particle size of the oil containing the vitamin A. They found that in a child with fibrocystic disease of the pancreas, using equivalent doses of vitamin ester, an aqueous preparation with submicroscopic particles of oil containing vitamin A brought about the highest rise in vitamin A concentration in the blood; an emulsion of vitamin A with particle size of the oil varying from 1 to 20 μ resulted in a smaller rise; and an oil whose particles when mixed with water were visible to the naked eye brought about no rise. It appears then that when the particle size of the oil, containing the vitamin A ester, is very small lipolysis is not a necessary preliminary to absorption.

The purpose of the present investigation was to assess the

significance of the plasma carotenoid and vitamin A levels in this disease and to determine the effect of treatment on these levels; also to study the absorption of an oily preparation of vitamin A ester.

PRESENT INVESTIGATION

Eleven children with fibrocystic disease of the pancreas were studied. The clinical diagnosis was confirmed either by demonstrating that a specimen of duodenal juice obtained by duodenal intubation had no proteolytic activity (Andersen and Early, 1942) or by finding an abnormally high concentration of sodium and chloride in the sweat (Di Sant 'Agnese et al.,1953). The stool proteolytic activity of nine of these children was tested (Emery, 1952) and in none was any activity present. These confirmatory tests were done by the author on all but two of the cases (Cases 1 and 3). The results of these tests are shown in Table 5 (page 70). Two of the children (cases 9 and 10) died and in both the diagnosis was confirmed at autopsy.

Four of the children (Cases 1 - 4) were investigated before any treatment had been given. The other seven children (Cases 5-11) had been treated for periods varying from one day to ten months with pancreatin before feeds and a daily dose of an aqueous multivitamin preparation (Abidec) which provided approximately 7,500 I.U. vitamin A palmitate. Treatment was stopped for two days in case 5 and six days in case 6 before the

date of the investigation. In cases 7 to 11 inclusive treatment was continued up to and including the day of the investigation.

The fasting plasma carotenoid and vitamin A levels were estimated and vitamin A tolerance tests were done on cases 1 to 6 inclusive. The plasma carotenoid and vitamin A levels of case 2 were re-estimated (2a) after treatment for sixteen days with a daily dose of 25,000 I.U. synthetic vitamin A palmitate in arachis oil (Avoleum B. D. H). The plasma carotenoid and vitamin A levels were estimated and vitamin A tolerance tests were done on cases 7 to 11 inclusive while they were on treatment. In case 7 these investigations were repeated after treatment had been withdrawn for forty-eight hours (7a).

For comparative purposes five infants who were marasmic from causes other than fibrocystic disease were also investigated. The fasting plasma carotenoid and vitamin A levels of these infants were estimated and in each case a vitamin A tolerance test was done.

All the children were afebrile when investigated.

RESULTS

A. Fibrocystic Disease of the Pancreas.

The results are shown in Table 6 (page 71) and Appendix 4.

Plasma Vitamin A.

The four cases (cases 1 to 4) studied before any treatment had been given had very low plasma vitamin A levels. A daily dose of 25,000 I.U. synthetic vitamin A palmitate in arachis oil for sixteen days raised the concentration of vitamin A in the plasma of case 2 to a level just below the range found in healthy children (2a). Of the patients studied after treatment had been instituted four (cases 6, 7, 8, and 10) had plasma vitamin A levels within the range found in healthy children, two (cases 9 and 11) had plasma vitamin A levels just below this range, and one (case 5) had a plasma vitamin A level just above this range.

Vitamin A Tolerance Test

Four hours after the test dose of vitamin A the concentration of vitamin A in the plasma of cases 1, 2, 3, 6, 9, 10 and 11 was only slightly above the pre-test level, while that of case 7 in both tests and that of case 8 was slightly below the pre-test level. In case 5 there was a marked rise in the plasma vitamin A level four hours after the test dose of the vitamin.

Plasma Carotenoids.

All the children had low plasma carotenoid levels.

B. Infants Marasmic from Diseases other than Fibrocystic Disease of the Pancreas.

The results are shown in Table 7 (page 72) and Appendix 5.

Plasma Vitamin A.

Three infants had plasma vitamin A levels within the range found in healthy children, while two infants had levels below this range.

Vitamin A Tolerance Test.

Four hours after the test dose of vitamin A the concentration of vitamin A in the plasma in cases 2 and 3 was considerably above the pre-test level, while that of case I was only slightly above, and that of cases 4 and 5 was below the pre-test level.

Plasma Carotenoids.

The plasma carotenoids were low in all five cases, but such low values are normal for the ages of the infants.

DISCUSSION

In fibrocystic disease of the pancreas the body stores of vitamin A are frequently depleted (Baar, 1953) and this is reflected in the low blood vitamin A levels of children with this disease before treatment has been instituted. It is interesting that the patient (case 2) in the present series who at the age of eight years had a plasma vitamin A level of 22 I.U. per 100 ml., and who presumably had been deficient in vitamin A all her life, showed no evidence of xerophthalmia. During the German occupation of Holland in the Second World War Jonxis (1946) never observed keratomalacia "although there were children who did not get any vitamin A for months and had no vitamin A in their blood". The possibility must be considered therefore that some unknown factor determines the appearance of the eye lesions in vitamin A deficient children.

The children with fibrocystic disease of the pancreas who had been treated with vitamin A supplements had normal or near normal plasma vitamin A levels. This was so even after treatment had been withdrawn for two days in two cases (cases 5 and 7) and six days in another (case 6) showing that it was not due to the post-absorptive rise after an oral dose of the vitamin A supplement.

Although vitamin A supplements restored the blood vitamin A level to normal or near normal, recurrent pulmonary infections

were not thereby prevented. Moreover the patient aged eight years who had a plasma vitamin A level of 22 I.U. per 100 ml. had minimal pulmonary involvement. The pulmonary infections would appear therefore to be unrelated to vitamin A deficiency. This conclusion is at variance with the opinion of some workers (Andersen, 1938; Baar, 1953) but in agreement with others (Shwachman et al., 1955).

In most cases of fibrocystic disease of the pancreas a large oral dose of an oily preparation of vitamin A ester causes only a small rise in the plasma vitamin A level. A review of the literature has revealed convincing evidence that this is due to the absence of pancreatic lipase from the intestine causing defective absorption of such preparations of vitamin A. On theoretical grounds, however, rapid removal of vitamin A from the blood stream might be responsible, at least in part, for the low rise in the concentration of vitamin A in the blood following a standardised oral dose of an oily preparation of vitamin A ester. That this is not so is shown by the results of the vitamin A tolerance tests on the children who had been treated for varying periods with an aqueous preparation of vitamin A palmitate. The body vitamin A stores of these children had been at least partially replenished as shown by their normal plasma vitamin A levels, yet except in case 5 the test dose of vitamin A palmitate caused at the most a small rise

in the plasma vitamin A level. The marked rise in the plasma vitamin A level in case 5 must indicate that in this case pancreatic lipase was present in the intestine although the duodenal juice showed no proteolytic activity. Such dissociation of enzyme activity in fibrocystic disease of the pancreas with normal absorption of vitamin A esters has been previously reported by Shwachman (1955). When the diagnosis of fibrocystic disease of the pancreas has been established, therefore, the vitamin A tolerance test, using an oily preparation of vitamin A ester, provides indirect evidence of the presence or absence of pancreatic lipase.

In fibrocystic disease of the pancreas the administration of pancreatin along with an oily preparation of vitamin A has been shown to improve the absorption of the vitamin. In the first test on case 7 forty-five grains of pancreatin were given thirty minutes before the test dose of vitamin A palmitate in arachis oil. However, instead of the pancreatin producing a normal rise in the plasma vitamin A level, the level fell four hours after the test dose. The cause of this unexpected result is obscure but may lie in the preparation of pancreatin used being deficient in lipolytic activity.

Although in the absence of pancreatic lipase absorption of oily preparations of vitamin A ester is defective, a small proportion of the ingested dose is absorbed. This is shown by

the significant rise in the plasma vitamin A level of one child (case 2) following large doses of synthetic vitamin A palmitate in arachis oil for sixteen days.

All the children with fibrocystic disease of the pancreas had low plasma carotenoid levels. Such low levels are normal before the introduction of mixed feeding but thereafter are presumably due to defective fat absorption in the absence of pancreatic lipase from the intestine. Two of the infants with fibrocystic disease of the pancreas (cases 6 and 11) had been having pancreatin therapy for several months. The low plasma carotenoid levels of these two infants suggests that pancreatin therapy had failed to improve materially their fat absorption.

In marasmus due to diseases other than fibrocystic disease of the pancreas the fasting plasma vitamin A level may be low, and only a small rise in the plasma vitamin A level may occur in the vitamin A tolerance test when an oily preparation of vitamin A ester is used (McCoord et al., 1948; Present Investigation). For this reason, the fasting plasma vitamin A level and the vitamin A tolerance test, in the form used in the present study, are of limited value in confirming a diagnosis of fibrocystic disease of the pancreas in young infants. Also the plasma carotenoid level is of no diagnostic value in infancy as the plasma carotenoid levels of healthy infants are low before the introduction of mixed feeding. In older infants and children

however the association of a very low fasting plasma vitamin A level, a low plasma carotenoid level and a low rise in the plasma vitamin A level in the vitamin A tolerance test would provide biochemical support for a clinical diagnosis of fibrocystic disease of the pancreas. Such findings would also indicate that, if the clinical diagnosis is correct, pancreatic lipase is absent from the intestine.

SUMMARY.

1. The literature relevant to a study of carotenoid and vitamin A metabolism in fibrocystic disease of the pancreas has been reviewed.

2. An investigation of eleven patients with fibrocystic disease of the pancreas showed that in this disease:

(a) Before treatment the fasting plasma vitamin A level was usually very low but that after treatment with an aqueous or oily preparation of vitamin A ester normal or near normal levels were found. The eye lesions of vitamin A deficiency did not occur despite the very low fasting plasma vitamin A levels. The occurrence of pulmonary infections was unrelated to the plasma vitamin A level.

(b) A small rise in the vitamin A level occurred in the vitamin A tolerance test even when after treatment the fasting plasma vitamin A level was normal. It is suggested that defective absorption is the sole cause of the abnormal vitamin A tolerance test.

(c) The plasma carotenoid levels were low.

3. The low plasma carotenoid and vitamin A levels and the abnormal vitamin A tolerance test are considered to be due to the absence of pancreatic lipase from the intestine. As evidence of absent pancreatic lipase these findings would support a clinical diagnosis of fibrocystic disease of the pancreas. Similar findings occurred, however, in young infants marasmic from causes other than fibrocystic disease of the pancreas.

4. One of the infants investigated had a high fasting plasma vitamin A level and a marked rise in the plasma vitamin A level in the vitamin A tolerance test. It was concluded that as occasionally happens in fibrocystic disease of the pancreas pancreatic lipase was being secreted normally in this case.

5. Pancreatin failed to improve the absorption of vitamin A ester in one patient. Two other patients had low plasma carotenoid levels despite treatment with pancreatin for several months.

TABLE 5.

Results of the Biochemical Tests used to confirm the clinical diagnoses of fibrocystic disease of the pancreas.

Case No.	Proteolytic Activity of the Duodenal Juice	Proteolytic Activity of the Stool.	Sweat Electrolytes m.eq./L	
			Na	Cl
1	Nil	Nil		
2	Nil	Nil	119.2	120.0
3	Nil	Not done	109.0	136.0
4	Nil	Nil	59.8	85.2
5	Nil	Nil	112.9	145.1
6	Nil	Nil		
7	Not done	Nil	125.3	155.6
8	Nil	Nil	108.5	178.7
9	Nil	Nil		
10	Nil	Nil		
11	Nil	Not done		

TABLE 6.

Plasma Carotenoid and Vitamin A Levels in Fibrocystic Disease of the Pancreas.

Case No.	Age	Wt. Kg.	Duration of Treatment	Plasma Carotenoids µgm. per 100 ml.	Plasma Vitamin A I.U. per 100 ml.	
					Pre-test dose of Vitamin A	4 hrs. after test dose of Vitamin A
1	7 mos.	4·6	Nil	3	14	62
2	8 yr.	18·2	Nil	10	20	27
2a	8 yr.	18·2	16 days	23	69	-
3	9 mos.	8·0	Nil	3	16	23
4	4 mos.	3·2	Nil	6	31	-
5	4 mos.	3·8	12 days	7	218	1585
6	10 mos.	8·9	8 mos.	6	104	156
7	1yr.7mos.	7·9	1 day	12	116	96
7a	1yr.7mos.	7·9	1 day	13	110	106
8	5 mos.	4·5	11 days	8	175	164
9	4 mos.	4·1	19 days	2	75	192
10	4 mos.	3·1	20 days	7	142	371
11	1yr.10mos.	11·9	10 mos.	12	71	304

TABLE 7

Plasma Carotenoid and Vitamin A levels of Infants marasmic from causes other than fibrocystic disease of the Pancreas.

Case No.	Age Mos.	Disease	Plasma Carotenoids µgm/100 ml.	Plasma Vitiman A I.U. per 100 m.l.	
				Pre-test Level	Level 4 hrs. after test dose of Vitamin A.
1	1	Gastro-enteritis	5	100	154
2	2	Gastro-enteritis	20	50	412
3	3	Bronchitis	14	153	758
4	4	Mongol	10	4	Nil
5	4	Marasmus	12	118	102

VITAMIN A STUDIES

in

COELIAC DISEASE

REVIEW OF THE LITERATURE

The earliest studies of vitamin A metabolism in coeliac disease were those of Chesney and McCoord in 1934 when, as noted earlier in this thesis, they employed a test which has since become known as the vitamin A absorption test or the vitamin A tolerance test. They estimated the serum vitamin A level of healthy children and of two children with coeliac disease before and 2, 4, 6, 9, 12 and 24 hours after a standard-:ised oral dose of an oily preparation of vitamin A (Haliver oil). The rise in the blood vitamin A level was much smaller in the children with coeliac disease than in the healthy children. This finding has been confirmed by subsequent workers (Clausen and McCoord, 1938; Breese and McCoord, 1939; May et al., 1940; Andersen and di Sant 'Agnese, 1953). Chesney and McCoord (1934) considered that this low rise was probably due to faulty absorption of vitamin A although they appreciated that a more rapid withdrawal from the blood stream or a re-excretion into the intestine might play a part.

One of the cardinal features of coeliac disease is steatorrhoea. Weijers and Van de Kamer (1953) have shown that under certain circumstances the increased faecal fat is entirely derived from non-dietary sources. There is no doubt, however,

that unabsorbed dietary fat usually contributes to the increase of faecal fat as Macrae and Morris (1931) have shown that increasing the fat intake increases the amount of fat in the faeces. The source of non-dietary fat in the faeces has not been determined. Frazer (1955) has pointed out that it may be fat excreted either into the duodenum in the bile, or into the lower intestine, or else it may be fat synthesised in the intestinal cells, in bacteria, and in other intestinal organisms. He considers that "Steatorrhoea is likely to be closely associated with faulty absorption with regard to increase of both dietary and non-dietary components in the faeces. The only other situation that might result in similar changes is excretion of food materials into the lower part of the bowel, for which there is no satisfactory evidence at the present time."

It has been generally assumed that oily preparations of vitamin A are absorbed in the same way as other fats and that their absorption parallels fat absorption. Legerton, Texter and Ruffin (1953) carried out fat balance studies and vitamin A tolerance tests on twenty-seven patients including normal subjects and patients with various gastro-intestinal diseases (eight patients with sprue, four with chronic pancreatitis, one with Whipple's disease, three who had gastric resection for ulcer, and three who had a resection of the small bowel for regional enteritis). They showed that in these diseases there was a fairly regular

relationship between the degree of fat absorption and the height of the plasma vitamin A level five hours after a standardised oral dose of vitamin A in an oily medium. Despite the assumption that the same relationship holds in coeliac disease (May et al., 1942), considerable evidence has accumulated to show that this is not so. Weijers and Van de Kamer (1953) compared the results of fat balance studies with the progression of the vitamin A tolerance curve in patients with coeliac disease. They showed that a rise in the serum vitamin A level of at least 100 I.U. per 100 ml. signified good fat absorption, but otherwise no conclusion could be drawn from the rise in the serum vitamin A level as to the severity of the fat absorption defect.

Follow-up studies in coeliac disease have shown that an abnormal vitamin A tolerance curve frequently persists after clinical improvement has occurred and even after the fat content of the stool has returned to normal. These follow-up studies relate to patients treated before Dicke (1950) demonstrated the beneficial effect of excluding wheat gluten from the diet of children with coeliac disease. Breese and McCoord (1939) studied ten children with coeliac disease before and after treatment with a high protein, low fat and low carbohydrate diet. After treatment, the blood vitamin A levels following a standard-:ised oral dose of fish liver oil were still markedly less than the controls. May and McCreary (1941) studied twenty-four

patients with coeliac disease, showing considerable improvement after treatment with a high protein, low fat and low carbohydrate diet for periods ranging from six weeks to a few years. Following a standardised oral dose of percomorph liver oil eleven patients had a normal rise of blood vitamin A, one after only three weeks treatment, while thirteen still showed a low rise, one after six years treatment. Di Sant 'Agnese (1953) reviewed twenty-three patients three to eleven years after their original admission with coeliac disease to the Babies Hospital, New York City. At the time of the study they were aged five to thirteen years and all of them were leading normal lives. Thirteen still had abnormal stools with occasional episodes of diarrhoea and pain. The glucose tolerance test was normal in all but four. Fourteen of eighteen tested had a small rise in the serum vitamin A level after a test dose of oleum percomorphum. Katsampes et al. (1955) studied twenty patients with coeliac disease aged seven to thirty-five years, who had been diagnosed six to twenty-five years previously. Twelve still had symptoms of the disease and seventeen had flat vitamin A ester absorption curves. In 1939, Hardwick reviewed the progress of seventy-three children who had been diagnosed as having coeliac disease in the Hospital for Sick Children, London, between 1923 and 1938. Twenty-two had died in hospital and four more in the intervening period. Thirty-seven patients were traced and of these

twenty-seven were in good health, six still had active disease and four had relapsed after improvement. In 1956, Lindsay et al. re-assessed twenty-five of the thirty-seven patients previously investigated by Hardwick. Vitamin A absorption tests were done on thirteen and ten showed a five hour vitamin A level below the lower limit of normal. Fat balances were done on nine of these thirteen patients. In only one was it definitely abnormal. Fat absorption exceeded 90% of the intake in the remaining eight, and in five of these it exceeded 95%. Four of the five whose fat absorption exceeded 95% had a small rise in blood vitamin A level in the vitamin A absorption test.

On the assumption that the rise in the plasma vitamin A level following a standardised oral dose of an oily preparation of vitamin A is a reliable index of fat absorption in coeliac disease May et al. (1942) carried out a series of interesting experiments to find out which of the factors then known to be concerned with fat absorption was responsible for the deficient absorption of vitamin A. They measured the rise in the blood vitamin A level of a child with coeliac disease following the introduction of a standard dose of an emulsified form of vitamin A into the duodenum. In the first experiment active intestinal peristalsis was induced by administering mecholyl. In the next experiment bile salts were given along with the vitamin A: olive oil and milk were also given in case they might be needed as a

vehicle for the transportation of vitamin A across the intestinal mucosa, and active gastro-intestinal peristalsis was again induced by administering mecholyl. In the final experiment the conditions were the same as in the second experiment but pancreatin was added to the test meal. In none of these experiments was there a greater rise in the blood vitamin A level than occurred in the vitamin A tolerance test. It was concluded that, as any fault in gastro-intestinal motility, emulsification and bile salts which could contribute to defective absorption of fat in coeliac disease had been eliminated, there must be "some defect in the ability of the intestinal mucosa to absorb vitamin A." It is interesting that these workers found that the absorption of vitamin A was improved within a period of three to six weeks by treatment with intra-muscular injections of crude liver extract and the B complex of vitamins. In 1950, Gibbs confirmed that the administration of pancreatin along with an oily preparation of vitamin A does not result in a normal rise in the blood vitamin A level in children with coeliac disease. This is not surprising as lipase is present in normal amounts in the duodenal juice of children with coeliac disease.

Further evidence that intraluminal factors are not responsible for the defective absorption of vitamin A in coeliac disease has been provided by the use of various preparations of vitamin A in the vitamin A tolerance test. McCoord et al. (1948) carried out

vitamin A tolerance tests on children with coeliac disease, using an oily preparation of vitamin A ester for some of the tests and an oily preparation of vitamin A alcohol for others. With both preparations flat tolerance curves were obtained. Danielson et al. (1949) showed that when vitamin A alcohol in 70% propylene glycol, vitamin A in a water paste emulsion or vitamin A alcohol or vitamin A acetate dispersed on dextri-maltose was given to patients with coeliac disease the rise in the blood vitamin A level was only slightly greater than that obtained with percomorph oil. In all instances the curves remained low and failed to reach the lower limit of a normal response. There was no consistent difference between the response to vitamin A alcohol and vitamin A acetate. Katsampes et al. (1953) investigated six children with coeliac disease and found that solutions in Wesson oil of vitamin A acetate, vitamin A aldehyde and vitamin A alcohol all caused a smaller rise than normal in the blood vitamin A level. Aqueous preparations of vitamin A give higher concentrations of vitamin A in the blood than oily products in children with coeliac disease, but the concentration is less than that produced by aqueous preparations of vitamin A in healthy children. (Kramer et al., 1947). These results obtained using various preparations of vitamin A in the tolerance test support the conclusions reached by May et al. (1942) that the low rise in the blood vitamin A is neither due to faulty emulsification

nor to a deficiency of lipase in the intestine. They also show that if there is defective absorption of vitamin A in coeliac disease it persists when the particle size of the vitamin A containing vehicle is reduced.

If the abnormal vitamin A tolerance test in coeliac disease were not due to defective absorption of the vitamin, then either excretion of vitamin A into the intestine or by the kidney, or a more rapid withdrawal of vitamin A from the blood would have to be responsible. The blood vitamin A level of children with coeliac disease frequently remains slightly above the fasting level for twelve hours after a dose of an oily preparation of the vitamin. This suggests that the abnormal vitamin A tolerance curve is not due to any mechanism lowering the blood vitamin A level but rather to defective absorption causing a small rise over a prolonged period.

It is interesting that, despite the small rise in the blood vitamin A level following a test dose of the vitamin, the majority of patients with coeliac disease have normal fasting blood vitamin A levels. Low fasting plasma vitamin A levels were found by Clausen and McCoord (1938) in nine of twelve children with untreated coeliac disease, but in the following year Breese and McCoord (1939) showed that, although the fasting plasma vitamin A levels of ten children with untreated coeliac disease were lower than the controls, the difference was not

statistically significant. This statistical conclusion has been supported by the results of two more recent investigations. May and McCreary (1941) obtained normal values in twenty-three children with coeliac disease and Andersen and di Sant 'Agnese (1953) normal or high values in thirty-five out of forty-two patients investigated. The latter workers however suggest that their findings may have been due to the prior administration of vitamin A.

In the follow-up study carried out by Lindsay et al. (1956) seven of the fourteen patients tested had rather low vitamin A levels. It may be significant that the only patient who still had steatorrhoea had the lowest level. This would suggest that when the vitamin A absorption defect persists for a long time the body stores of vitamin A become depleted and the blood vitamin A level then falls.

In contrast to their frequently normal blood vitamin A levels most patients with coeliac disease have a low concen-:tration of carotenoids in the blood (Clausen and McCoord, 1938; May and McCreary, 1941; Andersen and di Sant 'Agnese, 1953). This observation was first made in 1938 by Clausen and McCoord who stressed its diagnostic importance in terms which merit quotation: "The present observations indicate that when the plasma carotenoids are low in a person consuming a normal diet who, without fever or hepatic disease, is suffering from

malnutrition, and in whom diarrhoea may or may not be present, then the diagnosis of coeliac disease must be considered."

Follow-up studies have yielded conflicting results regarding the relation of the carotenoid level to clinical improvement. May and McCreary (1941) found that with clinical recovery the blood carotenoid levels usually rose to normal values. On the other hand, of the twenty-three patients tested by di Sant 'Agnese (1953) three to eleven years after their original admission to hospital with coeliac disease fourteen had low serum carotenoid levels.

The purpose of the present investigation was to assess the value of the fasting plasma carotenoid and vitamin A levels and the vitamin A tolerance test in confirming a diagnosis of coeliac disease, and to determine the effect of treatment with a gluten free diet on these levels. It was also hoped to obtain further information on the relationship between the faecal fat content and the vitamin A tolerance test in coeliac disease.

PRESENT INVESTIGATION

Twelve patients with coeliac disease were studied. Each case was diagnosed by a senior physician at the Royal Hospital for Sick Children, Glasgow. This is stressed as diagnosis depends on the recognition of the clinical features of the disease, since there is no laboratory test to give the diagnosis the 'stamp of certainty' (Sheldon, 1955). The fat content of

the stools in every case was above the accepted normal level; faecal fat was estimated on stool collections obtained while the patients were receiving the ordinary hospital diet except in cases 7, 8, and 9 where the patients were given a diet of known fat content. Fibrocystic disease of the pancreas was excluded by laboratory tests except in two children in whom a megaloblastic anaemia, which has not been reported in association with fibro-:cystic disease of the pancreas, was regarded as confirming the diagnosis. The results of these investigations are shown in Table 8 (page 90).

Nine children (cases 1 to 9) were investigated after treatment with a gluten free diet for periods ranging from one month to four years. Cases 1 to 5 inclusive had been either in the Royal Hospital for Sick Children, Glasgow, or in East Park Home, Glasgow, throughout their period of treatment with this diet. Cases 6 to 9 inclusive had been treated at home after an initial short period of hospital care. Three of these nine children (cases 1, 2, and 8) had also been investigated before any treatment had been instituted. Case 10 was investigated on admission to hospital with a severe relapse of the disease; he had been treated for two years with a high protein, low fat and low carbohydrate diet, for eighteen months with a gluten free diet and thereafter for five months with an ordinary diet in East Park Home. He was discharged on an ordinary diet but three

months after his return home the relapse occurred. Cases 11 and 12 were investigated when admitted to hospital with megaloblastic anaemia; the diagnosis of coeliac disease had been made in these cases at the age of one year and ten years respectively and both had been treated with a low fat, low carbohydrate, and high protein diet.

Investigation consisted in each instance in estimating the fasting plasma carotenoid and vitamin A levels and in doing a vitamin A tolerance test.

RESULTS

The results are shown in Table 9 (page 91) and appendix 6.

Plasma Vitamin A.

Of the three children studied before any treatment had been instituted, two (cases 1 and 2) had plasma vitamin A levels just below the range found in healthy children while the third (case 8) had a plasma vitamin A level at the lowest limit of this range. Following treatment with a gluten free diet the concentration of vitamin A in the plasma of all three children rose appreciably, although two of them had been on the diet for one month only. All the other children (cases 3, 4, 5, 6, 7 and 9) tested while on treatment with a gluten free diet had normal plasma vitamin A levels. The child (case 10) who relapsed after treatment with a gluten free diet had been discontinued had a plasma vitamin A level just below the range found in healthy children. The two

children who had been treated with a low fat, low carbohydrate and high protein diet and who had megaloblastic anaemia had normal plasma vitamin A levels.

Vitamin A Tolerance Test.

All the children who were not having a gluten free diet (cases 1, 2 and 8 before treatment, and cases 10, 11 and 12) had either a small rise or a small fall (case 10) in the plasma vitamin A level four hours after the test dose of the vitamin. Of the five children tested after treatment with a gluten free diet for longer than one year, three (cases 5, 8, 9) had a marked increase in the plasma vitamin A concentration four hours after the test dose of the vitamin while two had a moderate increase. Of the four children tested after treatment with a gluten free diet for less than one year, three (cases 1, 6 and 7) showed a small increase while the fourth showed a moderate increase.

Plasma Carotenoids.

All the children who were not having a gluten free diet (cases 1, 2 and 8 before treatment, and cases 10,11 and 12) had plasma carotenoid levels below the range found in healthy children. Of the four children who had been on a gluten free diet for less than one year three (cases 1, 6 and 7) had low plasma carotenoid levels and one (case 2) had a normal level. On the other hand, all the children who had been on a gluten free diet for more than one year had normal plasma carotenoid levels.

DISCUSSION

The results of the present investigation are in agreement with those of previous workers in showing that children with coeliac disease have low plasma carotenoid levels and normal or only slightly reduced plasma vitamin A levels; and that a standard oral dose of an oily preparation of vitamin A produces only a small rise in the plasma vitamin A level of such children. As these are invariable findings in untreated coeliac disease they provide reliable biochemical confirmation of the clinical diagnosis. They are not diagnostic, however, as similar findings have been reported in fibrocystic disease of the pancreas (Present Investigation), infective hepatitis, (Clausen and McCoord, 1938; Breeze and McCoord, 1940), malnutrition (McCoord et. al., 1948; Present Investigation), inflammatory lesions (May and McCreary, 1941), cretinism (May and McCreary, 1941), and in several skin diseases. Fortunately from the clinical standpoint only fibrocystic disease of the pancreas causes real difficulty in differential diagnosis and here the fasting plasma vitamin A level is usually lower than in coeliac disease. An important corollary is that a normal plasma carotenoid level or a high rise in the plasma vitamin A level following a standardised oral dose of an oily preparation of vitamin A excludes the diagnosis of coeliac disease. With the technique employed in the present investigation for the vitamin A tolerance test, none of the

children with untreated coeliac disease had a plasma vitamin A level as high as 500 I.U. per 100 ml. four hours after the test dose of the vitamin; a rise above this level probably therefore excludes the diagnosis, while a rise to 716·4 I.U. per 100 ml. the mean value of the healthy children would certainly do so.

The normal or only slightly reduced plasma vitamin A levels of the children with untreated coeliac disease are consistent with the rarity of vitamin A deficiency in this disease.

Investigation of the two patients with coeliac disease who had been treated with a high protein, low fat and low carbohydrate diet, and who were re-admitted to hospital with megaloblastic anaemia gave results similar to those found in untreated coeliac disease, yet both had normal fat absorption as judged by fat balance tests. This is further evidence to add to that already reviewed that in coeliac disease the rise in the plasma vitamin A level following an oral dose of an oily preparation of vitamin A does not parallel fat absorption. The absorption of carotenoids also does not parallel fat absorption. It is suggested that in coeliac disease there is a specific malabsorption of carotenoids and vitamin A.

The five children who had been on a gluten free diet for longer than one year had normal plasma carotenoid levels. Three of them showed a marked increase in plasma vitamin A concentration in the vitamin A tolerance test and two showed a moderate increase.

One child who had been on a gluten free diet for one month only had a normal plasma carotenoid level and showed a moderate increase in the plasma vitamin A concentration in the vitamin A tolerance test. It is concluded from these findings that the exclusion of gluten from the diet of children with coeliac disease restores the absorption of carotenoids and vitamin A to normal. The results obtained in the child who relapsed when the gluten free diet was stopped were similar to those found in untreated coeliac disease. At the present time it is not known for how long the gluten free diet should be continued. The persistence of normal plasma carotenoid levels and of a moderate or marked increase in the plasma vitamin A concentration in the vitamin A tolerance test after the diet had been stopped for some months would provide valuable biochemical confirmation that the disease had been cured.

Summary

1. The literature relevant to a study of carotenoid and vitamin A metabolism in coeliac disease has been reviewed.

2. Twelve children with coeliac disease were investigated. Three children who had received no treatment had low plasma carotenoid levels, normal or near normal fasting plasma vitamin A levels and a small rise in the plasma vitamin A level in the vitamin A tolerance test. Two children, who had developed a megaloblastic anemia while on treatment with a high protein, low fat and low

carbohydrate diet did not have steatorrhoea but had low plasma carotenoid levels and a small rise in plasma vitamin A in the vitamin A tolerance test. Nine children were investigated while on treatment with a gluten free diet. The five children who had been on this diet for more than a year had normal plasma carotenoid levels and a moderate or marked rise in plasma vitamin A in the vitamin A tolerance test.

3. It is suggested that carotenoids and vitamin A are defectively absorbed in coeliac disease, not as a secondary result of impaired fat absorption, but rather due to the existence of separate malabsorption defects; also that when gluten has been excluded from the diet of children with coeliac disease for at least one year carotenoids and vitamin A are normally absorbed.

TABLE 8.

LABORATORY TESTS CONFIRMING THE DIAGNOSIS OF COELIAC DISEASE.

Case No.	Faecal Fat Gm. per Cent	Fat Balance Test Per Cent Absorption	Proteolyte Activity Duodenal Juice	Proteolyte Activity Stool+	Sweat Electrolytes M. Eq. per Litre Sodium	Sweat Electrolytes M. Eq. per Litre Chloride
1	48·0	--	--	--	52·1	44·8
2	51·5	--	--	1:160	--	--
3	63·0	--	0·00125*	1:40	--	--
4	44·0	--	--	1:20	71·8	63·1
5	58·5	--	0·005*	1:80	--	--
6	54·3	--	--	1:160	27·2	23·4
7	--	52	1:50x	No Liq.	40·6	82·0
8	41·6	89	--	1:320	--	--
9	--	85	--	--	37·0	31·1
10	36·3	--	0·08*	--	--	--

* Minimum amount (ml.) of duodenal juice required to liquefy 2 ml. 7·5% gelatine (Andersen and Early, 1942).

x This ratio is the maximum dilution of duodenal juice which liquefied the gelatine of an x-ray plate.

\+ Maximum dilution of stool which liquefied 1 ml. 7·5% gelatine (Emery, 1952).

TABLE 9

PLASMA CAROTENOID AND VITAMIN A LEVELS IN COELIAC DISEASE.

Case No.	Duration on gluten free diet	Plasma Carotenoids µgm. per 100 ml.	Plasma Vitamin A I.U. per 100 ml.	
			Fasting	4 hrs. after test dose of Vitamin A
1	1 mo.	31 (13)	119 (73)	150 (104)
2	1 mo.	116 (26)	189 (62)	575 (202)
3	2 yrs.8 mos.	78	150	471
4	3 yrs.6 mos.	71	141	406
5	4 yrs.	71	97	2357
6	6 mos.	22	97	325
7	4 mos.	29	137	171
7a	5 mos.	32	122	327
8	1 yr.8 mos.	77 (23)	127 (83)	1562 (396)
9	1 yr.8 mos.	209	102	1866
10	-	10	81	75
11	-	29	137	175
12	-	14	94	95

The results shown in brackets are those obtained before treatment was instituted.

VITAMIN A STUDIES

in

RENAL DISEASE

REVIEW OF THE LITERATURE

A considerable amount of evidence has accumulated both from animal experiments and human studies which appears to connect renal disease with vitamin A deficiency or abnormalities of vitamin A metabolism.

When only small amounts of vitamin A are available to the rat the importance of the vitamin to the kidney is apparant, as under these circumstances the kidney has priority even over the liver in absorbing the vitamin (Johnson and Baumann, 1947; Eden and Moore, 1950). Vitamin A deficiency in rats frequently leads to fatal urinary infections often with the formation of calculi (Moore and Sharman, 1951), and children deficient in vitamin A are unduly susceptible to urinary infections (Bloch, 1924).

The blood vitamin A level is frequently high both in chronic nephritis (Clausen and McCoord, 1938; Hedberg and Lindquist, 1938; Popper et al., 1945) and in the nephrotic syndrome (Clausen and McCoord, 1938; Kagan et al., 1950b). Popper et al. (1945) found that patients with nephritis who had high plasma vitamin A levels usually had hypertension and azotaemia. In one of these patients who, it is stated, had chronic nephritis, they observed a parallel increase in plasma vitamin A and serum non protein nitrogen. It is not clear, however, whether all the patients investigated by these workers had chronic nephritis or whether

some had acute glomerulo-nephritis.

It has also been shown that both in 'azotaemic renal conditions of nephritic origin' and in the nephrotic syndrome the vitamin A tolerance test shows the same abnormality. In 'azotaemic renal conditions of nephritic origin' Popper et al. (1945) found that the rise in the plasma vitamin A level following a standardised oral dose of an oily preparation of vitamin A ester was greater than normal; also that the return of the vitamin A concentration in the blood to the pre-test level was delayed. Kagan et al. (1950b) demonstrated that the same type of curve occurred in the nephrotic syndrome with both vitamin A ester in oil and vitamin A alcohol in aqueous dispersion; these workers likened the curve to the diabetic glucose tolerance curve.

There is no vitamin A in normal human urine. In 1936, Boller and Brunner made the surprising discovery that the urine from patients suffering from certain diseases did nontain vitamin A. Boller et al. (1937) showed that chronic nephritis and nephrosis were two such diseases. Constant or irregular excretion of vitamin A in the urine was found in twenty-three out of twenty-five cases of chronic nephritis investigated by Hedberg and Lindquist (1938). These workers found that large oral doses of vitamin A neither increased the number of positive urine specimens nor increased the quantity of vitamin A excreted

There was however a relationship to the degree of hypertension and azotaemia. A rise in the non-protein nitrogen in the blood was almost always associated with an increased excretion of vitamin A in the urine. Furthermore only three of the patients who constantly excreted vitamin A in the urine had normal retinae. Lawrie et al. (1941) confirmed that some patients with chronic nephritis, nephrosis, and certain other diseases, particularly pneumonia excreted vitamin A in the urine. They found that urines containing vitamin A always contained protein although vitamin A was not present in every urine containing protein. From extensive chemical investigations they concluded that the vitamin A was associated with a non-heat-coagulable protein fraction. Only small amounts of lipoids were found in the urines containing vitamin A so that the excretion of the vitamin is highly selective.

Although these investigations suggest that the same abnormality of vitamin A metabolism occurs in chronic nephritis and the nephrotic syndrome, other investigations indicate that this is not so. Partitioning of the blood vitamin A into its ester and alcohol fractions has revealed that in 'azotaemic renal disease of the nephritic type' the high blood vitamin A is due to an increase in the alcohol fraction while in the nephrotic syndrome it is due to an increase in the vitamin A ester fraction (Popper et al., 1948).

The liver stores of vitamin A have been estimated post-mortem in chronic nephritis and in the nephrotic syndrome. Moore (1937) estimated the liver vitamin A reserves in adults dying from accidental causes and from a variety of diseases; the smallest reserves were found in patients dying from chronic nephritis and from kidney and bladder infections. Clausen and McCoord (1938) also found that the liver vitamin A reserves were low in two patients who died of chronic nephritis. Lawrie et al. (1941) consider that the amount of vitamin A excreted in the urine in chronic nephritis is insufficient to account for the reduction in liver reserves. Kagan and Kaiser (1952) found that the livers of three children who had died of the nephrotic syndrome contained considerably more vitamin A than the livers of patients who had died from other causes.

High blood carotenoid levels have been reported both in chronic nephritis (Clausen and McCoord, 1938) and in the nephrotic syndrome (Clausen and McCoord, 1938; Kagan et al.,1950b).

It is clear, therefore, that vitamin A metabolism is disordered in different ways in chronic nephritis and the nephrotic syndrome, although a high concentration of vitamin A in the blood, an abnormal vitamin A tolerance test and the excretion of vitamin A in the urine are common to both diseases.

In both chronic nephritis and the nephrotic syndrome the cause of the disordered vitamin A metabolism is uncertain. It

has been suggested that in the nephrotic syndrome the increased blood lipoid content increases the solubility of vitamin A esters in the blood so raising the blood vitamin A level and causing the abnormal vitamin A tolerance test (Popper et al.,1948). It is necessary to assume that the greater solubility of vitamin A in the blood increases the absorption of vitamin A from the gut and so explains the excessive storage of the vitamin in the liver. On the other hand, it has been suggested that in chronic nephritis there is an increased output of vitamin A alcohol by the liver to maintain a high blood vitamin A alcohol level despite urinary excretion of the vitamin. It is necessary, however, also to postulate a diminished rate of storage or an increased rate of absorption of the vitamin to explain the abnormal vitamin A tolerance test. The low liver stores in chronic nephritis make a diminished rate of storage the more likely possibility.

It is known that patients dying from kidney and bladder infections have low vitamin A liver reserves (Moore, 1937) but a study of the literature has revealed no investigations of the concentration of vitamin A in the blood in such infections.

The purpose of the present investigation was to determine the blood carotenoid and vitamin A levels in urinary infections and to compare these with the levels in nephritis and nephrosis; also to carry out vitamin A tolerance tests in these diseases.

PRESENT INVESTIGATION

Six children with acute pyuria (cases 1-6), one with renal tuberculosis (case 7), one with acute nephritis (case 8), one with chronic pyelonephritis (case 9), and three with nephrosis (cases 10-12) were investigated. One child with acute pyuria (case 3) was reinvestigated when she had a recurrence of pyuria (case 3a). The fasting plasma carotenoid and vitamin A levels of these children were estimated. A vitamin A tolerance test was done on all but case 10; the dose of vitamin A used in the test was 20,000 I.U. in cases 3, 4, 5, 6, 9 and 11, and 250,000 I.U. in cases 7, 8 and 12. All the children were afebrile when investigated.

RESULTS

The results are shown in Table 10 (page 101) and Appendix 7.

Fasting Plasma Vitamin A.

The children with acute pyuria had fasting plasma vitamin A levels within the normal range except for one child who had a high level. This child (case 6) developed renal tuberculosis about one year after the estimations were done. The child with active renal tuberculosis (case 7) had a fasting vitamin A level at the top limit of the normal range. The child with chronic pyelonephritis (case 9) and two of the children with nephrosis (cases 10 and 11) had levels above the normal range.

Vitamin A Tolerance Test

The plasma vitamin A level four hours after the test dose of the vitamin was always considerably above the pre-test level. The highest level occurred in a child who was having her second attack of pyuria (case 3a). The rise in the level bore no relation to the pre-test level. In the patients with acute pyuria the mean of the plasma vitamin A values four hours after the test dose is 1563 I.U. per 100 ml. while in the patients with nephrosis it is 1549·5 I.U. per 100 ml. Both values are much higher than the mean value of 716·4 for the healthy children investigated, although many of the patients with renal disease received a smaller test dose of vitamin A than the healthy children.

Plasma Carotenoids.

The plasma carotenoid levels were high in two of the three children with nephrosis. In the remainder of the patients the level was within the normal range.

Discussion

The results of the present investigation are in agreement with those of other workers who have shown that in the nephrotic syndrome the plasma carotenoid and vitamin A levels are high and the rise in the plasma vitamin A level following a standardised oral dose of the vitamin is greater than in healthy children.

The children with acute urinary infections when afebrile had

normal or high plasma vitamin A levels, which suggests that vitamin A deficiency plays no part in the aetiology of these infections.

The child with chronic pyelonephritis had a high fasting plasma vitamin A level. In view of the observation of Popper et al. (1945) that patients with chronic nephritis who had high plasma vitamin A levels usually had hypertension and azotaemia, it is interesting that this child with chronic pyelonephritis had a blood urea of 130 mg. per 100 ml. and a blood pressure of 190 systolic, and 150 diastolic.

When the vitamin A tolerance tests on the children with acute urinary infections are considered individually, only in the test on the child who was having her second attack of pyuria did the plasma vitamin A level four hours after the test dose of the vitamin rise above the range of levels found in healthy children. However, four hours after the test dose of the vitamin the mean plasma vitamin A level of the children with acute urinary infections was much higher than the mean level of healthy children, and slightly higher than the mean level of the nephrotic children. This could be due either to an increased rate of absorption from the gut or to a diminished rate of storage of the vitamin in acute urinary infections. The low liver stores of the vitamin found in adults who had died from urinary infections (Moore, 1937) indicate that a diminished rate of storage of the

vitamin is the probable explanation of the abnormal vitamin A tolerance test in these infections.

SUMMARY

1. The literature relating to vitamin A metabolism in renal disease is reviewed.

2. Six children with acute pyuria, one with renal tuberculosis, one with acute nephritis, one with chronic pyelonephritis and three with nephrosis were investigated.

3. The children with nephrosis had high plasma carotenoid levels, high fasting plasma vitamin A levels and a marked rise in the vitamin A tolerance test.

4. The child with chronic pyelonephritis, who had azotaemia and hypertension, had a high fasting plasma vitamin A level but only a moderate rise in the plasma vitamin A level in the vitamin A tolerance test.

5. The children with acute urinary infections had, when afebrile, normal or high fasting plasma vitamin A levels. This suggests that vitamin A deficiency plays no part in the aetiology of acute urinary infections. In the vitamin A tolerance tests on these children the mean plasma vitamin A level four hours after the test dose of the vitamin was much higher than the mean level of healthy children. It is suggested that this is due to a diminished rate of storage of vitamin A in children with acute urinary infections.

TABLE 10.

PLASMA CAROTENOID AND VITAMIN A LEVELS IN RENAL DISEASE

Case No.	Age Yrs.	Disease	Plasma Carotenoids µgm. per 100 ml.	Plasma Vitamin A I.U. per 100 ml.	
				Fasting	4 hrs. after test dose of Vitamin A
1	5/12	Acute Pyuria Hydronephrosis	38	185	1710
2	9/12	Acute Pyuria	36	121	1895
3	6	Acute Pyuria	56	75	560
3a	6	Recurrent Pyuria	83	175	3506
4	7	Acute Pyuria	83	193	1081
5	10	Acute Pyuria	78	160	752
6	11	Acute Pyuria	56	275	1439
7	10	Tuberculosis of Kidney	115	204	1529
8	12	Acute Nephritis	73	189	2177
9	8	Chronic Pyelonephritis	90	264	604
10	6	Nephrosis	113	410	-
11	6	Nephrosis	174	216	1135
12	8	Nephrosis	187	137	1964

VITAMIN A STUDIES

in

IDIOPATHIC HYPERCALCAEMIA

REVIEW OF THE LITERATURE

Under the title of 'Idiopathic hypercalcaemia in infants with failure to thrive' Lightwood (1952) described a syndrome affecting infants in the first year of life and characterised by anorexia, vomiting, constipation, hypotonia and failure to thrive, with raised serum calcium and blood urea levels. The clinical features of this syndrome closely resemble those of hyperchloraemic renal acidosis but there are some differences to which Hutchison (1955) has drawn attention. The hypercalcaemic infant is usually apathetic and has a pink complexion, while the acidotic infant is frequently irritable and pale. Again in idiopathic hypercalcaemia hypotonia may be very marked, whereas dehydration is often absent unless vomiting has been severe. Finally each syndrome has distinctive biochemical findings.

Hypercalcaemia in infancy is occasionally associated with physical and mental retardation, an abnormal facies, osteo-:sclerosis, hypertension and a loud praecordial systolic murmur. It is not yet known whether this is a severeform of idiopathic hypercalcaemia or a different disease entity.

Since 1952 many cases of idiopathic hypercalcaemia have been reported from children's hospitals in Great Britain and Northern Ireland (Creery and Neill, 1954; Harris, 1954; Lowe et al., 1954; Morgan et al., 1956; Forfar et al., 1956). In most

cases complete recovery has occurred but there have been a few deaths. Post-mortem studies have revealed nephrocalcinosis, but the renal lesions are not specific and similar changes have been found in other conditions including deaths ascribed to hyper-:vitaminosis D (Rhaney and Mitchell, 1956).

The known causes of hypercalcaemia have been investigated as possible aetiological factors. On an analogy with the milk and alkali syndrome of adults Creery (1953) suggested that alkali medication might be a causal factor, but later Creery and Neill (1954) noted that idiopathic hypercalcaemia could develop in the absence of such therapy. Hyperparathyroidism has been considered an unlikely cause in view of the normal plasma phosphorus in the absence of severe uraemia, and the tendency to a low plasma alkaline phosphatase level (Morgan et al., 1956). Cow's milk contains four to five times as much calcium as human milk and as no case has been reported in a wholly breast fed infant, the use of cow's milk probably contributes to the production of the hypercalcaemia. However the possible aetiological role of vitamin D has aroused the most interest. It has been pointed out that in recent times many infants have been given much more vitamin D than is strictly necessary for health, due to the fortification of many infant foods with vitamin D and the increased use of vitamin D supplements. (Hutchison, 1955). It is known, however, that the syndrome

of idiopathic hypercalcaemia can occur in infants whose intake of vitamin D has not been excessive. In 1952 Payne rejected hypervitaminosis D as the cause, but in the following year Lightwood (1953) put forward the idea that patients who develop idiopathic hypercalcaemia might be hypersensitive to vitamin D; this hypothesis has been supported by many subsequent workers (Lowe et al., 1954; Creery and Neill, 1954; Hutchison, 1955; Morgan et al., 1956). Calcium balance studies (Creery and Neill, 1954; Bonham Carter et al., 1955; Morgan et al., 1956) have shown that infants with idiopathic hypercalcaemia absorb a high proportion of dietary calcium. Morgan et al. (1956) consider that this finding supports the hypothesis that vitamin D is the cause.

Sinclair (1956) has found that rats deficient in essential fatty acids are more sensitive to vitamin D and believes that this may be because vitamin D is normally esterified with essential fatty acids, and therefore "in deficiency of essential fatty acids one might expect a rise in unesterified vitamin D (which is known to be more active than esterified) or in abnormally esterified vitamin". He suggests that a deficiency of essential fatty acids may similarly make infants hypersensitive to vitamin D and so cause idiopathic hypercalcaemia. He points out that this hypothesis would explain the geographical distribution of this disease. In Great Britain roller dried

cow's milk which has a low content of essential fatty acids is widely used in infant feeding, whereas in the United States of America evaporated milk, which is richer in essential fatty acids, is generally employed. Again the high essential fatty acid content of breast milk would explain the immunity of the wholly breast fed infant. Recently Forfar et al. (1956) have contested the theory that vitamin D is the cause of this disease. They consider that some of the features of idiopathic hypercal-:caemia are unlike the known effects of calciferol. They cite the absence of significant clinical change in at least some patients with idiopathic hypercalcaemia on administration of calciferol, and also the failure of calciferol to increase the hypercalcuria in these cases. In addition the long duration of the illness after withdrawal of vitamin D together with the increased retention of phosphorus and the hypophosphaturia found in this disease all suggest to them some other aetiology. Citrate metabolism in one of their infants was more in keeping with rickets than hypercalcaemia. Hypercholesterolaemia was present in several of their cases and a statistically significant correlation was found between the serum calcium and plasma cholesterol levels. In view of these findings they postulate that idiopathic hypercalcaemia results from a disturbance of cholesterol metabolism, possibly due to infection, with the production of a cholesterol derivative with hypercalcaemic and

toxic effects. Sinclair (1956) believes that a deficiency in essential fatty acids would cause the hypercholesterolaemia.

Vitamins A and D are closely related in nature and as both are fat soluble their metabolism is probably in some respects similar. The possible aetiological role of vitamin D in idiopathic hypercalcaemia suggested therefore that it would be of interest to investigate vitamin A metabolism in this disease.

Present Investigation

The fasting plasma carotenoid and vitamin A levels of fourteen infants with idiopathic hypercalcaemia were estimated. Vitamin A tolerance tests were done on all but one of these infants (case 13); the test on case 6 was done using 20,000 I.U. vitamin A per kilogram of body weight instead of the standard dose of 12,500 I.U. per kilogram of body weight. Additional fasting levels of two of these infants (cases 8a and 9a) were estimated later in their illness. Two of the infants (cases 1 and 3) had renal acidosis as well as idiopathic hypercalcaemia.

In view of the theories of causation of idiopathic hyper-:calcaemia three other patients were investigated. These were a child with vitamin A resistant rickets, who was being treated with large oral doses of vitamin D, an adult suffering from idiopathic hypercholesterolaemia, and an infant with miliary tuberculosis who developed hypercalcaemia while under treatment with streptomycin. Only the fasting plasma carotenoid and

vitamin A levels of the latter two patients were estimated. The child with vitamin D resistant rickets was treated with 100,000 I.U. vitamin D daily from 16th December 1955 to 19th March 1956 and with 300,000 I.U. vitamin D daily from 19th March 1956 to 9th July 1956. Thereafter treatment was withdrawn for two weeks before re-starting treatment with 100,000 I.U. vitamin D daily. The fasting plasma carotenoid and vitamin A levels of this child were estimated on 9th January, 1956, 2nd July, 1956 and 7th July, 1956. A vitamin A tolerance test was also done on 7th July,1956.

All the patients were afebrile when investigated.

RESULTS

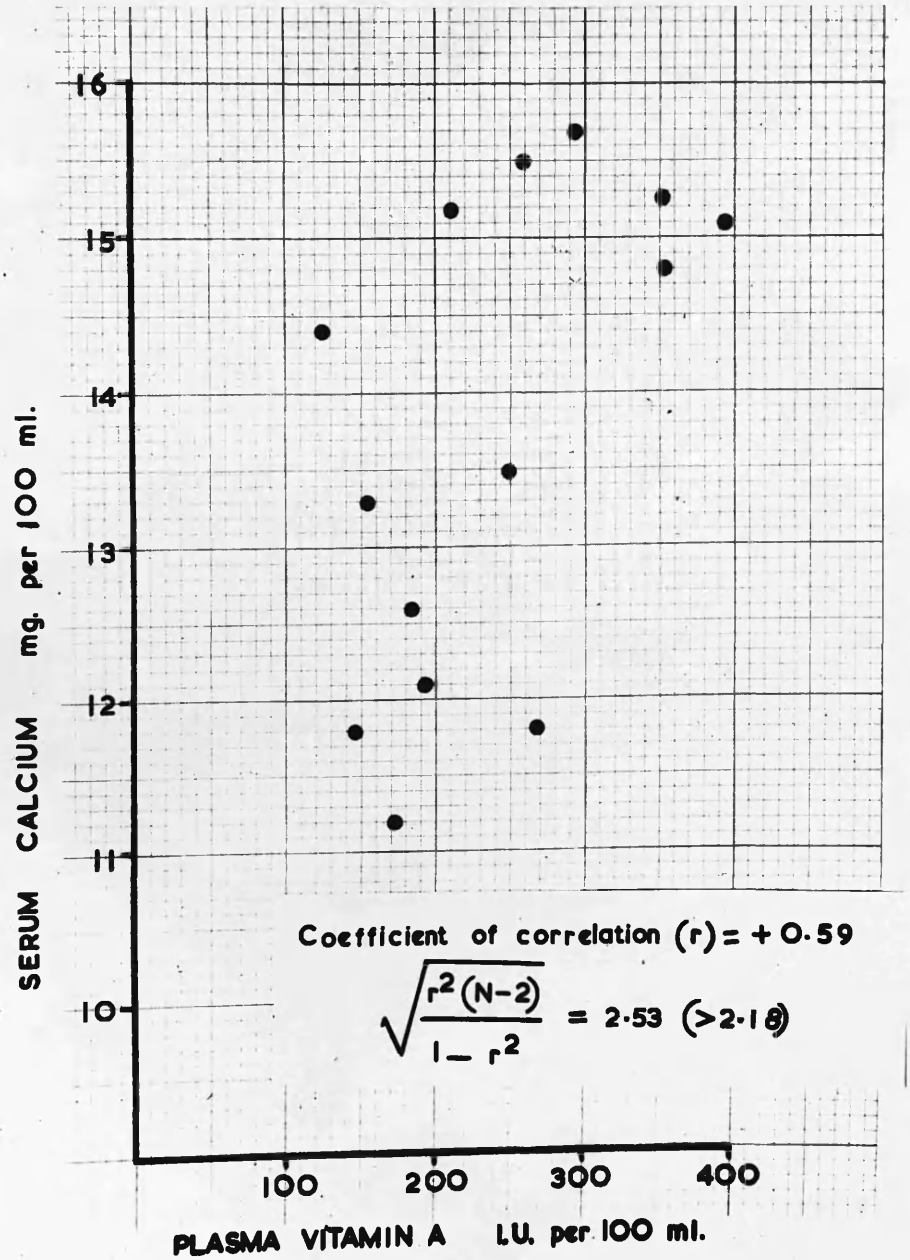
(A) Idiopathic Hypercalcaemia.

The results are shown in Table 11 (page 115) and Appendix 8.

Plasma Vitamin A

The fasting plasma vitamin A levels of eight of the infants with idiopathic hypercalcaemia (cases 2, 3, 4, 5, 7, 8, 11 and 12) were above the range found in healthy children. The plasma vitamin A levels of the other six infants were within the range found in healthy children but the levels of three of these (cases 1, 10 and 13) were at the upper limit of the normal range. Beginning recovery from the disease may explain the normal values in cases 1 and 6; they had been under medical supervision for five and four months respectively and both were starting to thrive

FIG. 1. CORRELATION OF PLASMA VITAMIN A AND SERUM CALCIUM LEVELS IN IDIOPATHIC HYPERCALCAEMIA



when their vitamin A levels were estimated. The symptomatology of case 14 is unusual and for that reason a short case history is appended.

Case 14. Date of Birth:- 28th May, 1955. Spontaneous delivery at full term. Birth weight 7lb. 1 oz. When two weeks old she was admitted to another hospital with a doubtful pneumonia. After three weeks she was discharged but was readmitted within twenty-four hours on account of fever. Thereafter she was kept in hospital until the age of five months as she continued to have recurrent febrile episodes associated with diarrhoea. Soon after discharge she became listless and anorexic and lost weight. Her stools which had been loose became constipated. These symptoms led to her admission to The Royal Hospital for Sick Children, Glasgow, on 6th December, 1955. Again while in hospital she had febrile episodes with diarrhoea and bronchitis. On admission the blood chemistry was normal but on 27th December, 1955, the serum calcium was 14.4 mg. per 100 ml.

In figure 1 the plasma vitamin A level of each case is plotted against the serum calcium level. These estimations were done within one week of each other except in case 1 where there was an interval of two weeks. The serum calcium level plotted is therefore the level at or near the date on which the plasma vitamin A was estimated, and not necessarily the highest serum calcium level recorded in each case. It is evident from figure 1 that the infants with the higher serum calcium levels

tend to have the higher plasma vitamin A levels. Statistics confirm this observation as there is a significant positive correlation between plasma vitamin A and serum calcium levels.

Vitamin A tolerance Test.

In every case the plasma vitamin A level four hours after the test dose of the vitamin was considerably above the fasting level. The mean of the levels four hours after the test dose is 1641 I.U. per 100 ml. compared with 716.4 I.U. per 100 ml. in healthy children.

Plasma Carotenoids.

Two infants (cases 5 and 9) had low plasma carotenoid levels but the rest of the infants had levels consistent with their ages.

(B) Vitamin D Resistant Rickets, Idiopathic Hypercholesterolaemia, and Miliary Tuberculosis with Hypercalcaemia.

The results are shown in Table 12 (page 116) and Appendix 8a.

Plasma Vitamin A.

The concentration of vitamin A in the plasma of the child with vitamin D resistant rickets rose from 129 I.U. per 100 ml. (case 15) to 177 I.U. per 100 ml. (case 15a) during vitamin D therapy.

As the values for adults are slightly higher than those for children (Aron, 1949) the plasma vitamin A level of the adult with idiopathic hypercholesterolaemia is probably at the upper limit of normal.

The plasma vitamin A level of the infant with miliary tuberculosis and hypercalcaemia is at the top of the range found in healthy children.

Vitamin A Tolerance Test.

Four hours after the test dose of vitamin A the plasma vitamin A level of the child with vitamin D resistant rickets (case 15b) was considerably above the pre-test level but was within the range of levels found four hours after the test dose in healthy children.

Four hours after the test dose of vitamin A the plasma vitamin A level of the adult with idiopathic hypercholesterolaemia was slightly below the pre-test level.

Plasma Carotenoids.

The child with vitamin D resistant rickets and the infant with miliary tuberculosis and hypercalcaemia had normal plasma carotenoid levels.

The adult with idiopathic hypercholesterolaemia had a rather high level compared with normal adult values (Moore and Leitner, 1949).

DISCUSSION

The plasma vitamin A level of a healthy individual is kept almost constant despite variations in the dietary intake of the vitamin. In healthy infants massive overdosage with vitamin A for about six months is required to produce the symptoms of

hypervitaminosis A with an increased concentration of vitamin A in the blood persisting for some weeks after stopping the vitamin (Caffey, 1950). When doses of smaller magnitude are given to infants over six months of age for periods of up to five months the plasma vitamin A level estimated forty-eight hours after stopping the vitamin is within normal limits (Lewis et al., 1941b). None of the infants with idiopathic hypercalcaemia had received massive doses of vitamin A and all vitamin A concentrates had been withheld for at least forty-eight hours before the date on which the estimations were made. Hence vitamin A overdosage was not responsible for the high plasma vitamin A levels of these infants.

McCoord et al. (1948) showed that after a standardised oral dose of an oily preparation of vitamin A, the rise in the plasma vitamin A level of malnourished infants is smaller than in healthy infants. This finding has been confirmed in the present investigation. It contrasts with the greater rise than normal observed in the infants with idiopathic hypercalcaemia.

The plasma vitamin A level of the child with vitamin D resistant rickets rose slightly during vitamin D therapy. Despite this increase in the fasting level the rise in the plasma vitamin A level following the test dose of the vitamin was within the normal range. This suggests that vitamin D is not directly responsible for the alterations in vitamin A metabolism in

idiopathic hypercalcaemia.

Hypercholesterolaemia in an adult was associated with a rather high plasma vitamin A level, but, in contrast to the finding in idiopathic hypercalcaemia, there was no rise in the level following the test dose of vitamin A.

Sinclair (1956) has suggested that a deficiency of essential fatty acids might raise the concentration of unesterified vitamin A in the blood. Presumably this vitamin A fraction could not be stored in the liver or elsewhere and so would accumulate in the blood. As partition studies were not done it is not known whether the increase in the blood vitamin A level in idiopathic hypercalcaemia is due to the ester or the alcohol fraction. However, it is an attractive hypothesis that infants with idiopathic hypercalcaemia have a disturbance of lipoid metabolism which causes viatmin A, and vitamin D in active form, to accumulate in the blood.

A disorder of vitamin A metabolism similar to that found in idiopathic hypercalcaemia occurs in the nephrotic syndrome (Kagan et al., 1950b; Present Investigation) and an abnormal vitamin A tolerance test has been found in urinary infections (Present Investigation). The post-mortem finding of renal calcinosis in an infant, who died of miliary tuberculosis after streptomycin had failed to control the infection, led to the discovery of hypercalcaemia in another infant with miliary

tuberculosis who was having streptomycin therapy. This latter infant (case 17) had a plasma vitamin A level at the upper limit of normal. As infants with idiopathic hypercalcaemia frequently have pyuria and sometimes develop renal calcinosis, the vitamin A derangement may be secondary to the renal lesion.

A low carotenoid intake due to anorexia and vomiting probably explains the low plasma carotenoid levels of two of the infants investigated.

SUMMARY

1. The theories of causation of idiopathic hypercalcaemia have been reviewed.

2. Fourteen infants with idiopathic hypercalcaemia were investigated. Eleven had high fasting plasma vitamin A levels and eight had levels higher than any healthy child investigated in the present study. There was a significant positive correlation between the plasma vitamin A and serum calcium levels of these fourteen infants. Vitamin A tolerance tests were done on thirteen infants. The mean of the plasma vitamin A levels four hours after the test dose of vitamin A was much higher than that of the healthy children investigated in the present study.

3. It is concluded that vitamin A metabolism is disordered in idiopathic hypercalcaemia and the possible causes of this are discussed.

TABLE 11

PLASMA CAROTENOID AND VITAMIN A LEVELS
IN IDIOPATHIC HYPERCALCAEMIA.

Case No.	Age mos.	Wt. Kg.	Serum Calcium		Date of Estimn. of plasma Carotenoid and Vitamin A level	Plasma Carotenoid level µg. per 100 ml.	Plasma Vitamin A I.U. per 100 ml.	
			Date of Estimn.	Mg. per 100 ml.			Fasting	4 hrs. after test dose Vitamin A
1	7	6·5	1.11.54	11·2	16.11.54	31	173	1035
2	8	6·6	4.11.54	15·1	10.11.54	43	395	1764
3	10	5·8	22.12.54	13·5	22.12.54	66	250	1217
4.	14	6·5	12.11.54	15·3	17.11.54	69	352	920
5	11	7·0	29.10.54	15·2	5.11.54	27	212	1346
6	9	5·5	10.3.53	13·3	11. 3.53	87	156	600
7	4	5·4	19.9.55	14·8	21. 9.55	62	354	3304
8	8	5·6	13.9.55	15·5	9. 9.55	44	260	3505
8a	10	6·7	21.11.55	15·8	21.11.55	66	187	-
9	10	7·5	12.10.55	11·8	12.10.55	28	148	1152
9a	11	8.1	9.11.55	11·0	9.11.55	9	167	-
10	9	7·2	27.1.56	12·6	6. 2.56	35	183	2049
11	10	8·2	5.12.55	11·8	5.12.55	38	267	2258
12	8	4·8	13.1.56	15·7	14.1.56	59	296	1506
13	7	8·2	28.6.56	12·1	28.6.56	24	196	-
14	7	6·3	27.12.55	14·4	27.12.55	30	123	683

TABLE 12

PLASMA CAROTENOID AND VITAMIN A LEVELS IN VITAMIN D RESISTANT RICKETS, IDIOPATHIC HYPERCHOLESTEROLAEMIA, AND MILIARY TUBERCULOSIS WITH HYPERCALCAEMIA.

Case No.	Disease	Duration of Vitamin D Therapy	Plasma Carotenoid Levels µgm. per 100ml.	Plasma Vitamin A I.U. per 100 ml.	
				Fasting	4 hrs. after test dose of Vitamin A.
15	Vitamin D Resistant Rickets	24days	78	129	-
15a	"	6½ mos.	73	177	-
15b	"	6½ mos.	56	168	1345
16	Idiopathic Hypercholesterolaemia	-	211	208	200
17	Miliary Tuberculosis with Hypercalcaemia	-	33	200	-

CONCLUSIONS.

We may now summarise the main conclusions which have been reached as a result of this investigation.

(1) Although the fasting plasma carotenoid and vitamin A levels and the plasma vitamin A level four hours after the test dose of vitamin A in the vitamin A tolerance test vary considerably from one healthy child to another, levels outwith the normal range occur in certain diseases.

(2) Fibrocystic disease of the pancreas, coeliac disease, nephrosis, chronic pyelonephritis, acute urinary infections and idiopathic hypercalcaemia belong to this group of diseases.

(3) The abnormal vitamin A metabolism is the result, and not the cause of these diseases.

(4) Because of defective absorption of carotenoids and vitamin A esters, low fasting plasma carotenoid and vitamin A levels and a small rise in the plasma vitamin A level in the vitamin A tolerance test occur in those cases of fibrocystic disease of the pancreas in which pancreatic lipase is absent from the intestine. As indirect evidence of absent pancreatic lipase these findings would therefore support a clinical diagnosis of fibrocystic disease of the pancreas. Similar findings occur, however, in young infants marasmic from causes other than fibrocystic disease of the pancreas.

(5) Low plasma carotenoid levels and a small rise in the plasma vitamin A level in the vitamin A tolerance test occur in

untreated coeliac disease. However, the fasting plasma vitamin A level is usually normal or near normal. It is suggested that carotenoids and vitamin A are defectively absorbed in this disease not as a secondary result of impaired fat absorption but rather due to the existence of separate malabsorption defects. When gluten has been excluded from the diet of children with coeliac disease for at least one year carotenoids and vitamin A are normally absorbed. It is suggested that in untreated coeliac disease the fasting plasma carotenoid and vitamin A levels and the vitamin A tolerance test provide valuable biochemical confirmation of the clinical diagnosis, and that following treatment with a gluten free diet their return to normal might be used as a test of cure.

(6) In acute urinary infections the abnormal vitamin A metabolism is possibly due to defective storage of the vitamin in the liver.

(7) In the syndrome of idiopathic hypercalcaemia of infants there is a statistically significant positive correlation between the fasting plasma vitamin A level and the serum calcium level. The vitamin A tolerance test is also abnormal in this disease. This abnormal metabolism of a fat-soluble vitamin supports the conception that vitamin D metabolism is disordered in this disease.

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APPENDIX 1.

Estimation of Carotenoid and vitamin A content of plasma.

Reagents:

1. N. potassium hydroxide in 90% ethyl alcohol. This solution must be freshly prepared by adding 1 ml. of 11 N. potassium hydroxide to 10 ml. absolute alcohol, centrifuging and using the relatively clear supernatant fluid.
2. Benzene.

Apparatus: (1) Spectrophotometer with cuvettes of 0.5 centimetres width.

(2) Ultra-violet lamp. This lamp was tested with a spectrophotometer and found to emit light of wavelengths between 250 and 400 millimicrons.

(3) Graduated 2 ml. pipettes.

(4) Bottles with ground glass stoppers.

(5) Centrifuge tubes.

(6) Small Pyrex tubes with cork stoppers.

Method:

Two millilitres of plasma are pipetted into a small bottle with a glass stopper and 2 ml. of N. potassium hydroxide in 90% ethyl alcohol added. The bottle is then shaken for five minutes before being placed in a water bath at 60°C. for twenty minutes. The bottle is next allowed to cool and 2 ml. benzene added. It is then shaken for five minutes. The contents of the bottle

are transferred into a centrifuge tube and centrifuged at 3000 revolutions per minute for ten minutes. The benzene layer is then transferred into a small pyrex tube with a cork stopper and from there to the spectrophotometer cuvette. The cuvettes used had a width of 0.5 centimetres. Readings are taken first at a wavelength of 460 millimicrons and a slit width of 4 and then, using the arc lamp, at a wavelength of 328 millimicrons and a slit width of 15. The benzene extract is then transferred back into the small stoppered pyrex tube and exposed to ultraviolet light for one hour. Following exposure to ultraviolet light the solution is transferred back into the spectrophotometer cuvette and a second reading taken at a wavelength of 328 millimicrons and a slit width of 15.

Construction of standard curves.

(a) Vitamin A.

Solutions of pure vitamin A acetate in benzene were prepared containing 100, 250, 500, 750 and 1000 international units vitamin A acetate per 100 ml. benzene. The optical densities of these solutions, at a wavelength of 328 millimicrons, were determined using the spectrophotometer and the results are shown in Table A. When these optical densities are plotted against the corresponding dilutions of vitamin A acetate the curve obtained is almost a straight line. All calculations have been based on a solution of 500 I.U. vitamin A in 100 ml.

benzene having an optical density of 0.120 and on the concentration of vitamin A in a benzene solution varying in direct proportion with the optical density of the solution.

For comparison, similar dilutions in benzene of synthetic vitamin A palmatate in arachis oil were prepared and their optical densities determined by spectrophotometry before and after irradiation with ultra-violet light for one hour. These results are shown in Table B. and will be seen to correspond with those obtained using pure vitamin A acetate.

(b) Carotene

Solutions of international standard beta-carotene in benzene were prepared containing 60, 80, 120, 180 and 240 micrograms beta-carotene per 100 ml. benzene. The optical densities of these solutions at a wavelength of 460 millimicrons were determined using the spectrophotometer and the results are shown in Table C. When these optical densities are plotted against the corresponding dilutions of beta-carotene the curve obtained is almost a straight line. All calculations have been based on a solution of 60 micrograms Beta-carotene in 100 ml. benzene having an optical density of 0.070 and on the concentration of carotenoids in a benzene solution varying in direct proportion with the optical density of the solution.

TABLE A.

Table showing spectrophotometric readings obtained with various dilutions in benzene of pur vitamin A acetate.

I.U. Vitamin A. acetate per 100 ml. benzene.	Optical Density at 328 millimicrons.		
	Specimen 1.*	Specimen 2.*	Average.
100	0.020	0.020	0.020
250	0.061	0.055	0.058
500	0.120	0.120	0.120
750	0.185	0.178	0.181
1000	0.238	0.238	0.238

*Duplicate solutions were prepared and separate estimations made.

TABLE B.

Table showing Optical Densities of various dilutions in benzene of vitamin A palmitate in arachis oil.

I.U. Vitamin A palmitate per 100 ml. benzene.	Optical Density at 328 millimicrons.						
	Specimen 1.*			Specimen 2.*			Average
	A.	B.	A-B.	A.	B.	A-B.	A-B
100	0.026	0.003	0.023	0.040	0.013	0.027	0.025
250	0.070	0.020	0.050	0.076	0.016	0.060	0.055
500	0.130	0.008	0.122	0.132	0.013	0.119	0.120
750	0.222	0.042	0.180	0.207	0.021	0.186	0.183
1000	0.260	0.028	0.232	0.265	0.024	0.241	0.236
1500	0.370	0.037	0.333	0.370	0.037	0.333	0.333

* Duplicate solutions were prepared and separate estimations made.

'A' is reading prior to irradiation.

'B' is reading after irradiation with ultra-violet light for one hour.

TABLE C.

Table showing optical densities of various dilutions in benzene of a sample of international standard beta-carotene.

Beta-carotene micrograms per 100 ml. benzene.	Optical density at 460 millimicrons.
60	0.070
80	0.096
120	0.149
180	0.208
240	0.276

Appendix 2.

Vitamin A tolerance Test.

After withdrawal of a blood sample for estimation of the patient's plasma carotenoid and vitamin A levels a standardised oral dose of vitamin A is given. The preparation of vitamin A used is synthetic vitamin A palmitate in arachis oil (Avoleum B.D.H.) and this is given in a dose of 12,500 I.U. per kilogram of body weight up to a maximum dose of 250,000 I.U. Four hours after the dose of vitamin A a further sample of blood is withdrawn for estimation of the plasma carotenoid and vitamin A levels. The patients receive their ordinary meals throughout the test.

(In the present study the plasma carotenoid and vitamin A levels before giving the test dose of vitamin A have been termed 'fasting' when vitamin A concentrates had been withheld for at least forty-eight hours before the estimations were made.)

APPENDICES 3 - 8a.

In these appendices the detailed figures of all the estimations of the plasma carotenoid and vitamin A levels are shown. The following abbreviations are used:-

Case No.....Case Number

Wt. Kg.....Weight in kilograms.

Date of Est^{n}.....Date of Estimation.

Plas. Spec.....Plasma Specimen.

a.....Fasting plasma specimen, or plasma specimen before the test dose of vitamin A in the vitamin A absorption test.

b.....Plasma specimen four hours after the test dose of vitamin A in the vitamin A absorption test.

µgm.....Micrograms.

I.U.....International units.

ml.....Millilitres.

M.....Millimicrons.

O.D.....Optical density.

A.....Optical density at 328 millimicrons before irradiation with ultra-violet light.

B.....Optical density at 328 millimicrons after irradiation with ultra-violet light for one hour.

A - B.....The difference between the optical density before and after irradiation with ultra-violet light.

APPENDIX 3. PLASMA CAROTENOID AND VITAMIN A LEVELS OF HEALTHY CHILDREN

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I. U. per 100 ml.
1	7 wk.	M	3.0	No. 55	a	0.020	17	0.060	0.035	0.025	104
2	3 mo.	M	4.9	Ap.'53	a	0.018	15	0.070	0.033	0.037	154
					b	0.017	14	0.198	0.052	0.146	608
					b	0.023	20	0.212	0.052	0.160	667
3	4 mo.	M	7.3	Ap'53	a	0.022	19	0.050	0.020	0.030	125
					b	0.024	21	0.264	0.070	0.194	808
4	7 mo.	F	6.1	Ja.'56	a	0.022	19	0.073	0.043	0.030	125
					a	0.022	19	0.073	0.043	0.030	125
					b	0.022	19	0.163	0.041	0.122	508
					b	0.022	19	0.165	0.032	0.133	554
5	15 mo.	M	7.0	Mr'53	a	0.063	54	0.040	0.012	0.028	117
					a	0.060	51	0.048	0.020	0.028	117
					b	0.066	57	0.068	0.022	0.046	192
					b	0.058	50	0.062	0.020	0.042	175
6	18 mo.	F	9.9	Jl.'56	a	0.061	52	0.081	0.044	0.037	154
					a	0.061	52	0.082	0.040	0.042	175
					b	0.076	65	0.164	0.052	0.112	467
					b	0.075	64	0.161	0.039	0.122	508

APPENDIX 3. PLASMA CAROTENOID AND VITAMIN A LEVELS OF HEALTHT CHILDREN

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
7	19mo.	M	9.0	My.'56	a	0.078	67	0.064	0.037	0.027	112
					b	0.078	67	0.247	0.070	0.177	737
8	19mo.	M	9.4	Ju.'56	a	0.083	71	0.056	0.027	0.029	121
					a	0.081	69	0.052	0.028	0.024	100
					b	0.085	73	0.110	0.036	0.074	308
					b	0.090	77	0.112	0.034	0.078	325
9	2 yr.	M	10.9	De.'54	a	0.055	47	0.076	0.034	0.042	175
					b	0.045	39	0.093	0.047	0.046	192
10	3 yr.	M	15.3	De.'54	a	0.073	62	0.069	0.022	0.047	196
					b	0.076	65	0.182	0.063	0.119	496
					b	0.075	64	0.181	0.053	0.128	533
11	3 yr.	F	-	De.'54	a	0.110	94	0.093	0.052	0.041	171
12	3 yr.	M	-	Ja.'55	a	0.068	58	0.065	0.030	0.035	146
					a	0.070	60	0.090	0.048	0.042	175
13	4 yr.	M	13.5	My.'56	a	0.154	132	0.097	0.044	0.053	221
					a	0.155	133	0.098	0.072	0.026	108
					b	0.048	127	0.388	0.060	0.328	1367
					b	0.142	122	0.400	0.072	0.328	1367

APPENDIX 3. PLASMA CAROTENOID AND VITAMIN A LEVELS OF HEALTHY CHILDREN

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
14	4 yr.	M	15.0	De.'54	a	0.050	43	0.062	0.045	0.017	71
					a	0.050	43	0.062	0.034	0.028	117
					b	0.056	48	0.365	0.065	0.300	1250
					b	0.056	48	0.370	0.067	0.303	1262
15	5 yr.	F	16.8	My.'56	a	0.124	106	0.061	0.037	0.024	100
					a	0.110	94	0.063	0.039	0.024	100
					b	0.110	94	0.383	0.142	0.241	1004
					b	0.118	101	0.331	0.096	0.235	979
16	5 yr.	M	20.0	Mr.'53	a	0.060	51	0.045	0.016	0.029	121
					b	0.064	55	0.070	0.024	0.046	192
17	6 yr.	M	-	Ja.'55	a	0.091	78	0.059	0.030	0.029	121
					a	0.093	80	0.080	0.042	0.038	158
18	6 yr.	F	-	Ju.'56	a	0.062	53	0.060	0.037	0.023	96
					a	0.066	57	0.067	0.040	0.027	112
19	7 yr.	F	-	Ja.'55	a	0.178	153	0.080	0.032	0.048	200
20	8 yr.	F	-	Ja.'55	a	0.155	133	0.071	0.032	0.039	162
21	8 yr.	F	-	Ja.'55	a	0.076	65	0.062	0.035	0.027	112
					a	0.073	63	0.073	0.032	0.041	171

APPENDIX 3. PLASMA CAROTENOID AND VITAMIN A LEVELS OF HEALTHY CHILDREN

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids O.D. at 460 M.	Carotenoids µgm. per 100 ml.	Vitamin A Optical Density at 328 M. A	Vitamin A Optical Density at 328 M. B	Vitamin A Optical Density at 328 M. A - B	Vitamin A I.U. per 100 ml.
22	8 yr.	F	-	Ja'55	a	0.070	60	0.047	0.030	0.017	71
					a	0.069	59	0.060	0.037	0.023	96
23	8 yr.	M	37.0	De.'54	a	0.097	83	0.107	0.070	0.037	154
					b	0.097	83	0.185	0.073	0.112	467
24	9 yr.	M	-	Ja.'55	a	0.167	143	0.075	0.044	0.031	129
25	9 yr.	F	-	Au.'56	a	0.078	67	0.070	0.034	0.036	150
					a	0.076	65	0.065	0.029	0.036	150
26	10 yr.	F	-	Ja.'55	a	0.106	91	0.067	0.044	0.023	96
					a	0.108	93	0.069	0.043	0.026	108
27	10 yr.	M	-	Au.'56	a	0.064	55	0.049	0.024	0.025	104
					a	0.066	57	0.049	0.023	0.026	108
28	10 yr.	F	-	Au.'56	a	0.123	105	0.080	0.046	0.034	142
					a	0.115	99	0.064	0.031	0.033	137
29	10 yr.	F	-	Jl.'56	a	0.085	73	0.056	0.030	0.026	108
					a	0.093	80	0.063	0.033	0.030	125
30	10 yr.	M	26.8	Ap.'56	a	0.050	43	0.076	0.042	0.034	142
					a	0.049	42	0.080	0.045	0.035	146
					b	0.057	49	1.040	0.517	0.523	2179
					b	0.055	47	1.040	0.570	0.470	1958

APPENDIX 4. PLASMA CAROTENOID AND VITAMIN A LEVELS IN FIBROCYSTIC DISEASE OF THE PANCREAS.

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
1	7 mo.	F	4.6	3.10.55	a	0.002	2	0.024	0.018	0.006	25
					a	0.003	3	0.032	0.031	0.001	4
					b	0.003	3	0.032	0.015	0.017	71
					b	0.004	3	0.035	0.022	0.013	54
2	8 yr.	F	18.2	16.4.56	a	0.010	9	0.029	0.027	0.002	8
					a	0.015	13	0.043	0.035	0.008	33
					b	0.010	9	0.034	0.029	0.005	21
					b	0.013	11	0.038	0.030	0.008	33
				22.5.56	a	0.035	30	0.062	0.045	0.017	71
					a	0.020	17	0.045	0.029	0.016	67
3	9 mo.	F	8.0	10.12.56	a	0.007	6	0.029	0.024	0.005	21
					a	0.006	5	0.031	0.028	0.003	12
					b	0.000	0	0.028	0.024	0.004	17
					b	0.002	2	0.033	0.026	0.007	29
4	4 mo.	F	3.2	22.11.56	a	0.007	6	0.024	0.017	0.007	29
					a	0.007	6	0.031	0.023	0.008	33

APPENDIX 4. PLASMA CAROTENOID AND VITAMIN A LEVELS IN FIBROCYSTIC DISEASE OF THE PANCREAS

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A- B	I.U.per 100 ml.
5	4 mo.	M	3.8	16.9.53	a	0.004	3	0.058	0.025	0.033	137
					a	0.014	12	0.094	0.022	0.072	300
					b	0.005	4	0.435	0.047	0.388	1617
					b	0.011	9	0.436	0.063	0.373	1554
6	10 mo.	M	8.9	4.10.54	a	0.008	7	0.058	0.033	0.025	104
					b	0.007	6	0.056	0.020	0.036	150
					b	0.007	6	0.081	0.042	0.039	162
7	19 mo.	M	7.9	21.7.56	a	0.015	13	0.041	0.016	0.025	104
					a	0.016	14	0.044	0.013	0.031	129
					b	0.014	12	0.045	0.016	0.029	121
					b	0.008	7	0.030	0.013	0.017	71
7a	19 mo.	M	7.9	23.7.56	a	0.014	12	0.046	0.023	0.023	96
					a	0.013	11	0.045	0.015	0.030	125
					b	0.014	12	0.052	0.025	0.027	112
					b	0.020	17	0.054	0.030	0.024	100
8	5 mo.	F	4.5	23.11.55	a	0.007	6	0.062	0.025	0.037	154
					a	0.007	6	0.067	0.020	0.047	196
					b	0.012	10	0.088	0.058	0.030	125
					b	0.012	10	0.078	0.029	0.049	204

APPENDIX 4. PLASMA CAROTENOID AND VITAMIN A LEVELS IN FIBRYCYSTIC DISEASE OF THE PANCREAS.

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	Mgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
9	4 mo.	M	4.1	27.10.54	a	0.002	2	0.050	0.032	0.018	75
					b	0.002	2	0.062	0.018	0.044	192
10	4 mo.	M	3.1	22.11.54	a	0.007	6	0.046	0.012	0.034	142
					a	0.007	6	0.047	0.013	0.034	142
					b	0.007	6	0.100	0.017	0.083	346
					b	0.012	10	0.115	0.022	0.093	396
11	22 mo.	F	11.9	22.9.54	a	0.016	14	0.037	0.020	0.017	71
					b	0.013	11	0.123	0.050	0.073	304

APPENDIX 5. PLASMA CAROTENOID AND VITAMIN A LEVELS IN MARASMIC INFANTS

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids O.D. at 460 M.	Carotenoids µgm. per 100 ml.	Vitamin A Optical Density at 328 M. A	Vitamin A Optical Density at 328 M. B	Vitamin A Optical Density at 328 M. A - B	Vitamin A I.U. per 100 ml.
1	1 mo.	F	3.1	23.3.53	a	0.006	5	0.040	0.018	0.022	92
					a	0.006	5	0.038	0.012	0.026	108
					b	0.007	6	0.064	0.025	0.039	162
					b	0.006	5	0.057	0.022	0.035	146
2	2 mo.	F	3.6	30.6.53	a	0.022	19	0.041	0.029	0.012	50
					b	0.024	21	0.142	0.043	0.099	412
3	3 mo.	M	3.1	4.5.53	a	0.008	7	0.041	0.010	0.031	129
					a	0.035	30	0.110	0.060	0.050	208
					b	0.015	13	0.213	0.031	0.182	750
					b	0.008	7	0.215	0.031	0.184	767
4	4 mo.	M	4.0	8.6.53	a	0.012	10	0.036	0.035	0.001	4
					b	0.012	10	0.046	0.046	Nil	Nil
5	4 mo.	F	3.0	20.3.53	a	0.011	9	0.040	0.010	0.030	125
					a	0.014	12	0.050	0.023	0.027	112
					b	0.018	15	0.051	0.026	0.025	104
					b	0.016	14	0.044	0.020	0.024	100

APPENDIX 6. PLASMA CAROTENOID AND VITAMIN A LEVELS OF CHILDREN WITH COELIAC DISEASE

Case No.	Age Yrs.	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids O.D. at 460 M.	Carotenoids µgm. per 100 ml.	Vitamin A Optical Density at 328 M. A	B	A - B	Vitamin A I.U. per 100 ml.
1	5	M	13.3	3.10.56	a	0.014	12	0.036	0.020	0.016	67
					a	0.015	13	0.036	0.017	0.019	79
					b	0.017	15	0.054	0.030	0.024	100
					b	0.016	14	0.056	0.030	0.026	108
1	5	M	15.2	7.11.56	a	0.035	30	0.054	0.026	0.028	117
					a	0.035	30	0.063	0.034	0.029	121
					b	0.039	33	0.060	0.025	0.035	146
					b	0.039	33	0.061	0.024	0.037	154
2	2	M	9.2	27.1.56	a	0.031	27	0.050	0.036	0.014	58
					a	0.030	26	0.053	0.037	0.016	67
					b	0.025	21	0.071	0.026	0.045	187
					b	0.035	30	0.110	0.058	0.052	217
2	2	M	9.7	2.3.56	a	0.144	123	0.130	0.086	0.044	183
					a	0.137	117	0.102	0.055	0.047	196
					b	0.125	107	0.226	0.082	0.144	600
					b	0.135	116	0.257	0.125	0.132	550

APPENDIX 6. PLASMA CAROTENOID AND VITAMIN A LEVELS OF CHILDREN WITH COELIAC DISEASE

Case No.	Age Yrs.	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids O. D. at 460 M.	Carotenoids µgm. per 100 ml.	Vitamin A Optical Density at 328 M. A	B	A - B	Vitamin A I.U.per 100 ml.
3	6	F	23	19.12.56	a	0.092	79	0.071	0.033	0.038	158
					a	0.096	82	0.079	0.045	0.034	142
					b	0.088	75	0.159	0.047	0.112	467
					b	0.089	76	0.151	0.037	0.114	475
4	9	F	23	19.12.56	a	0.087	75	0.084	0.055	0.029	121
					a	0.083	71	0.069	0.030	0.039	162
					b	0.082	70	0.134	0.035	0.099	412
					b	0.081	69	0.145	0.049	0.096	400
5	5	F	20.2	3.12.56	a	0.076	65	0.059	0.039	0.020	83
					a	0.078	67	0.060	0.033	0.027	112
					b	0.090	77	0.670	0.088	0.582	2425
					b	0.086	74	0.653	0.103	0.550	2290
6	7	M	22	14.12.56	a	0.029	25	0.054	0.034	0.020	83
					a	0.024	21	0.053	0.026	0.027	112
					b	0.024	21	0.113	0.037	0.076	317
					b	0.024	21	0.112	0.032	0.080	333

APPENDIX 6. PLASMA CAROTENOID AND VITAMIN A LEVELS OF CHILDREN WITH COELIAC DISEASE

Case No.	Age Yrs.	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
7	11	F	23	5.9.56	a	0.032	27	0.058	0.028	0.030	125
					a	0.035	30	0.061	0.025	0.036	150
					b	0.033	28	0.065	0.024	0.041	171
					b	0.035	30	0.071	0.030	0.041	171
7	11	F	23.28	10.10.56	a	0.037	32	0.056	0.030	0.026	108
					a	0.037	32	0.063	0.030	0.033	137
					b	0.036	31	0.107	0.032	0.075	312
					b	0.037	32	0.117	0.035	0.082	342
8	2	M	11.5	5.11.54	a	0.026	22	0.057	0.037	0.020	83
					b	0.028	24	0.125	0.030	0.095	396
8	2	M	16.0	30.7.56	a	0.085	73	0.056	0.026	0.030	125
					a	0.087	75	0.058	0.027	0.031	129
					b	0.095	81	0.442	0.076	0.366	1525
					b	0.095	81	0.450	0.066	0.384	1600
9	3	M	14.0	17.8.56	a	0.240	206	0.056	0.033	0.023	96
					a	0.235	201	0.066	0.040	0.026	108
					b	0.250	214	0.537	0.122	0.415	1729
					b	0.250	214	0.633	0.152	0.481	2004

APPENDIX 6. PLASMA CAROTENOID AND VITAMIN A LEVELS OF CHILDREN WITH COELIAC DISEASE

Case No.	Age Yrs.	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
					a	0.007	6	0.034	0.016	0.018	75
10	11	M	30.8	23.3.55	a	0.017	15	0.043	0.022	0.021	87
					b	0.011	9	0.038	0.020	0.018	75
11	11	F	30.2	20.10.54	a	0.038	32	0.083	0.050	0.033	137
					b	0.032	27	0.080	0.038	0.042	175
					a	0.018	15	0.042	0.020	0.022	92
12	13	M	30.0	25.10.54	a	0.015	13	0.040	0.017	0.023	96
					b	0.017	14	0.040	0.020	0.020	83
					b	0.018	15	0.047	0.021	0.026	108

APPENDIX 7. PLASMA CAROTENOID AND VITAMIN A LEVELS OF CHILDREN WITH RENAL DISEASES

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids O.D. at 460 M.	Carotenoids µgm. per 100 ml.	Vitamin A Optical Density at 328 M. A	Vitamin A Optical Density at 328 M. B	Vitamin A Optical Density at 328 M. A - B	Vitamin A I.U. per 100 ml.
1	5 mo.	M	7.9	29.4.53	a	0.042	36	0.076	0.033	0.043	179
					a	0.045	38	0.074	0.028	0.046	192
					b	0.047	40	0.530	0.126	0.404	1683
					b	0.043	37	0.537	0.120	0.417	1737
2	9 mo.	M	6.4	8.3.53	a	0.043	37	0.049	0.020	0.029	121
					b	0.043	37	0.580	0.133	0.447	1862
					b	0.041	34	0.617	0.154	0.463	1929
3	6 yr.	F	18.9	18.5.53	a	0.063	54	0.050	0.031	0.019	75
					b	0.067	57	0.178	0.043	0.135	521
					b	0.067	57	0.178	0.034	0.144	600
3a	6 yr.	F	20.9	19.8.53	a	0.089	76	0.075	0.035	0.040	167
					a	0.089	76	0.081	0.037	0.044	183
					b	0.117	100	1.255	0.390	0.865	3604
					b	0.093	79	1.158	0.340	0.818	3408
4	7 yr.	F	20.6	8.5.53	a	0.109	93	0.077	0.033	0.044	183
					a	0.101	86	0.080	0.031	0.049	204
					b	0.091	78	0.315	0.056	0.259	1079
					b	0.088	75	0.300	0.040	0.260	1083

APPENDIX 7. PLASMA CAROTENOID AND VITAMIN A LEVELS OF CHILDREN WITH RENAL DISEASES

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
5	10 yr.	F	25.3	6.5.53	a	0.085	73	0.057	0.027	0.030	125
					a	0.093	79	0.077	0.030	0.047	196
					b	0.093	79	0.235	0.071	0.164	683
					b	0.095	81	0.266	0.069	0.197	821
6	11 yr.	M	24.6	15.5.53	a	0.051	44	0.098	0.040	0.058	242
					a	0.057	49	0.098	0.024	0.074	308
					b	0.063	54	0.395	0.048	0.347	1446
					b	0.089	76	0.395	0.051	0.344	1433
7	10 yr.	M	29.0	21.7.54	a	0.135	116	0.105	0.060	0.045	187
					a	0.136	117	0.120	0.067	0.053	221
					b	0.132	113	0.505	0.138	0.367	1529
8	12 yr.	M	30.7	13.4.53	a	0.088	75	0.060	0.021	0.039	162
					a	0.084	72	0.071	0.019	0.052	217
					b	0.087	75	0.625	0.122	0.503	2137
					b	0.084	72	0.650	0.118	0.532	2217

APPENDIX 7. PLASMA CAROTENOID AND VITAMIN A LEVELS OF CHILDREN WITH RENAL DISEASES.

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas Spec.	Carotenoids		Vitamin A			
						O. D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
9	8 yr.	F	19.0	25.9.53	a	0.097	83	0.091	0.031	0.060	250
					a	0.097	83	0.100	0.033	0.067	279
					b	0.120	102	0.185	0.040	0.145	604
					b	0.110	94	0.185	0.040	0.145	604
10	6 yr.	F	21.4	20.11.54	a	0.147	126	0.217	0.123	0.094	392
					a	0.127	109	0.245	0.142	0.103	429
11	6 yr.	M	21.5	218.53	a	0.180	154	0.124	0.080	0.044	183
					a	0.220	188	0.145	0.085	0.060	250
					b	0.205	176	0.420	0.143	0.277	1154
					b	0.210	180	0.460	0.192	0.268	1117
12	8 yr.	M	36.3	7.12.56	a	0.206	177	0.113	0.080	0.033	137
					a	0.206	177	0.138	0.105	0.033	137
					b	0.234	201	0.637	0.170	0.467	1946
					b	0.226	194	0.666	0.190	0.476	1983

APPENDIX 8. PLASMA CAROTENOID AND VITAMIN A LEVELS IN IDIOPATHIC HYPERCALCAEMIA.

Case No.	Age	Sex	Wt. Kg.	Date of Estn	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
1	7 mo.	F	6.5	16.11.54	a	0.035	30	0.061	0.033	0.028	117
					a	0.037	32	0.090	0.035	0.055	229
					b	0.037	32	0.280	0.046	0.234	975
					b	0.036	31	0.325	0.062	0.263	1096
2	8 mo.	M	6.6	10.11.54	a	0.052	45	0.116	0.029	0.087	362
					a	0.047	40	0.119	0.016	0.103	429
					b	0.048	41	0.510	0.098	0.412	1717
					b	0.053	45	0.530	0.095	0.435	1812
3	10 mo.	M	5.8	22.12.54	a	0.082	70	0.115	0.055	0.060	250
					b	0.074	63	0.370	0.078	0.292	1217
4	14 mo.	M	6.5	17.11.54	a	0.073	63	0.146	0.080	0.066	275
					a	0.080	69	0.165	0.062	0.103	429
					b	0.080	69	0.320	0.095	0.225	937
					b	0.088	75	0.350	0.133	0.217	904
5	11 mo.	F	7.0	5.11.54	a	0.033	28	0.080	0.027	0.053	221
					a	0.031	27	0.083	0.034	0.049	204
					b	0.033	28	0.383	0.065	0.318	1325
					b	0.030	26	0.390	0.062	0.328	1367

APPENDIX 8. PLASMA CAROTENOID AND VITAMIN A LEVELS IN IDIOPATHIC HYPERCALCAEMIA

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U.per 100 ml.
6	9 mo.	F	5.5	11.3.53	a	0.091	78	0.059	0.021	0.038	158
					a	0.093	80	0.071	0.034	0.037	154
					b	0.109	93	0.180	0.035	0.145	604
					b	0.112	96	0.184	0.041	0.143	596
7	4 mo.	M	5.4	21.9.55	a	0.070	60	0.127	0.042	0.085	354
					a	0.075	64	0.130	0.045	0.085	354
					b	0.070	60	0.935	0.160	0.775	3229
					b	0.075	64	0.961	0.150	0.811	3379
8	8 mo.	M	5.6	9.9.55	a	0.051	44	0.113	0.055	0.058	242
					a	0.050	43	0.117	0.050	0.067	279
					b	0.051	44	1.055	0.193	0.862	3592
					b	0.053	45	0.985	0.150	0.835	3479
8a	10 mo.	M	6.7	21.11.55	a	0.077	66	0.103	0.058	0.045	187
9	10 mo.	M	7.5	12.10.55	a	0.030	26	0.058	0.023	0.035	146
					a	0.035	30	0.061	0.025	0.036	150
					b	0.030	26	0.331	0.056	0.275	1146
					b	0.035	30	0.342	0.064	0.278	1158
9a	11 mo.	M	8.1	9.11.55	a	0.010	9	0.055	0.015	0.040	167

APPENDIX 8. PLASMA CAROTENOID AND VITAMIN A LEVELS IN IDIOPATHIC HYPERCALCAEMIA

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas. Spec.	Carotenoids O.D. at 460 M.	Carotenoids µgm. per 100 ml.	Vitamin A Optical Density at 328 M. A	B	A - B	Vitamin A I.U. per 100 ml.
10	9 mo.	M	7.2	6.2.56	a	0.042	36	0.096	0.054	0.042	175
					a	0.037	32	0.086	0.040	0.046	192
					b	0.042	36	0.592	0.103	0.489	2037
					b	0.042	36	0.610	0.115	0.495	2062
11	10 mo.	M	8.2	5.12.55	a	0.043	37	0.113	0.055	0.058	242
					a	0.043	37	0.115	0.045	0.070	292
					b	0.046	39	0.650	0.113	0.537	2237
					b	0.046	39	0.665	0.118	0.547	2279
12	8 mo.	M	4.8	14.1.56	a	0.070	60	0.111	0.040	0.071	296
					b	0.069	59	0.412	0.058	0.354	1475
					b	0.069	59	0.434	0.065	0.369	1537
13	7 mo.	M	8.2	28.6.56	a	0.034	29	0.075	0.029	0.046	192
					a	0.022	19	0.070	0.022	0.048	200
14	7 mo.	F	6.3	27.12.55	a	0.031	27	0.041	0.012	0.029	121
					a	0.034	29	0.045	0.015	0.030	125
					b	0.038	33	0.205	0.043	0.162	675
					b	0.037	32	0.211	0.045	0.166	692

APPENDIX 8a. PLASMA CAROTENOID AND VITAMIN A LEVELS IN VITAMIN D RESISTANT RICKETS (Case 15), IDIOPATHIC HYPERCHOLESTEROLAEMIA (Case 16), AND MILIARY TUBERCULOSIS WITH HYPERCALCAEMIA (Case 17).

Case No.	Age	Sex	Wt. Kg.	Date of Estn.	Plas Spec.	Carotenoids		Vitamin A			
						O.D. at 460 M.	µgm. per 100 ml.	Optical Density at 328 M. A	B	A - B	I.U. per 100 ml.
15	9 yr.	F		9.1.56	a	0.091	78	0.080	0.052	0.028	117
					a	0.091	78	0.089	0.055	0.034	142
15	10 yr.			2.7.56	a	0.079	69	0.073	0.034	0.039	162
					a	0.090	77	0.102	0.056	0.046	192
15	10 yr.			7.7.56	a	0.060	51	0.071	0.032	0.039	162
					a	0.068	58	0.070	0.028	0.042	175
					b	0.062	53	0.365	0.050	0.315	1312
					b	0.073	63	0.398	0.067	0.331	1379
16	Adult	F		6.7.56	a	0.253	217	0.162	0.112	0.050	208
					b	0.240	211	0.168	0.120	0.048	200
17	6 mo.	M	4	1.2.55	a	0.039	33	0.072	0.024	0.048	200

BIBLIOGRAPHY

The bibliography is divided into two sections for convenience. The first section subserves Part I entitled "Historical Introduction" and the second section Part II entitled "Author's Investigations."

References quoted in both parts are contained in Section I of the bibliography.

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