SIGHT AND LIFE MANUAL ON VITAMIN A DEFICIENCY DISORDERS (VADD)

by

Donald S. McLaren, MD, PhD, FRCP

Honorary Head, Nutritional Blindness Prevention Programme, International Centre for Eye Health, London, UK

and

Martin Frigg, PhD

Secretary, Task Force SIGHT AND LIFE
PO Box 2116, 4002 Basel, Switzerland

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PO Box 2116
4002 Basel
Switzerland
Phone: +41 61 688 7494
Fax: +41 61 688 1910
E-mail: sight.life@roche.com
Further information:
http://www.sightandlife.org

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FOREWORD TO THE SECOND EDITION

Roche founded the Task Force SIGHT AND LIFE in 1986 to participate actively in the fight against preventable blindness. SIGHT AND LIFE is dedicated to the prevention and eradication of xerophthalmia and all forms of vitamin A deficiency that impair children's health in developing countries.

SIGHT AND LIFE is supporting a broad range of efforts aiming at preventing vitamin A-related blindness and child mortality in developing countries. One of the main activities is to donate vitamin A capsules for rapid intervention, but the Task Force also provides educational material, supports research projects and gives technical assistance. It helps funding projects and provides educational grants. With its Newsletter, SIGHT AND LIFE is offering a platform for the dialogue of experts engaged in the fight against vitamin A deficiency. The publication is covering all relevant topics from most advanced scientific findings in biochemistry to the practical problems of teaching gardening of green leafy vegetables in remote areas.

This handbook is the latest in the diverse range of information tools produced over the last few years by SIGHT AND LIFE. Its primary aim is to present the knowledge contained in existing lecture and pictorial material, some of which has been reworked, in a form that practitioners can put to direct use. The result is a handbook for those working in the "vitamin A front line" in developing countries who need an information tool that presents the complexities in a clear and understandable fashion without trivializing or oversimplifying the issues. Particularly when used together with the slide set, the handbook will serve as an aid to further education and in prevention campaigns.

In Professor Donald S. McLaren we are extremely fortunate to have found an author whose profound knowledge and many years of experience have made him an internationally recognized expert. Dr Martin Frigg, Secretary of SIGHT AND LIFE, contributed the Task Force's fund of accumulated know-how to the publication and helped ensure that the presentation of the material was geared to the needs of the target audience.

Three years after the initial publication, almost 5000 copies of the *SIGHT AND LIFE Manual* have found their way to an interested public of specialists all over the world. It is quite encouraging to see that after such a relatively short time a second, revised edition was necessary: It clearly shows, better than many words, that the body of knowledge on vitamin A deficiency, how it affects people and how it can be fought has grown very fast. And the publication of a thoroughly revised second edition also shows the determination of both authors to share all these new insights with everybody willing to be part of the efforts to reduce vitamin A deficiency disorders all over the world.

Dr Andres F. Leuenberger
Chairman
Task Force SIGHT AND LIFE

PREFACE TO THE FIRST EDITION

The reader of this book should know at the beginning something of the purpose for which it has been written. It is designated a Manual, or Handbook, and indeed is intended to be the kind of book that will be “at hand” as a guide to those interested in and working in this field. There was a time when such a book might be called a Vade Mecum – “come with me”, a companion for study.

For those lecturing and communicating about vitamin A, a slide collection and the accompanying notes have also been prepared for SIGHT AND LIFE at the same time. These are designed to amplify the other material, just as the notes enlarge on the topics that can only be hinted at in the slide presentation.

The Manual takes a very practical approach, dealing with those problems that are of concern to health and nutrition workers, especially those in the fields of child survival and protection of vision. If read through chapter by chapter it will provide a comprehensive and up-to-date account of the subject. The Manual may also be used, to some extent, as a reference text and to assist this process a detailed Table of Contents and Index are provided. However, it has not been the intention that the information provided should be considered to be exhaustive. The story of vitamin A and carotenoids in nature, of the consequences to man of consuming a deficient diet, and of the measures now being taken to eradicate the problem is a fascinating one. It is hoped that many readers will have their appetites whetted to delve much more deeply into the subject. For those readers, in addition to the key references that appear in alphabetical order at the end of the book, a short list of publications for further reading has been provided.

It will also prove helpful to the reader at the outset to appreciate the reasoning behind the choice of the title of the Manual. The term Vitamin A Deficiency (VAD) embraces all forms and degrees of deficiency, including the most severe, in which the function and structure of the eye are affected. All stages of the eye changes are covered by the term Xerophthalmia (X). It is only in the past two decades or so that the threat to health and survival of lesser degrees of VAD has become apparent. Those responsible for setting up SIGHT AND LIFE at Roche are to be commended for their insight when they named it in this way, cov-

ering the implications for morbidity and mortality of milder degrees of VAD, but also including X, which itself embraces the blinding consequences of severe deficiency.

It is now becoming increasingly apparent that VAD is responsible for a wide variety of disorders. Vitamin A Deficiency Disorders (VADD) is proposed as a comprehensive term to cover all aspects of the deficiency state.

So it is that interest in a relatively uncommon blinding disease of children (albeit still the most common cause of blindness worldwide in that age group) that was primarily the responsibility of ophthalmologists and paediatricians has expanded in recent years to include concern about one of the major public health factors in developing countries threatening survival and well-being in young children and other vulnerable groups.

We commence our study with consideration of the roles played in nature by vitamin A and its precursor carotenoids and learn how the chemical and physical characteristics of their molecular structure largely determine their functions. Of central importance for our purposes is knowledge about the food sources, both vegetable and animal, of vitamin A, and these are considered in some detail, together with the various factors that may influence the concentration of nutrients and their availability from the diet. Ultimately a lasting solution to the problem of VADD can only come by ensuring that the dietaries of those at risk provide adequate vitamin A for their needs.

We then turn our attention to what happens to vitamin A in the body once it has been ingested and to what we know about how it fulfils its various functions at the molecular level, an area of knowledge that is the subject of intense research. This leads logically to the need for a balance to be struck between the body's requirements for vitamin A to carry out the various functions under a variety of conditions and the intake from the diet. These requirements are customarily expressed in terms of Recommended Dietary Allowances (RDA). As a prelude to discussion of VADD itself in its various forms, there then follows an account of the existing methodologies for the assessment of vitamin A status and their application for drawing up guidelines for defining the existence and extent of a problem of VAD. Recently much attention has been given to the development of indicators for assessing subclinical VAD and it will be necessary to point out some of the difficulties being encountered in this complex field.

There then follow three chapters that describe the ocular manifestations of vitamin A deficiency (xerophthalmia), the contribution of VAD to mortality and morbidity, especially in young children, and its at present only partially understood role in growth retardation, impairment of the immune response, defective haemopoiesis and some other disorders. Two further chapters describe the global prevalence of VADD, actively under review by the World Health Organization, and its epidemiology.

The final section is devoted to the all-important subject of the control of VADD. This is dealt with under seven headings – treatment, prophylaxis, prevention and management of infectious diseases, fortification, dietary modification, plant breeding, and disaster relief.

Finally, we are very grateful to all those authors of the original work which we have used and for their contribution to this en-

deavour. In particular it will be evident how dependent we have been on the timely publication of the monumental book *Vitamin A Deficiency: Health, Survival, and Vision* by Alfred Sommer and Keith West of Johns Hopkins University. Where for our purposes tables or figures have been simplified, readers requiring full particulars should refer to the original sources, which are cited here. It would be appreciated if readers would inform us of any errors they may come across. The intention is to keep the Manual under regular revision and updating, and suggestions in this regard would be welcome.

Donald S. McLaren and Martin Frigg

PREFACE TO THE SECOND EDITION

The first edition of the *SIGHT AND LIFE Manual on Vitamin A Deficiency Disorders (VADD)* was produced in September 1997. Just over three years later this second edition is appearing. During that relatively short time supplies of the earlier edition have been exhausted, and many people have been able to read the Manual in their own language, in Spanish with widespread distribution in Latin America and in Chinese. The translation into Farsi is ready to be printed, and this second edition is being translated into French. Other translations may appear in the future. The Slide Set issued in more limited fashion at the same time has also been widely appreciated.

There are several reasons why a new and revised edition would appear to be justified at this time. In certain sections in particular, advances in recent years have been so great that the information had to be considered to be seriously out of date. For example, it has recently become much better understood exactly how vitamin A carries out its varied functions in virtually every part of the body. In the cell nucleus acid compounds of vitamin A (all-*trans* retinoic acid and other related retinoids) bind to and activate specific nuclear receptors to induce or repress hundreds of genes. These retinoids are now recognized to have the functions of a hormone belonging to what has been termed the Steroid-Thyroid-Retinoid Superfamily.

The complexity of the many factors determining the bioavailability of provitamin A carotenoids in the body is only now beginning to be appreciated. Techniques have recently been developed which enable the determination with some accuracy of the fraction of ingested carotenoid that is available

for utilization by the body. In general it appears that estimations in the past have been over-optimistic and this seems to have been especially true in the case of dark green leafy vegetables. These have been regarded as the mainstay of the approach to the improvement of the intake of vitamin A from vegetable sources in developing countries. This clearly has important implications for the design of dietary interventions.

During this time too attention has been drawn to the potential importance of vitamin A and β-carotene supplementation during pregnancy to maternal health and survival and also to that of the fetus and very young infant. It has become quite clear that stronger emphasis needs to be given to the role of vitamin A status in the area of maternal as well as child health. In the first edition only passing reference was made to those aspects of vitamin A and carotenoid metabolism that do not refer directly to VADD. Research in some of these areas has recently increased to such an extent that a new chapter on "Vitamin A in general medicine" has been included. This chapter covers topics such as secondary or endogenous vitamin A deficiency, hypervitaminosis A, therapeutic use of retinoids, and the role of non-provitamin and provitamin carotenoids in the prevention and treatment of diseases such as cancer, heart disease and some eye diseases.

Finally, we have tried to accede to the request of some readers that the final chapter on control of VADD be given greater prominence. After all this is the focus and climax of all the effort that has been building up since the early days when eventually the international community acknowledged that there was a serious problem and set about tackling it.

The opportunity to revisit this theme has been an enlightening and encouraging experience. It is good to know that research and intervention efforts in this field continue unabated. The reader will find here a wide range of challenges to both head and heart. The wonders of the colours in the world around us will take on new meanings. The intricacies of the fundamental processes of life will never cease to amaze. The needless destruction and blighting of innocent lives through the lack of a few units of a vitamin on display all around us will continue to move our hearts and hands until the need has been ended.

Donald S. McLaren and Martin Frigg

October 2000

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VITAMIN A IN NATURE

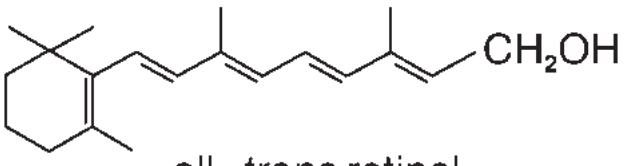
Introduction

Strictly speaking the term Vitamin A should be restricted to the chemical substance all-*trans* retinol (see Glossary, *Cis/trans*), but it is justified in the present context to follow a less strict definition. The alcohol, retinol ($C_{20}H_{30}O$, see Figure 1.1), is usually represented by some closely related forms in nature.

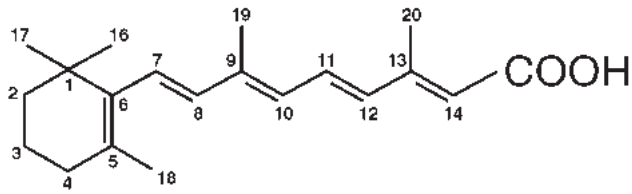
Thus it is common practice to say that vitamin A is stored in the liver, or that vitamin A is required for the normal functioning of the rod cells in the retina. In actual fact retinyl palmitate is the usual storage form of the vitamin, and 11-*cis* retinal is the very specific form of vitamin A that acts as the prosthetic group attached to the protein opsin to form rhodopsin, or visual purple, required for normal night vision.

In almost all tissues vitamin A functions are carried out by the acid form, retinoic acid (RA). Specifically this is the all-*trans*-retinoic acid form, but recently other functional acid derivatives have emerged (see p 29). As a result of the recent revolutionary advances in our knowledge of the mechanisms of action of vitamin A at the cellular level in the form of RA this compound is now classified as a hormone (see p 29).

Vitamin A, in all its closely related forms, is only present in nature as a result of enzymic action that takes place on certain precursor



all–*trans* retinol



all–*trans* retinoic acid

Figure 1.1. Formulae of retinol and retinoic acid.

compounds within the bodies of most vertebrate animals. These precursors of vitamin A comprise a small proportion of a large group of compounds known as carotenoids.

Occurrence of carotenoids

It will be most instructive for us to commence our exploration of vitamin A in nature with a consideration of the much more prevalent and diverse carotenoids (Karrer, Jucker, 1950). Carotenoids are the most widespread of all groups of naturally occurring pigments. They are red, orange or yellow in colour and are found in many plants and animals. Nature produces about 100 million tons of carotenoid pigments per year. Most of this is fucoxan-

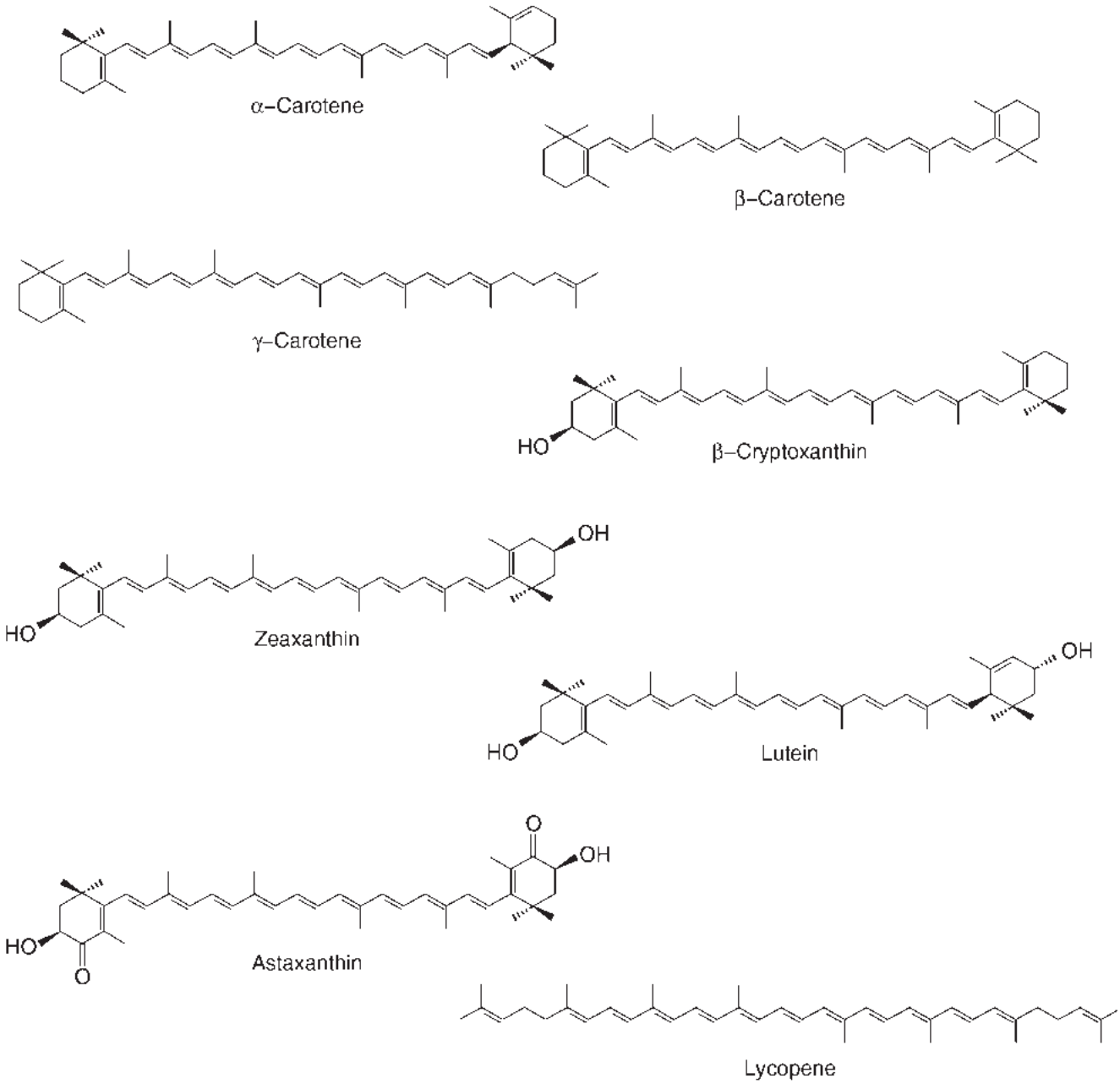


Figure 1.2. Formulae of some common carotenoids.

thin, the characteristic pigment of many marine brown algae and the most abundant of all carotenoids. In the leaves of green plants lutein, violaxanthin and neoxanthin are the three main carotenoids and none is capable of being converted to vitamin A. Until the present more than 600 carotenoids, exclusive of *cis-trans* isomers, have been isolated and fully characterized. They share common structural features, such as the polyisoprenoid structure and a series of centrally-located bonds (Figure 1.2) (Olson, 1994). Most of the carotenoids are xanthophylls, which have one or more oxygen groups on the ring or in the chain. Xanthophylls are responsible for the glorious autumnal tints in leaves of deciduous trees. Carotenoids of this group also contribute to the striking plumage of birds such as the cock-of-the-rock, the flamingo and many others. The attractive colours of many

red and yellow fruits and vegetables are also attributable to their carotenoid content. These are only a few of the most evident examples of carotenoids in nature. They also occur in algae, fungi, yeasts, moulds, mushrooms, bacteria and in all classes of plants and animals, including mammals. No animal is able to synthesize carotenoids. Some may alter them slightly by oxidative metabolism during digestion and absorption. Many mammals accumulate carotenoids in their tissues, and there is recent evidence that this may possibly prove to have a function in some rare instances (see Chapter 10).

Animals differ greatly in the way that carotenoids from their diet accumulate in their tissues, especially in adipose tissue (Bauerenfeind, 1981). The reason for these differences is not understood. Man accumulates carotenoids indiscriminately and they remain *in situ* when fat is mobilised as in starvation. Cattle and horses accumulate mainly carotenes. Pigs and goats accumulate few carotenoids or none. Birds accumulate oxycarotenoids.

Occurrence of vitamin A

Certain carotenoids are capable of conversion to vitamin A. This is known to occur in insects, fish, reptiles, birds and mammals. Among the latter, members of the carnivorous cat family do not have this ability. These provitamin A carotenoids, as they are known, number more than 50 and are listed in Bauernfeind (p 179, 1981). Of these, all-*trans* b-carotene makes by far the largest contribution to vitamin A activity in foodstuffs. a-carotene, g-carotene and b-cryptoxanthin (3-hydroxy-b-carotene) contribute to a lesser extent.

For provitamin A activity to occur, there are certain molecular structural requirements. The compound must include at least one unsubstituted β-ionone ring and a polyene side chain. The other end of the molecule may have a cyclic or an acyclic structure. It may be lengthened but not shortened to less than an 11-carbon polyene chain. Chain lengthening decreases activity.

Table 1.1 gives a selection of provitamin A activities of carotenoids expressed in terms of that of β-carotene, which is considered as 100%.

Much interest has recently arisen in the occurrence and metabolism of 9-*cis* β-carotene. This is because it has been reported to be a precursor of 9-*cis* retinoic acid, which is the ligand for the nuclear retinoid X receptors (see Chapter 3). It has been estimated that its provitamin A activity may be as high as 57% of that of all-*trans* β-carotene. It is present in significant amounts in most dietaries but appears to undergo isomerization to a considerable extent before entering the

Table 1.1. Relative provitamin A activity of various carotenoids (Simpson, Tsou, 1986)

Carotenoid	Activity (%)
β-carotene	100
α-carotene	50–54
γ-carotene	42–50
3,4-dehydro-β-carotene	75
β-carotene-5,6-epoxide	21
α-carotene-5,6-epoxide	25
3-oxo-β-carotene	52
3-hydroxy-β-carotene (cryptoxanthin)	50–60
4-hydroxy-β-carotene	48
β-2'-apo-carotenal	active
β-8'-apo-carotenal	72
lycopene	inactive
lutein	inactive
3,3'-dihydroxy-β-carotene (zeaxanthin)	inactive

bloodstream. It may therefore not be a rich source of 9-*cis* retinoids for tissues (You, Parker, Goodman et al, 1996).

The richest sources of vitamin A in nature are livers of some fish, most notably those of halibut, cod and shark. Those animals that are at the end of a long food chain in which first carotenoids and then vitamin A itself are progressively concentrated at each stage build up the highest concentrations. Very high levels occur in polar bear and bearded seal without apparently harming the animal. However, it has long been known that polar explorers and their sled dogs have fallen sick when feeding on the liver of these animals. This is an instance of acute hypervitaminosis A or vitamin A toxicity (McLaren, 1993) (see Chapter 10). If large doses of vitamin A or one of the synthetic retinoids are taken during early pregnancy the fetus may be damaged. The subject of safe use of vitamin A is considered later (see Chapter 11). Kidney, plasma, milk and those tissues where vitamin A is known to have its main functions, such as the eye and epithelial tissues, have very low concentrations in comparison with those found in the liver.

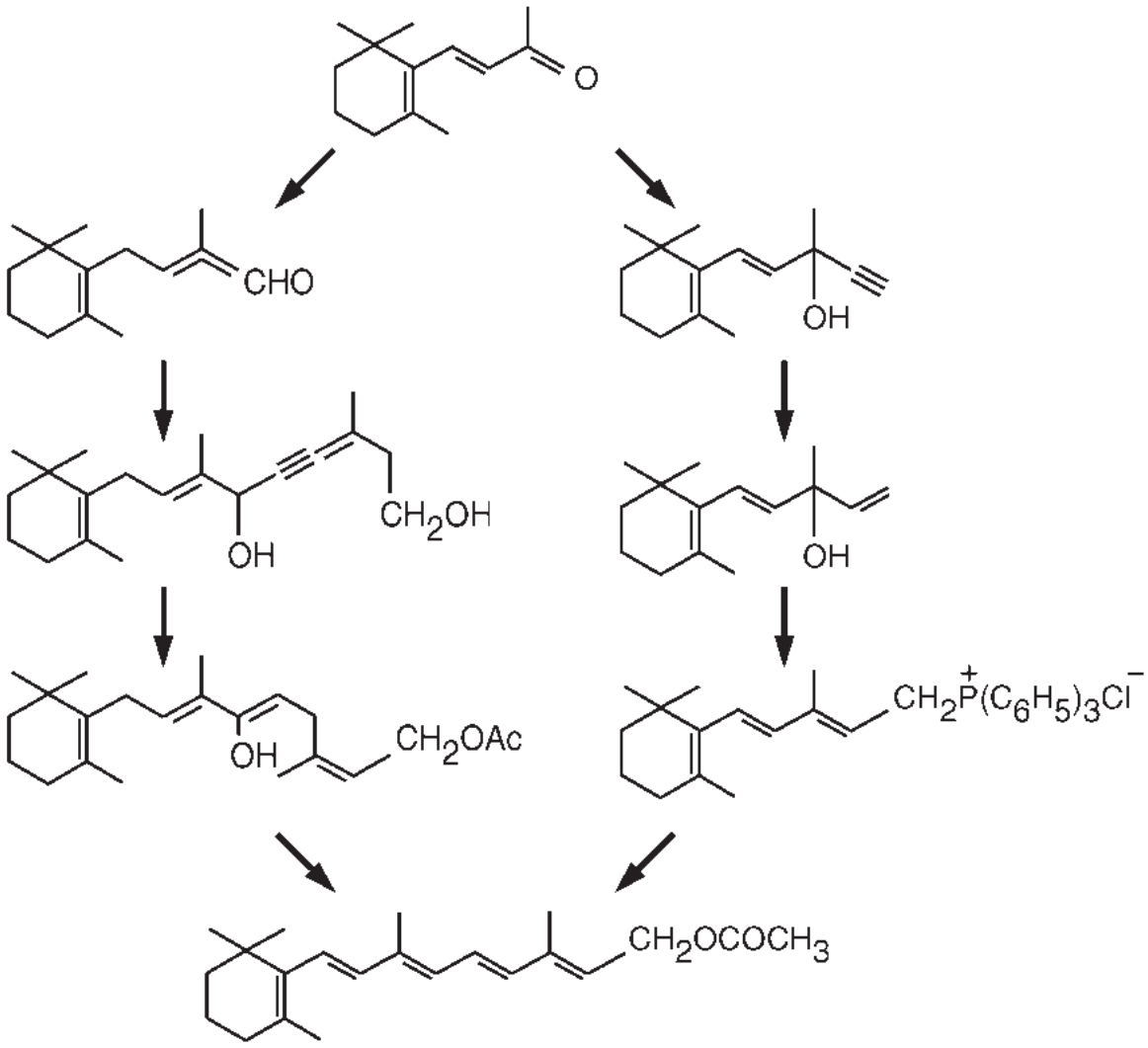


Figure 1.3. Major commercial routes to the synthesis of retinyl acetate (bottom) from, β-ionone (top). The Isler (Roche) procedure is on the left, the Wittig (BASF) method on the right (Olson, 1999).

Vitamin A in liver and other tissues is mainly present in esterified form. Pharmaceutical preparations which are identical with natural sources, both chemically and biologically, exist also in the form of fatty acid esters: retinyl acetate, propionate or palmitate, which are more stable than retinol.

In recent years there has been intense interest in the therapeutic use of retinoids in some dermatological disorders and their possible prophylactic value in epithelial cancers and other diseases (see Chapter 10).

Vitamin A has been added to a wide variety of foods in order to improve their nutritional value (Bauernfeind, 1981). Food fortification with vitamin A is one of the measures available for control of VADD (see Chapter 11). The major commercial forms are the acetate or palmitate esters, which are relatively stable and have high solubility in oils and other commercial preparations. Esters can be incorporated into a carrier matrix, e.g. gelatine, which protects them from oxidation as in cooking. Such forms have been used frequently in food fortification and animal feeds. Under good storage conditions a high vitamin A activity is retained for a considerable period of time.

As a footnote it is of interest that vitamin A has been found in the plant kingdom (Stoeckenius, Bogomolni, 1982). The report of a new retinaldehyde-containing pigment, bacteriorhodopsin, in the membrane of the purple bacterium *Halobacterium halobium* caused great interestΔ Its basic function is, under the influence of light, to assist in the pumping of protons across membranes, thus possibly aiding the active transport of nutrients. The structure and functions of bacteriorhodopsin are very similar to those of rhodopsin; as are several other related pigments in Halobacteria (Olson, 1991).

Chemistry

The structural formulae of β-carotene and vitamin A were established by Karrer and colleagues at the University of Zurich between 1928 and 1931. Isler and co-workers, at Roche, were responsible for a commercially feasible synthesis of β-carotene and vitamin A. Karrer was awarded the Nobel prize for chemistry in 1937. Figure 1.3 shows the major commercial routes for the synthesis of vitamin A.

Carotenoids (Britton, 1995)

In nature only plants and microorganisms are capable of synthesizing carotenoids. Acetate is the starting compound. Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, which coalesces with acetyl-CoA. After several other steps the important biological five-carbon isoprenoid unit, isopentenyl diphosphate (IDP, see Figure 1.4) is formed. Successive condensations result in ten, twenty, and ultimately forty-carbon units. The last of these forms phytoene, from the tail-to-tail linkage of two C_{20} geranylgeranyl diphosphate molecules to form the basic 40-carbon acyclic carotenoid structure (see Figure 1.4).

One classification system subdivides the carotenoids into acyclic, monocyclic and bicyclic derivatives. Respective parent compounds of these categories are lycopene, γ-carotene and β-carotene. In former times the prefix “neo” was used to designate a carotenoid stereoisomer with at least one *cis* configuration in the double-bond chain. Nowadays the position of a *cis* double bond can normally be assigned unambiguously by spectroscopic methods. The prefix “apo” designates a carotenoid that has been derived from another carotenoid by loss of a structural element through degradative action. The prefix “pro” designates some poly-*cis* carotenoids.

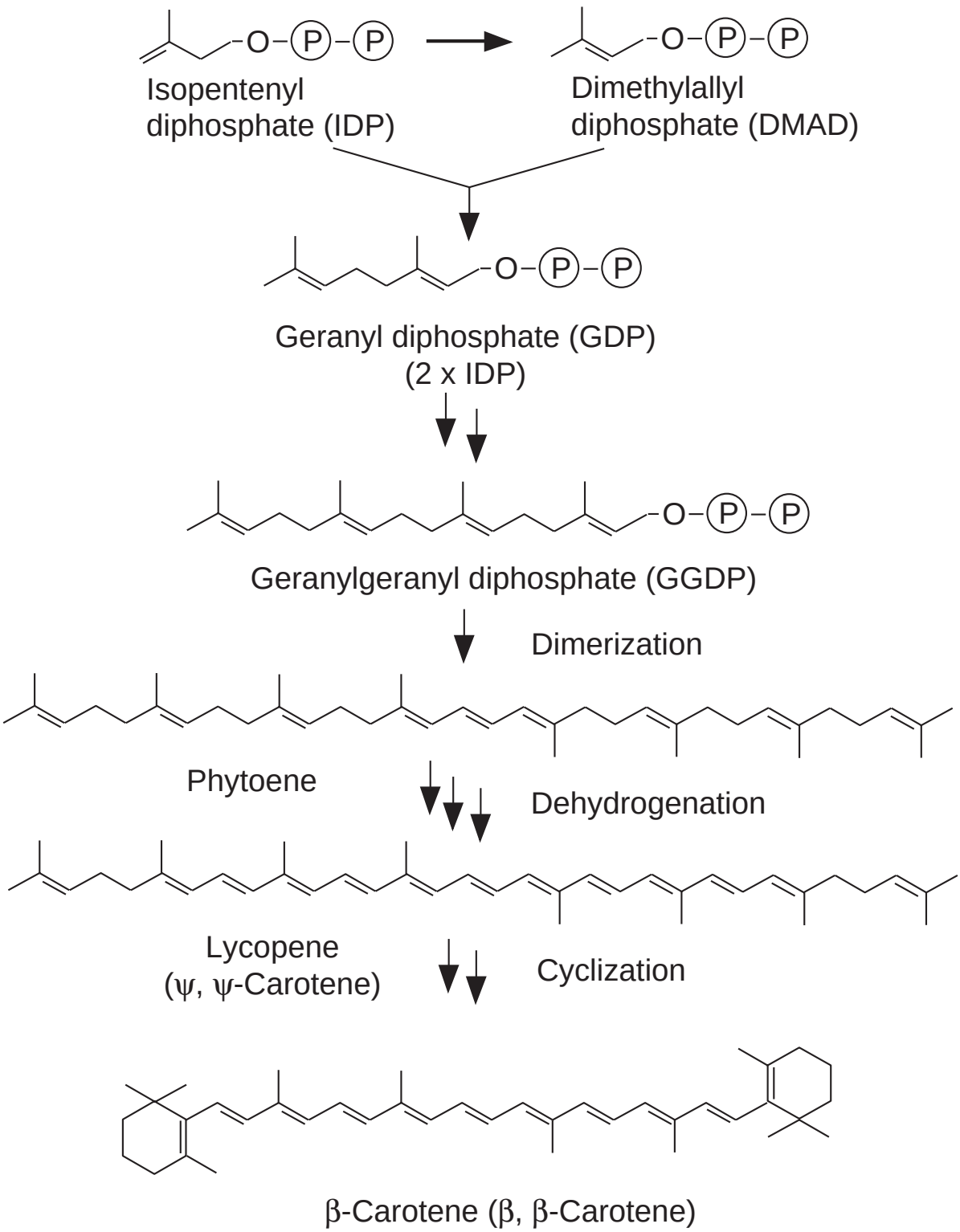


Figure 1.4. Carotenoid biosynthesis from isoprenoid units (IDP, DMAD).

Because carotenoids possess many conjugated double bonds – usually 9 to 13 – each can form many geometric isomers. The total theoretically possible number of compounds in the whole class runs into hundreds of thousands. Until recently attention has been focussed on a few dozen carotenoids that are in the all-*trans* configuration. The presence of a *cis* double bond predisposes to increased thermodynamic instability.

The most characteristic feature of carotenoid structure is the long system of alternating double and single bonds, the polyene chain, that forms the central part of the molecule. It is this that gives carotenoids their distinctive molecular shape, chemical reactivity and light-absorbing properties. Usually, the most stable form of the polyene chain is a linear, extended conformation, as in lycopene.

The ability of carotenoids to absorb visual light is related to the presence of delocalized π-electrons. In plants energy transfer can take place from excited carotenoids to generate excited chlorophyll, which is active in photosynthesis. In leaves carrying out photosynthesis the physical structure of the chloroplast, the subcellular structure containing the carotenoid, facilitates this transfer of energy to chlorophyll (Britton, 1995).

Under conditions of high light intensity the triplet state of chlorophyll can accumulate and cause damage. Carotenoids can counteract this effect in two ways: by deactivating the triplet state of chlorophyll or by converting singlet-state oxygen to its ground triplet state and again allowing release of the transferred energy as heat (Britton, 1995).

Because of their high degree of unsaturation carotenoids can extract or donate electrons, resulting in radical anions and cations which can react with oxygen or other molecules, showing both antioxidant and prooxidant properties under various conditions. It is important to realize that these properties appear to be in no way related to provitamin function. At the present time a great deal of research effort is being directed towards the potential role of carotenoids and other antioxidant nutrients in the prevention of major chronic diseases such as cancer and coronary heart disease (see Chapter 10).

As a group, carotenoids are nonpolar and extremely hydrophobic with virtually no solubility in water. They are therefore restricted to hydrophobic areas in cells, such as the inner core of membranes. The formation of carotenoid-protein complexes in nature allows them access to an aqueous medium such as blood plasma.

The many activities of carotenoids have been classified into functions, actions, and associations (Olson, 1996) and this topic is explored on this basis in Chapter 3.

Vitamin A

Vitamin A is now considered to be a subgroup of a class of compounds known as retinoids. All retinoids are derived from a monocyclic parent compound containing five carbon-carbon double bonds and a functional group at the end of the acyclic portion. The term Vitamin A is used generically for all β-ionone derivatives (other than carotenoids) that have the biological activity of all-*trans* retinol.

Both vitamin A and carotenoids are soluble in most organic solvents but not in water. Like other hydrophobic substances their transport within the body, which is over 60% water, poses problems which have been overcome by a variety of means (see Chapter 3). Their sensitivity to oxidation, isomerization, and polymerization leads to their ready destruction especially when adsorbed as a thin surface film in the presence of light and oxygen. This has important implications for the storage and analysis of biological tissues.

Methods of analysis

Analytical procedures

Vitamin A has several physical properties which have been exploited in its analysis in the past. These include a characteristic ultraviolet (UV) absorption with an absorption maximum of 325 nm and greenish fluorescence at 470 nm when excited at 325 nm. A blue chromophore, which is unfortunately transient, forms on exposure to certain Lewis acids, such as antimony trichloride (Carr-Price reaction) and trifluoroacetic acid (Neeld-Pearson reaction). Carotenoids also have characteristic UV absorption spectra. Nowadays vitamin A and carotenoids are most generally measured by high-performance liquid chromatography (HPLC). Straight-phase HPLC is most suitable for separating *cis* and *trans* isomers, and reverse-phase HPLC best separates compounds of different polarity. To prevent oxidation and polymerization, samples should be immediately analyzed or stored frozen in the dark at -70ºC (Furr, Barua, Olson, 1992).

Recently there has been increased interest in the use of plasma retinol-binding protein (RBP) as a surrogate for plasma retinol. This has been measured by radial immunodiffusion (Almekinder, Manda, Kumwenda et al, 1999) and in a rapid field test using dried blood spots by fluorometry (Craft, 1999), and also by HPLC (Craft, Haitema, Brindle et al, 2000).

Bioassay procedures

Biological tests have played a very important part in assessing vitamin A activity. These include the classical growth response tests in vitamin A-deficient rats, liver storage assays in rats and chicks, and the vaginal smear technique. Cell culture systems have been introduced for assessing the biological activity of retinoids (Olson, 1991).

Carotenoid analysis in foods

Broadly speaking there are two main methods for estimating carotenoid content of foodstuffs – open-column method and HPLC (Rodriguez-Amaya, 1999). The open-column method is simpler and much less costly and quite satisfactory for the main four or five carotenoids in a sample.

FOOD SOURCES

Human communities rely on a very wide range of plant and animal foods to meet their dietary requirements for vitamin A. Ovo-lacto vegetarians and those who eat no foods of animal origin (vegans) can usually obtain all their nutritional needs from plant sources alone, with the exception of vitamin B_{12}. Animal products are usually expensive and are rarely relied on almost exclusively to meet requirements. Figure 2.1 shows the food supply of vitamin A for the period 1979–81 in the world as a whole and in 6 regions. It is not thought that the data have altered substantially since. It should be noted that Asia and Africa, where the most serious problems of VADD occur, place the greatest reliance on vegetable sources. Furthermore, inter-regional variations are greater for preformed vitamin A intake than for total vitamin A availability. These facts should have some influence on food policy as it bears upon the prevention of VADD.

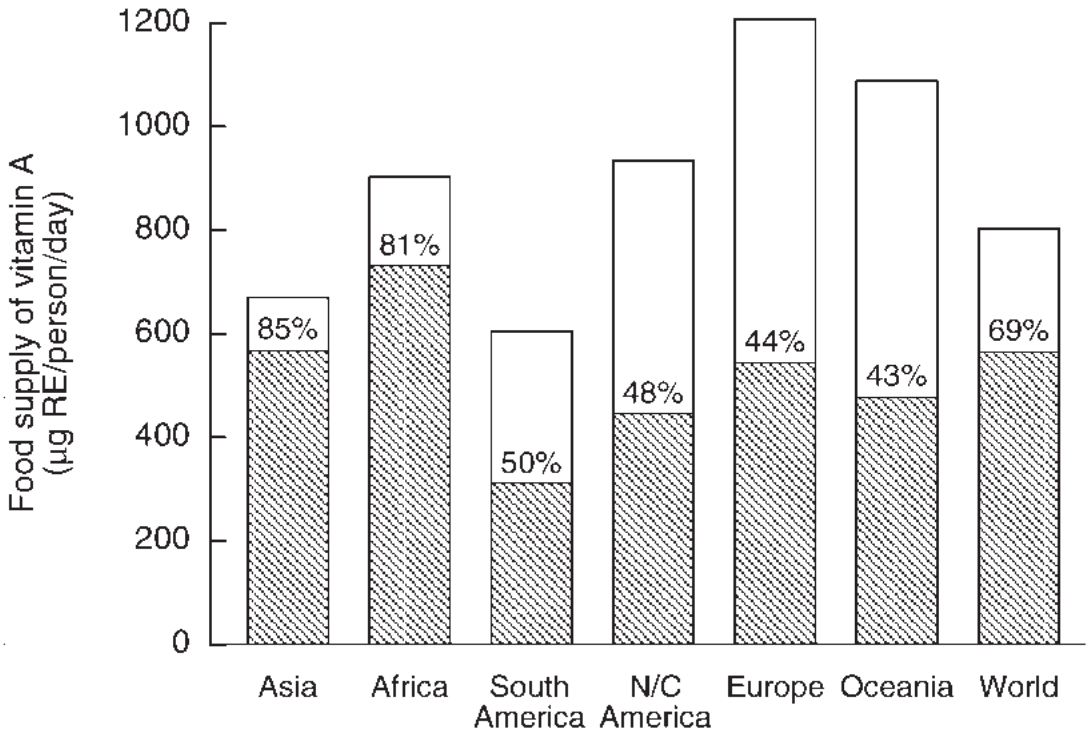


Figure 2.1. Food supply of total vitamin A partitioned by the percentage available from provitamin A carotenoids (cross-hatched segments, % indicated) and preformed vitamin A food sources (open segments), for the period 1979–81 (FAO/WHO Expert Consultation, 1988).

The data in Figure 2.1 are for provitamin A carotenoid intake. Although similar data for nonprovitamin carotenoid intake are not available, from what is known of the carotenoid composition of common fruits and vegetables it is probable that the intake of nonprovitamin carotenoids is even greater than that of provitamin carotenoids. The significance of this for human health has only been becoming evident in recent years (see Chapter 10).

Units of vitamin A activity

Earlier analytical methods failed to distinguish between individual carotenoids and as a result nonprovitamin carotenoids, were frequently included along with those with vitamin A activity when concentrations were reported in food composition tables for fruits and vegetables. For example, one analysis showed that as little as 7% of the carotene value estimated was actually β-carotene or provitamin A. The introduction of HPLC paved the way for the solution of this problem but it has taken time for accurate analyses to be made and for the results to replace the older, inaccurate values in food composition tables (see Table 2.1). It is clearly important to check the method of analysis before utilizing values for provitamin A carotenoid activity in publications.

Another problem that has to be addressed with regard to the vitamin A activity of foodstuffs is the need to make allowances for the differences in activity between retinol itself and β-carotene and also the need to differentiate between the activity of β-carotene and other provitamin carotenoids. The reasons for these differences will be considered later (see p 16). The generally accepted position on the relationships between different expressions of vitamin A activity up until the present time is as shown in Table 2.2.

The values for provitamin activity are approximations because the bioavailability and bioconversion of carotenoids may be influenced by many different factors. The doubt that has recently been cast on the efficacy of dark green leaves and some other sources of provitamin A has stimulated much research activity in this field (see p 18).

Table 2.1. Provitamin A values in tomatoes: comparison of different analytical methods (Simpson, Chichester, 1981)

Methodology	Average value µg/g
β-carotene by HPLC	1.218
β-carotene and lycopene by HPLC	11.001
AOAC* method	18.063

* Association of Official Analytical Chemists

Provitamin A carotenoid sources

Dark green leafy vegetables, yellow fruits, orange roots – mainly carrots – and the oils of palms are the main sources of provitamin A. Among leaves only those that are dark green are really good sources. This is because their carotenoid content in chloroplasts is roughly proportional to the concentration of chlorophyll with which they are associated for photosynthesis. Edible dark green leaves are readily available in most areas where VADD are a problem. The species vary considerably from place to place and Table 2.3 shows just a few examples of typical provitamin A values for some that are commonly consumed and have proved useful in intervention programmes. More exhaustive lists of similar data on a national or regional basis are becoming increasingly available and some examples are included in this Manual in Further Reading.

Table 2.2. International Units (IU) and Retinol Equivalents (RE)

Compound	µg/IU	IU/µg	RE/µg	µg/RE
all-*trans* retinol[a]	0.300	3.33	1.000	1.0
all-*trans* retinyl acetate	0.344	2.91	0.873	1.15
all-*trans* retinyl palmitate	0.549	1.82	0.546	1.83
all-*trans* β-carotene[b]	1.800	0.56	0.167	6.0
Mixed carotenoids[c]	3.600	0.28	0.083	12.0

a: Molecular weight = 286.44; 1 µmol = 286.44 µg, 10 µg/dL = 0.35 µmol/L, 1 µg = 0.00349 µmol; 60 mg = 200,000 IU = 209.5 µmol
b: Molecular weight = 536.85
c: Provitamin A carotenoids other than β-carotene

Vegetables

Current analyses tend to report the content of each carotenoid identified, irrespective of presence or absence of provitamin activity. This has the additional value of indicating the value of the foodstuff as a source of antioxidant activity. Table 2.4 shows the lutein and β-carotene concentrations in green vegetables.

Table 2.3. Examples of common vegetable/fruit-carotenoid sources

	µg RE/100 g edible portion
Mango (golden)	307
Papaya (solo)	124
Cucurbita (mature pulp)	862
Buriti palm (pulp)	3,000
Red palm oil	30,000
Carrot	2,000
Dark green leafy vegetables	685
Tomato	100
Apricot	250
Sweet potato, red and yellow	670

In leafy vegetables β-carotene and lutein are the major carotenoids and together account for over 80% of all carotenoids. α- and γ-carotene, cryptoxanthin and lycopene are minor components.

Carrots are increasingly being grown in parts of the developing world and may vary considerably in carotenoid content (see Table 2.5). A characteristic feature is the relatively high concentration of α-carotene, usually about half of that of β-carotene.

In tomatoes lycopene usually far exceeds the concentration of β-carotene, although some varieties rich in β-carotene have been developed. Tomato and tomato products are by far the best source of lycopene, which is of considerable interest in relation to some prevalent diseases worldwide (see Chapter 10).The main carotenoids in pumpkin are α-carotene, β-carotene and lutein.

Table 2.4. Lutein and β-carotene concentrations in green vegetables (μg/100 g fresh weight) (Ong, Tee, 1992)

Vegetable	Lutein	β–Carotene
Green, leafy* (4 types)	–	330–5,030
Green, nonleafy (6 types)	–	217–763
"Cruciferous" vegetables (5 types)	280–34,200	80–14,600
Leafy vegetables (32 types)	–	1,000–44,400
Tuberous vegetables and beans (16 types)	–	40–1,700
Green, leafy* (7 types)	250–10,200	1,000–5,600
Other vegetables (19 types)	trace–440	11–430
Green, leafy* (27 types)	73–29,900	97–13,600
Green, nonleafy (8 types)	142–460	74–569

* Values from different references

Fruits

The vitamin A activity of fruits is generally lower than that of leafy vegetables and their carotenoid content is more complex. Their greater acceptability, especially to young children, is an advantage as far as intervention programmes are concerned (see Table 2.6). The "sweet paste" of the buriti *(Mauritia vinifera)* fruit in north and central Brazil is almost as rich in provitamin A carotenoids as is red palm oil (see below).

Roots and tubers

Fewer analyses have been carried out on roots and tubers than on vegetables or fruits and most of the varieties examined have had low contents (see Table 2.7). However, the higher values for some pigmented varieties suggest that plant breeding for these might be of importance.

Vegetable oils

Carotenoids are present in most oils that are consumed but usually in low concentration. Red palm oil (*Elaeis* sp. see Table 2.8) contains the highest carotenoid concentration in the vegetable kingdom and has been extensively studied, for its unsaturated fatty acid content as much as for its high β-carotene content. It is also a rich source of other dietary substances such as vitamin E, ubiquinones and phytosterols. Different species have different concentrations (see Table 2.8) as do different oil extracts (see Table 2.9). It is the main cooking oil in most regions of west and central Africa, but the tree is also cultivated in parts of Asia and of South America. Red palm oil used in cooking imparts a distinctive taste to food that may not be readily accepted by some people. Highly refined red palm oil is increasingly being made available and is much more readily accept-

able than the crude form. Many authorities see a major role for this oil in the control of VADD in the future (Kritchevsky, 2000; Nagendran, Unnithan, Choo et al, 2000; and Rao, 2000), see Chapter 11.

Other sources

Hen's eggs are often considered to be a rich source of provitamin A because of the rich colour, but the major pigments are lutein and zeaxanthin, and β-carotene makes up less than 7% of the total. Some fish flesh is brightly coloured, but most of the pigment are xanthophylls.

Table 2.5. α- and β-carotene concentrations in carrots (μg/100 g fresh weight) (Ong, Tee, 1992)

Carrot	α-Carotene	β-Carotene
Raw	2,000–5,000	4,600–12,500
Canned	3,200–4,800	7,000–11,000
Frozen	8,400–8,800	26,000–28,100
Raw	3,790	7,600
Raw, A+ hybrid	10,650	18,350
Freshly cooked, A+ hybrid	15,000	25,650
Canned	2,800	4,760
Line B6273		
Lyophilized	3,400	6,000
Raw	3,200	5,200
Frozen	3,100	5,100
Line B9692		
Lyophilized	6,100	13,800
Raw	6,600	11,700
Frozen	6,600	11,600
HCM line		
Lyophilized	20,300	28,200
Raw	20,600	25,100
Frozen	20,400	25,500
Raw		
19 cultivars	2,200–4,900	4,600–10,300
Raw	3,410	6,770

Table 2.6. Carotenoid concentrations in fruits (μg/100 g fresh weight) (Ong, Tee, 1992)

Fruit	Lutein	Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
Banana	20–40	0	0	60–160	40–100
Berries, grapes, black currant	20–200	0	0	0–60	6–150
Mango	–	–	–	–	63–615
Orange, mandarin	20–30	7–300	–	20	25–80
Papaya, watermelon	0	450–1,500	2,000–5,300	0	228–324
Starfruit	60	1,070	0	0	28

Natural extracts containing carotenoids have long been used for colouring foods to make them appear more attractive. These have included extracts from leaves, carrots and red palm oil. β-carotene was the first synthetic carotenoid to be used as a food colour and others are β-apo-8'-carotenal and canthaxanthin. The former two have vitamin A activity and therefore they also contribute to nutrient intake.

Table 2.7. Carotenoid concentrations in selected roots and tubers (μg/100 g fresh weight) (Ong, Tee, 1992)

Root or tuber	Lutein	Cryptoxanthin	Lycopene	β–Carotene
Sweet potato				
Different varieties	–	0	–	5–551
	–	–	–	1–4
Yellow variety	25	0	42	19
Orange variety	7	27	147	1,140
Cassava				
White variety	2	3	1	20
Yellowish	–	–	–	40–790
Potato	13–60	Trace	Trace	3–40
Taro	3–31	1	1–3	2–16

Table 2.8. Total carotenoids (ppm, estimated at 446 nm) from various oil palm species (Ong, Tee, 1992)

E.o.	4,347
E.o. x *E.g. (D)*	1,846
E.o. x *E.g. (P)*	1,289
E.o. x *E.g. (D)* x *E.g. (P)*	864
E.g. (P)	380
E.g. (D)	948
E.g. (T)	610

E.o., Elaeis oleifera; E.g., Elaeis guineensis; D, Dura; P, Pisifera; T, Tenera

Table 2.9. Carotenoid contents of palm oil extracts (ppm) (Ong, Tee, 1992)

Crude palm oil	630–700
Crude palm olein	680–760
Crude palm stearin	380–540
Second pressed oil	1,800–2,400
Residual oil from fibre	4,000–6,000

Bioavailability of carotenoids

Of all the sections in this book this one has probably undergone the most significant developments since the 1st edition was written in 1996–97. The advances that are taking place in our understanding of the bioavailability of provitamin A carotenoids is having a profound impact on the approaches that are being adopted to the control of VADD (see Chapter 11).

Although it is all too easy to pass judgement with the benefit of hindsight it does nevertheless seem quite astounding that decisions that were taken on little or no evidence in the mid 1960s were allowed to remain unchallenged and have formed the basis for action for the subsequent 30 years and more. The Joint FAO/WHO Expert Group (1976) introduced the term Retinol Equivalent and assigned β-carotene 1/6 and other provitamin A carotenoids 1/12 the value of preformed vitamin A on the very scanty evidence available at that time (Hume, Krebs, 1949). The concept has proved to be of immense help in assessing the equivalence of different sources of vitamin A activity. The actual values remained untested, despite a strong call from the committee for research, and led to a false sense of security until challenged in the mid 1990s.

In fairness it should be recalled that in the 1960s and long thereafter attention in this field was focussed on xerophthalmia and its prevention. Intermittent large-dose supplementation was the prevailing control measure for this emergency situation. The growing and consumption of dark green leafy vegetables (DGLV) was encouraged as a long-term solution, but it received little attention at international meetings. Most sources of food composition data at this time did not distinguish between provitamin and non-provitamin carotenoids.

In the mid 1980s evidence began to accumulate that widespread subclinical vitamin A deficiency could lead to a significant increase in the risk of mortality and morbidity in young children. It began to become clear that while consumption of dark green leaves and yellow fruits could protect against severe VAD leading to xerophthalmia it might not be capable of preventing subclinical vitamin A deficiency in a substantial proportion of the young child population of developing countries.

The group at the Department of Human Nutrition at Wageningen, Netherlands, has been at the forefront of the new investigations into the bioavailability of dietary carotenoids, nonprovitamin A as well as provitamin A. They have also led in the study of the many and complex factors that determine bioavailability.

de Pee, West, Muhilal et al (1995) in Indonesia found that an additional daily portion of dark green leafy vegetables given to lactating women did not improve vitamin A status (as measured by serum retinol, breast milk retinol, and serum β-carotene). In those who received a similar amount of β-carotene on a wafer there was significant improvement. A review of previous studies (de Pee, West, 1996) found many of those with positive results had design faults and more recent, better controlled studies tended to be negative. Later work showed that in central Java vegetables played a lesser part in maintaining vitamin A status than had been thought (de Pee, Bloem, Gorstein et al, 1998) and orange fruits were more effective in improving vitamin A status than dark green leafy vegetables (de Pee, West, Permaesih et al, 1998). The calculated β-carotene : retinol equivalence in leafy vegetables and carrots was 26:1 (compare below). A study in Guatemala, where 50 g of cooked carrots were added to the diet of children aged 7–12 years failed to find evidence of nutritional benefit (Bulux, Quan de Serrano, Giuliano et al, 1994). A study in Ghana (Takyi, 1999) found that consumption of DGLV with added fat significantly increased serum retinol levels in children, but even so just over 50% remained with inadequate vitamin A status.

On the other hand, more recently (Tang, Gu, Hu et al, 1999) in China the effect on vitamin A body stores of children 3–7 years old of two vegetable diets was studied by a double stable isotope method. Vitamin A labelled with two different kinds of deuterium (D4 and D8) was administered before and after the intervention to estimate changes in total body vitamin A stores that occurred. One group received their daily customary intake of 56 g of green-yellow vegetables and 224 g of light-coloured vegetables, and the other received 238 g of green-yellow vegetables and 34 g of light-coloured vegetables. Only the latter diet maintained adequate vitamin A stores. It was calculated from the isotope studies that provitamin A carotenoids (mainly β-carotene) provided an estimated vitamin A equivalence of 27:1 on average (see above). It should be noted that to achieve this effect more than 1/4 kg of vegetables was consumed each day by these young children, as young as 3 years. Another study of the bioavailability of β-carotene from oriental vegetables found higher bioavailabilities, but still much less than from purified β-carotene beadlets (Huang, Tang, Chen et al, 2000).

Factors affecting bioavailability

The Wageningen group has introduced a mnemonic (SLAMENGHI) to assit in the discussion of this complicated subject (Table 2.10) (see also Further Reading). It should be pointed out that the question of bioavailability applies not only to provitamin A carotenoids, but also to nonprovitamin A carotenoids, such as lycopene, lutein and others whose possible role in prevention or treatment of a number of diseases is discussed in Chapter 10.

Before something is said on each of these aspects the use of another term may be mentioned. Bioconversion is not the same as bioavailability, it relates to the efficiency with which the carotenoid in question is converted to vitamin A in the body. There is little information on this subject. The 1976 FAO/WHO Expert Group assumed that β-carotene has twice the efficiency of other provitamin A carotenoids.

*"S"*pecies of carotenoids

The naturally occurring configuration of carotenoids in plants is usually the all-*trans* isomer. It is more readily absorbed in man than the 9-*cis* form. A significant proportion of 9-*cis* β-carotene is converted to the all-*trans* form before entering the blood stream.

Molecular *"L"*inkage

Esters of carotenoids are common in fruit and vegetables but their absorption has been studied little. Esters of lutein are reported to be more bioavailable.

*"A"*mount of carotenoids consumed in a meal

In a meta-analysis that included 31 studies in which a daily β-carotene supplement of <50 mg lasted <1 year it was found that the duration of β-carotene supplementation was a significant predictor of β-carotene response (Castenmiller, West, 1998).

*"M"*atrix in which the carotenoid is incorporated

In green leaves carotenoids exist within chloroplasts as pigment-protein complexes which require disruption of the cells for the carotenoid to be released. In other vegetables and fruits carotenoids are sometimes found in lipid droplets from which they may be readily released. Cooking assists in the release, but if excessive may lead to oxidative destruction of the carotenoid. The carbon-carbon double bonds of carotenoids are subject to oxidation by oxygen in the air, and heat may bring about structural changes including isomerization of all-*trans* carotenoids to *cis* forms.

Table 2.10. Effect of food matrix and processing on bioavailability of carotenoids (after Boileau, Moore, Erdman Jr, 1999)

Very high bioavailability	
Formulated natural or synthetic carotenoids	Formulated carotenoids in water-dispersible beadlets
Natural or synthetic	Carotenoids – oil form
Papaya, peach, melon	Fruits
Squash, yam, sweet potato	Tubers
Tomato juice	Processed juice with fat-containing meal
Carrots, peppers	Mildly cooked yellow/ orange vegetables
Tomato	Raw juice without fat
Carrots, peppers	Raw yellow/orange vegetables
Spinach	Raw green leafy vegetables
Very low bioavailability (<10%)	

*"E"*ffectors of absorption and bioconversion

Dietary components may influence absorption. A minimum of 5 g fat daily is required for adequate micelle formation. This appears to be sufficient for optimal absorption of α-carotene, β-carotene and vitamin E, but lutein esters require more (Roodenburg, Leenen, van het Hof et al, 2000). Adequate protein and zinc intake assists maintenance of vitamin A status. Vitamin E, as an antioxidant, protects vitamin A from being oxidized. Fibre, chlorophyll and nonprovitamin carotenoids, which are commonly present in the diet, tend to reduce bioavailability. There is evidence that alcohol ingestion interferes with the conversion of β-carotene to vitamin A.

*"N"*utrient status of the host

Absorption of carotenoids is influenced by vitamin A status. If it is low, conversion of carotenoids to vitamin A is likely to be increased. There is evidence that zinc deficiency impairs the efficiency of β-carotene conversion to vitamin A.

*"G"*enetic factors

As clinical genetics advances it is likely that further cases of rare gentic defects as the cause of vitamin A deficiency will be reported. To date three types of defect are known: enzymatic failure to cleave β-carotene in the small intestinal mucosa (McLaren, Zekian, 1971), heterozygotic reduction of plasma RBP (Matsuo, Matsuo, Shiraga et al, 1988), and mutations in the gene for retinol-binding protein (Biesalski, Frank, Beck et al, 1999) (see also Chapter 10).

*"H"*ost-related factors

The serum response to β-carotene is higher in women than in men but the reason is not known. In several ways men are more susceptible to develop VAD than women. Age does not appear to be a factor. Diseases that interfere with intestinal absorption, especially of lipids, are likely to impair carotenoid bioconversion. In terms of public health, especially in developing countries, intestinal parasites such as *Ascaris lumbricoides* and *Giardia lamblia* are of great importance (see Chapter 9).

Mathematical *"i"*nteractions

This final letter of the mnemonic refers to the possible synergistic or even antagonistic effect that two or more factors might have when acting together. At present this is a theoretical concept only, as data are lacking, but it will have to be borne in mind as knowledge progresses.

Remarks

As might be expected, better results from interventions tend to occur when more than one adverse factor is counteracted. For example, increased fat intake and anthelminthic drug treatment have enhanced the effectiveness of food-based programmes (Jalal, Nesheim, Agus et al, 1998).

The methodology for the accurate determination of provitamin A carotenoid bioactivity in man is still in an early and rapidly growing stage of its development. There are two main approaches; techniques that rely on changes in one or more indicators of vitamin A status, and those that provide a more direct estimate of absorption and/or conversion efficiency of single doses of provitamin A carotenoids (Parker, 2000). It seems unlikely that precise values will be set, as in the past, but it should be possible eventually to provide reliable, practial information for application in interventions for the control of VADD. There is evidently a great deal more painstaking research that needs to be done (Traber, 2000). Meanwhile, efforts to promote the consumption of

green leaves and yellow fruits remain a major element of control programmes.

Preformed vitamin A

Mention has already been made of fish liver oils as highly concentrated sources of vitamin A. They are used as pharmaceuticals rather than as items of diet. Fish liver is often discarded along with other soft organs, but if utilized could form a valuable source of the vitamin in many parts of the developing world. The storage form is vitamin A_1 alcohol (retinol) in salt water fish. In fresh water fish it is vitamin A_2 alcohol (3-dehydroretinol), which has about 40% of the activity of retinol. Because of the teratogenic effects of large doses of vitamin A the consumption of liver is contraindicated during pregnancy (Ministry of Agriculture, Fisheries and Food, UK, 1995). Most mammalian livers consumed, such as those of calves, ox, lambs or chicken, have concentrations that are comparable to those in cod liver oil. Milk, butter, cheese and eggs are all moderate sources. Table 2.11 gives a selection of preformed vitamin A concentrations of some common foods.

Table 2.11. Examples of common animal vitamin A sources (μg retinol/100 g edible portion)

Fatty fish liver oils	
Halibut	900,000
Cod	18,000
Shark	180,000
Herring and mackerel	50
Dairy produce	
Butter	830
Margarine, vitaminized	900
Eggs	140
Milk	40
Cheese, fatty type	320
Meats	
Liver of sheep and ox	15,000
Beef, mutton, pork	0–4

Vitamin A has been added successfully to many foods (Bauernfeind, 1981). What is termed food fortification (or occasionally nutrification) is one of the major long-term approaches to the control of the problem of VADD and is discussed further in Chapter 11.

VITAMIN A IN HEALTH

In recent years knowledge of the physiology, biochemistry and molecular biology of vitamin A has advanced enormously. More is probably known than for any other vitamin in man.

Digestion and absorption

Dietary preformed vitamin A and carotenoids are released from protein in the stomach by proteolysis. They then aggregate with lipids and pass into the upper part of the small intestine. Dietary fat and protein and their hydrolytic products stimulate, through the secretion of the hormone cholecystokinin, the secretion of bile. This emulsifies lipids and promotes the formation of micelles which have lipophilic groups on their inside and hydrophilic groups on their outside. In this way the absorption of fat is facilitated. Bile salts stimulate pancreatic lipase and other esterases that hydrolyze retinyl esters in in-

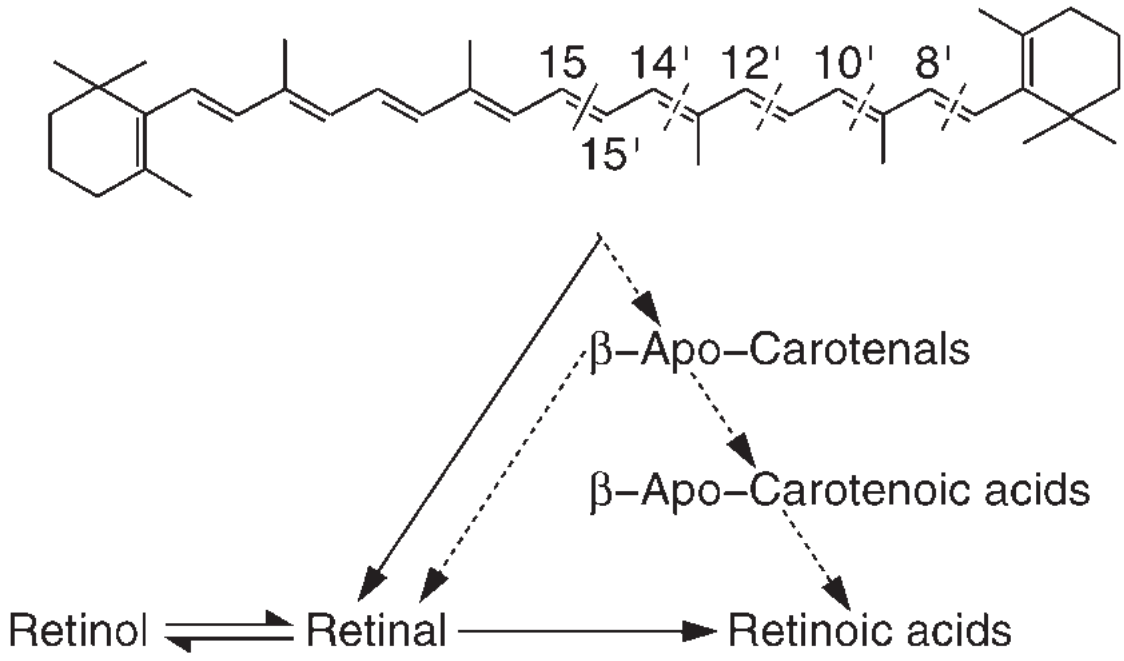


Figure 3.1. Formation of retinoids from β-carotene via the proposed central (solid line) and excentric cleavage (dashed lines) pathways (Stahl, Sies, Sundquist, 1994).

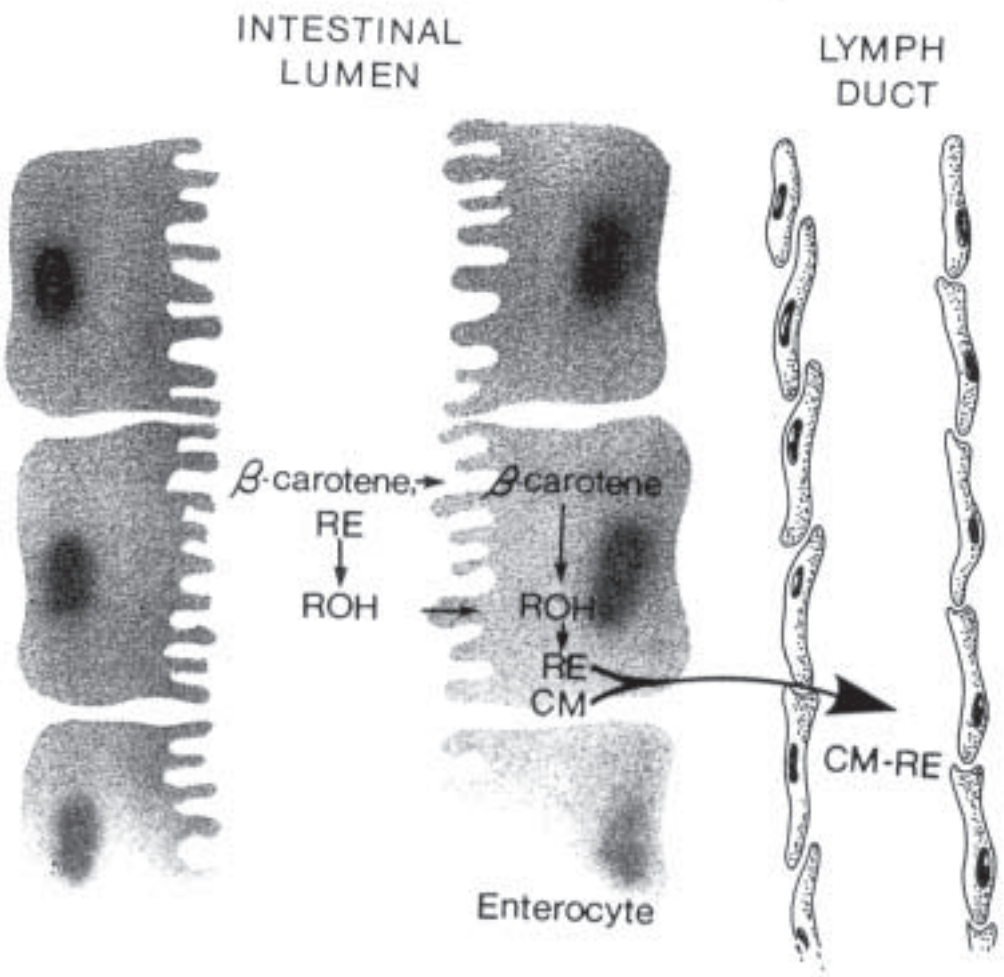


Fig 3.2. Absorption of preformed vitamin A and provitamin A from the small intestine. RE, retinyl ester; ROH, retinol; CM, chylomicron (Blomhoff, 1994).

testinal mucosal cells (enterocytes). Retinol, the product of the hydrolysis, is well absorbed (70–90%) by intestinal mucosal cells.

Provitamin A carotenoids pass into the mucosal cells unchanged. A proportion of each, along with non-provitamin carotenoids, passes through unchanged into the lymph and the blood. The remainder undergoes cleavage of the molecule by a specific enzyme 15,15'-dioxygenase within the intestinal mucosal cell. This process can also take place within the liver and some other tissues (Goodman, Huang, Shiratori, 1966). Symmetrical cleavage of the β-carotene molecule yields two molecules of retinal, which is mostly reduced and esterified to retinyl ester. Some cleavage is asymmetrical and less retinal is produced (see Figure 3.1). In practice β-carotene and other provitamin A carotenoids have only a fraction of the activity of retinol, as was pointed out earlier (see Tables 1.1 and 2.2).

Within the mucosal cells retinol is esterified before incorporation into chylomicrons (see Figure 3.2). In this process a specific cellular retinol-binding protein (CRBPII) carries the lipid-soluble retinol through the aque-

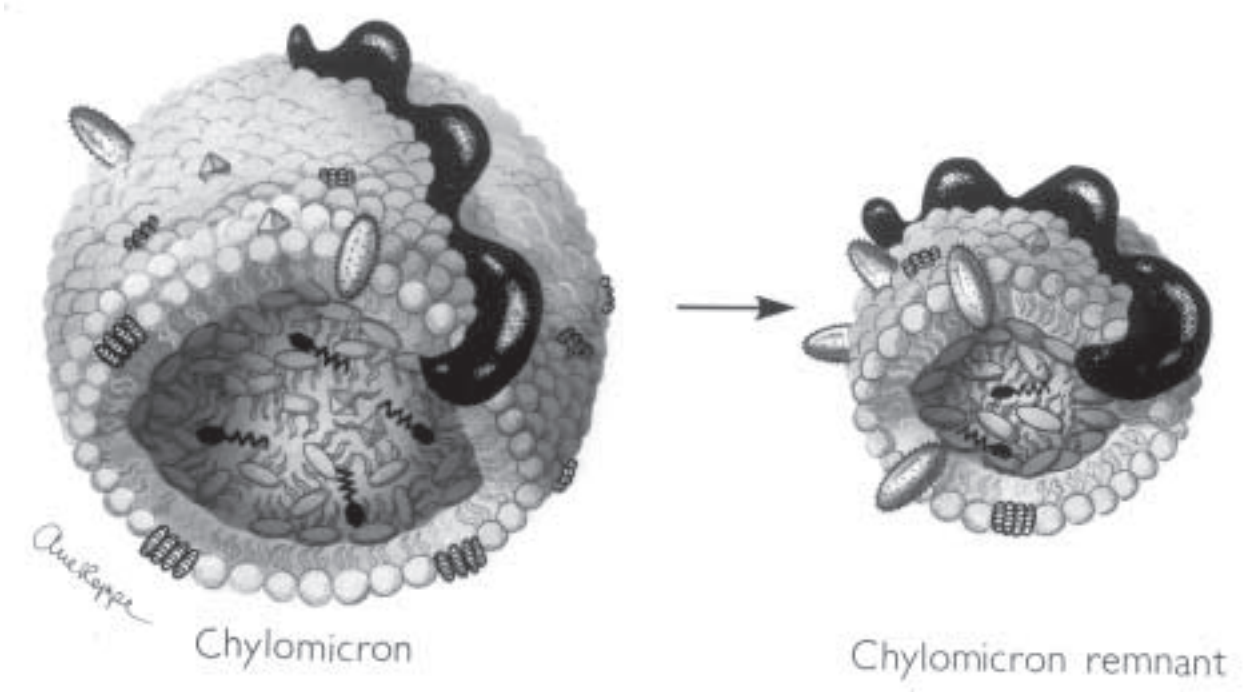


Figure 3.3. Schematic drawing of chylomicron and chylomicron remnant (Blomhoff, 1994).

ous media and delivers it to the enzyme lecithin:retinol acyltransferase (LRAT) (Ong, Kakkad, MacDonald, 1987). This enzyme appears to be the main intestinal enzyme that normally esterifies retinol and then delivers it to the chylomicrons.

In terms of intake of vitamin A in the diet, about 10% is not absorbed, 20% appears in the faeces through the bile, 17% is excreted in the urine, 3% is released as CO_2 and 50% is stored in the liver (Olson, 1994).

Transport to the liver

Chylomicrons consist of aggregates of thousands of molecules of triglycerides and phospholipids with fat-soluble vitamins, including retinyl esters and carotenoids, cholesterol esters and some apolipoproteins (see Figure 3.3).

They pass into the lymph and then the general circulation and are broken down to some extent to produce chylomicron remnants. Almost all retinyl esters remain with the chylomicron remnants, which are mainly cleared by the liver. Recent work has demonstrated that they also deliver retinyl esters to lung and some other tissues, and also to some cancer cells (Blomhoff, 1994). In these sites they are converted to retinoic acid, which can be used for regulation of gene expression (see later).

Metabolism in the liver

In the liver (see Figure 3.4) most vitamin A from chylomicron remnants in the form of retinyl esters is taken up by hepatic parenchymal cells (hepatocytes). There the esters are hydrolyzed and after processing in endosomes retinol is transferred to the endoplasmic reticulum. There it binds to retinol-

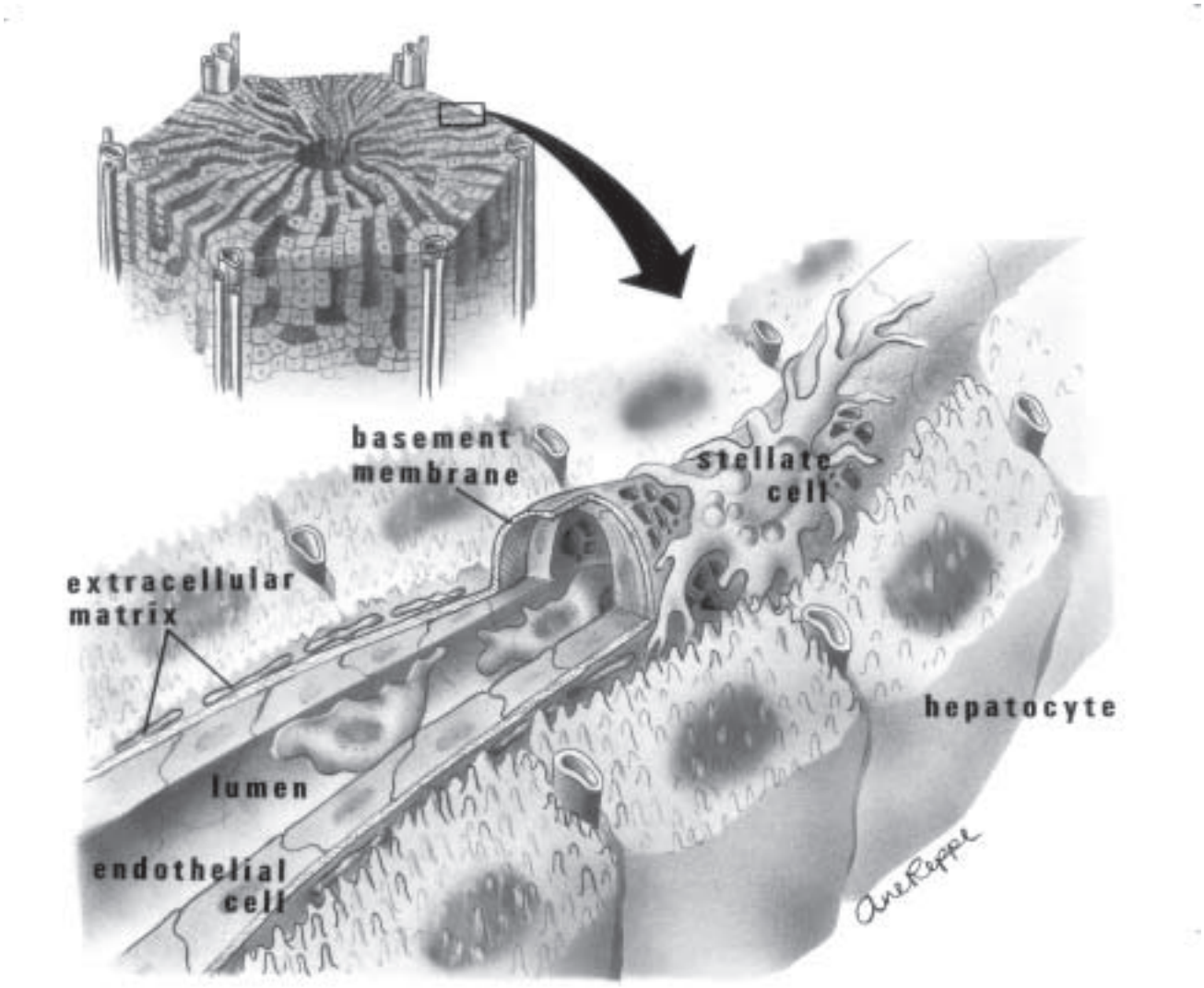


Figure 3.4. Schematic diagram of the liver structure (Blomhoff, 1994).

binding protein (RBP) and after the complex enters the Golgi complex it is secreted from the cell.

In the absence of retinol RBP is retained by the liver and tends to accumulate (Rask, Valtersson, Arundi et al, 1983). This ill-understood phenomenon forms the basis of relative dose-response tests (RDR) of vitamin A status (see Chapter 4).

Most of the retinyl ester taken up by hepatocytes from chylomicron remnants is transferred as retinol attached to RBP to another type of liver cell called stellate cells (Wake, 1994). Storage of vitamin A as retinyl esters seems to be one of the main functions of this type of liver cell. They also produce collagen type III, which when excessive may contribute to a form of cirrhosis of the liver (see also Chapter 10). 50–80% of vitamin A in the body is in the liver. 90–95% of this is in the stellate cells and 98% of this is in the form of retinyl esters, mostly as palmitate. This store is normally sufficient to last for several months. Bound to RBP retinol is released from both stellate and parenchymal cells. When liver reserves of vitamin A are low parenchymal cells are the major site of storage (Batres, Olson 1987). Some parenchymal

cells contain much more vitamin A than others. These are respectively termed “heavy” or “light” according to their density (Batres, Olson, 1987a).

Cells identical to the liver stellate cells also occur in many other tissues of the body, including the lung, intestine and kidney (Nagy, Holven, Roos et al, 1997). Recently it has been shown that adipose tissue is an important storage site for retinol, as well as for β-carotene (Wei, Lai, Patel et al, 1997). About 15–20% of the rat’s store of retinol is in adipocytes. This comes from chylomicron-bound esters and not from circulating RBP as is the case of that in the liver. Furthermore, free retinol is secreted rather than retinol bound to RBP. The secretion of vitamin A from the liver is a complicated process and by tracer kinetics in animal studies several pools have been identified (Green, Green, 1996).

Transport to other cells

Retinol in plasma bound to RBP is almost entirely associated also with another protein, transthyretin (TTR) (Ingenbleek, Young, 1994). The formation of this complex reduces the loss of retinol in glomerular filtrate in the kidney. RBP has been fully characterized and is a single polypeptide chain with a molecular weight of 21,230. Its three-dimensional structure is such that it contains a specialized hydrophobic pocket within which the fat-soluble retinol fits (Sivaprasadarao, Findlay, 1994).

Table 3.1. Properties and functions of some selected lipocalins (after Sivaprasadarao, Findlay, 1994)

Protein	Size*	Tissue	Fluid	Ligand	Property/Function
β-Lactoglobulin	2 x 162	Mammary gland	Milk	Retinol (?)	Possible involvement in transport of vitamin A via gut receptor
Serum retinol-binding protein (RBP)	183	Liver and other tissues	Serum	Retinol, retinal, retinoic acid	Retinol transport, delivery of retinol to tissues via a receptor
Complement C8γ	182	Liver	Serum	Retinol, retinoic acid	Component of complement
Apolipo-protein D (ALPD)	169	Adrenal, kidney, pancreas, liver	Serum, gut secretion	Lecithin, cholesterol	Lipid transport, enhances lecithin: cholesterol acyl-transferase activity

* Number of residues

In well-nourished adults total RBP concentration in plasma is 1.9–2.4 μmol/l (40–50 μg/ml). The value for children is about 60% of that of adults. Protein-energy malnutrition, infections and parasitic infestations lower concentrations of RBP in plasma and these must be used with caution in assessing vitamin A status (see Chapter 4).

RBP is a member of a family of proteins, the knowledge of which is rapidly growing. It includes proteins that bind to other lipid-soluble molecules. This group of proteins is called the lipocalin superfamily. Table 3.1 shows some of these.

RBP is synthesized in hepatocytes, but probably also in many other tissues.

In healthy subjects mean values and ranges of serum retinol as a function of age and sex are shown in Table 3.2. In most age ranges, levels are higher in males.

Retinol is extensively recycled between plasma, liver and other tissues. At present it is thought that most of the irreversible utilization of retinol is a kind of detoxification, and only a small fraction is functional. Small amounts of other retinoids can be detected in plasma, including all-*trans* retinoic acid.

There is a variety of molecules for the extracellular transport of retinoids and carotenoids (see Table 3.3). It will be noted that there are no specific carrier proteins for carotenoids.

Concentrations of carotenoids in plasma (see Figure 3.5) are highly dependent on diet. In the case of provitamin A carotenoids usual levels in plasma are significantly higher in women than in men (Olmedilla, Granado, Blanco et al, 1994), in contrast to retinol.

Table 3.2. Serum retinol concentrations (μmol/L) as a function of age and sex in American residents, 1971–74* (Olson, 1996)

Age (years)	Total	Males	Females
3–5	1.28 (0.73–2.0)	1.29 (0.70–2.1)	1.26 (0.77–2.0)
n	1414	725	689
6–11	1.31 (0.84–1.9)	1.30 (0.84–1.9)	1.32 (0.84–1.9)
n	1857	930	927
12–17	1.58 (1.0–2.3)	1.62 (1.1–2.3)	1.53 (1.0–2.2)
n	2035	1026	1009
18–44	1.94 (1.2–2.9)	2.08 (1.4–3.0)	1.80 (1.1–2.8)
n	7035	2164	4871
45–74	2.20 (1.3–3.3)	2.29 (1.4–3.5)	2.11 (1.3–3.2)
n	6111	2911	3200

* The 5th and 95th percentile values are given in parentheses. Derived from Pilch, 1987

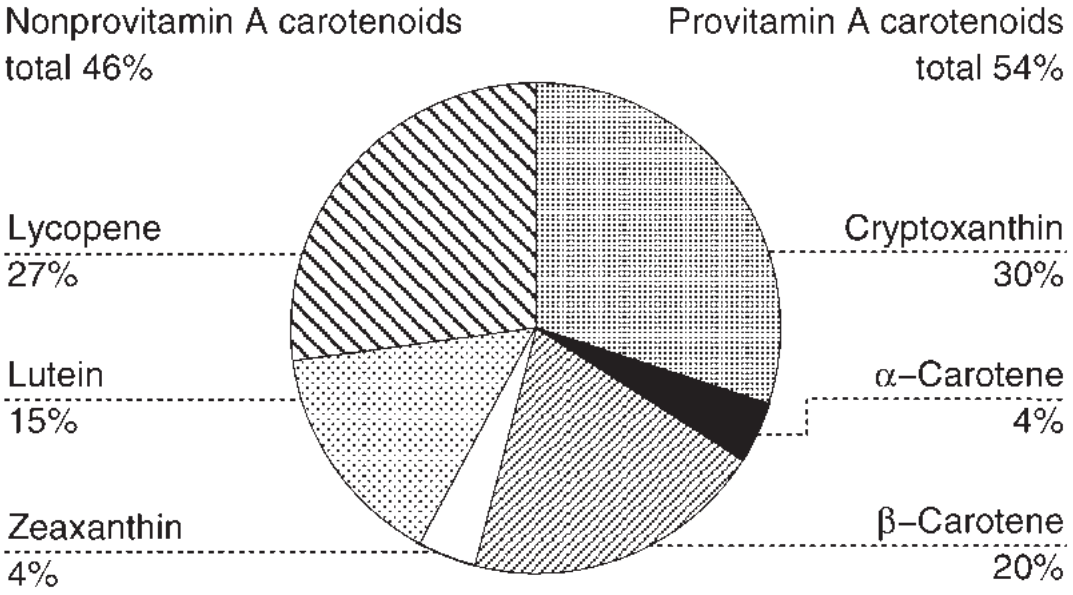


Figure 3.5. Pie diagram of a typical carotenoid pattern in plasma.

Table 3.3. Extracellular transport molecules for retinoids and carotenoids (Blomhoff, 1994)

	Approximate MW (kD)	Main ligand	Suggested function
Chylomicrons and their remnants	20,000–200,000	Retinyl esters and carotenoids	Lymph and plasma transport of newly absorbed vitamin A and carotenoids
VLDL and LDL	2,000–20,000	Carotenoids	Plasma transport of carotenoids
RBP	21	Retinol	Blood plasma and interstitial fluid transport
IRBP	140	Retinol, retinal	Intercellular transport in visual cycle
EBP	20	Retinoic acid	Intercellular transport in epididymis

VLDL, very low-density lipoproteins; LDL, low-density lipoproteins; RBP, retinol-binding protein; IRBP, interphotoreceptor retinol-binding protein; EBP, epididymal-binding protein

Cellular uptake

Some retinol may enter cells by diffusion but the evidence is that most is taken up by membrane RBP receptors which are being characterized at the present time (see Figure 3.6 and Table 3.4). Carotenoids are mainly carried in plasma by low-density lipoproteins (LDLs) and enter cells bearing the LDL receptor.

Cellular retinoid-binding proteins (Li, Norris, 1996)

Within cells there are many proteins that specifically bind retinoids and direct them to specific enzymes (see Table 3.4). These proteins, like RBP, also belong to a family with other members. In recent years mounting evidence has established key functions for CRBP in retinoid metabolism. It acts as a kind of chaperone in protecting retinol within the cell and interacts with certain retinoid-metabolizing enzymes (Napoli, 2000).

Gene function and retinoids

It is known that the functions of vitamin A in different tissues, with the exception of the visual process (see p 30), are mediated by its acid derivatives (Pfahl, Chytil, 1996). All-*trans* retinoic acid (RA) is noncovalently bound to three nuclear RA receptors (RARa, RARb, RARg,) in gene transcription regulation. When activated the nuclear receptors for vitamin A are able to bind to “response elements” in specific genes to increase or de-

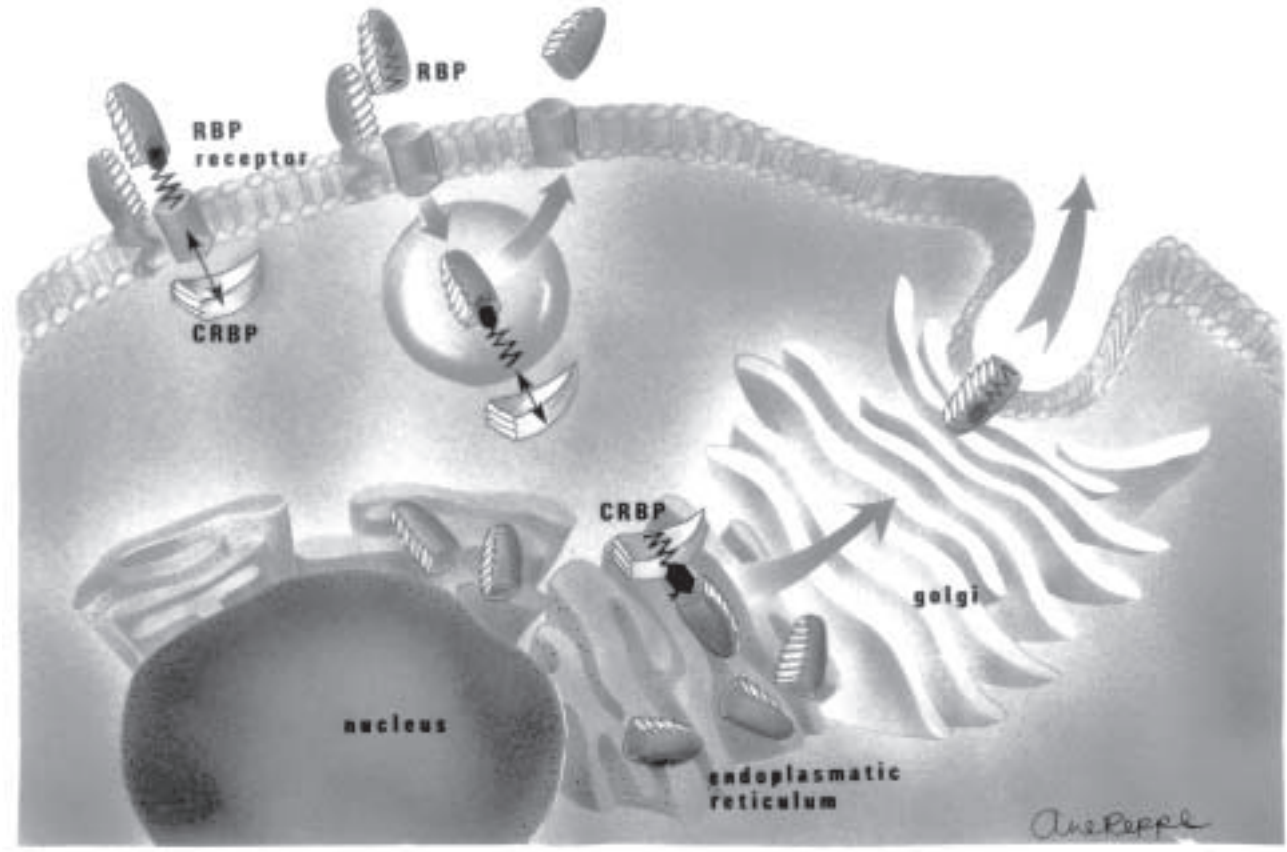


Figure 3.6. Hypothesis for cellular RBP metabolism. RBP-retinol may be recognized by a cell surface receptor. Retinol may be transferred to CRBPs either at the cell surface or after internalization into endosomes. CRBP may also deliver normal retinol to newly synthesized RBP in the endoplasmic reticulum (Blomhoff, 1994).

Table 3.4. Intracellular retinoid-binding proteins (Blomhoff, 1994)

	Approximate MW (kD)	Main ligand	Suggested function
CRBP(I)	16	Retinol	Donor for LRAT reaction and oxidative enzymes; regulates retinyl ester hydrolysis
CRBP(II)	16	Retinol	Donor for LRAT reaction
CRABP(I)	16	Retinoic acid	Regulates free retinoic acid concentration; donor for catabolizing enzymes
CRABP(II)	15	Retinoic acid	Regulates free retinoic acid concentration; donor for catabolizing enzymes
CRALBP	36	Retinal	Enzymatic reactions in the visual cycle

CRBP, cellular retinol-binding protein; CRABP, cellular retinoic acid-binding protein; CRALBP, cellular retinal-binding protein; LRAT, lecithin:retinol acyl transferase

crease the level of expression of the gene. The response elements are nucleotide sequences which in the DNA compose the genes.

Soon afterwards it was shown that 9-*cis* RA binds and activates three other nuclear receptors (RXRa, RXRb, RXRg). RA only binds RAR receptors, whereas 9-*cis* RA can bind all six nuclear receptors but *in vivo* it is mainly bound to RXRs. Considerable surprise resulted when the discovery was announced of a new metabolite of retinol, all-*trans*-oxo-retinol, with the functions of receptor activator and differentiation agent (Achkar, Derguini, Blumberg et al, 1996). It binds and transactivates RARs but not RXRs.

The expression of these six receptors varies between cells, but most if not all cells express at least one of these receptors. Several hundred genes have so far been shown to be induced or repressed by retinoids. Table 3.5 gives a representative listing of vitamin A-responsive genes thought to be activated through the action of RARs and/or RXRs.

These retinoid hormone receptors are now recognised to be part of a hormone receptor superfamily whose members interact. It has been called the “Steroid-Thyroid-Retinoid Superfamily”. Table 3.6 gives a partial listing of these nuclear receptors and their respective activating substances. If these nuclear receptors are to recognise response elements within responsive genes they must act in pairs (dimers). A member of the RXR family must serve as a partner if the dimer is to function. It is therefore evident that vitamin A, usually in the form of RA, and the RXRs can be regarded as regulators of several hormone response systems.

Major sources of RA in the diet are its precursors retinol and retinyl esters. The diets of animals and man contain little RA itself. How-

Table 3.5. Partial and representative listing of vitamin A-responsive genes thought to be regulated through the action of RARs and/or RXRs, (Blaner, 1998).

Gene	Gene function/role
Oxytocin	Reproduction
Growth hormone	Growth
Phosphoenol pyruvate carboxykinase	Gluconeogenesis
Class I alcohol dehydrogenase	Alcohol oxidation
Transglutaminase	Cell growth and cell death
Laminin B_1	Cellular interactions
Matrix Gla Protein	Bone growth and strength
Keratin	Skin
Cellular retinol-binding protein type I	Vitamin A metabolism
RAR-beta	Vitamin A action
Hox 1.6	Fetal development
Dopamine D_2 receptor	Central nervous system

ever, it has been shown that both rat and human intestinal cytosol can produce RA from β-carotene (Napoli, 1994). This suggests a new source for the generation of RA within the body.

Cytochrome P450 (CYP) enzymes constitute a large superfamily with hundreds of members. They catalyze the oxidative metabolism of many endogenous compounds as well as numerous foreign chemicals. It has been proposed that P450s regulate steady-state levels of ligands for the nuclear hormone receptor superfamily which includes retinoids (Sonneveld, van der Saag, 1998). RA is metabolized through oxidation to more polar metabolites. Recently a novel cytochrome P450 enzyme (CYP26) with specific RA 4-hydroxylase activity has been cloned from man and some animal species. It fulfils all the requirements of an enzyme which could control levels of active retinoids in cells and target tissues. CYP26 may play a role in embryonic development (see below and Chapter 10) and in cancer (see Chapter 10). The great advances made in the understanding of the hormonal control retinoids exert over gene function have highly significant implications for the management and treatment of many diseases that occur throughout the world (see Chapter 10).

Functions of vitamin A

In recent years knowledge of the functions of vitamin A has increased greatly. Table 3.7 indicates the major functions of vitamin A as far as man is concerned. In view of the fact that nuclear receptors for retinoids have been identified in virtually every type of cell it could be argued that in some way vitamin A plays a part in every bodily process. For our present purposes those included in Table 3.7 may be concentrated upon.

Vision

In the outer segment of rod cells in the retina 11-*cis* retinal combines with the membrane-bound protein, opsin, to form rhodopsin, in-

Table 3.6. Partial listing of members of the "Steroid-Thyroid-Retinoid Superfamily" of nuclear receptors and their activators (Blaner, 1998).

Nuclear receptor	Activating substance
Retinoid X receptor (RXR)	9-*cis* retinoic acid
Retinoic acid receptor (RAR)	all-*trans* retinoic acid
Vitamin D receptor (VDR)	1,25-dihydroxy vitamin D
Thyroid hormone (TR)	Triiodo-thyronine (T3)
Estrogen receptor (ER)	Estrogen
Progesterone receptor (PR)	Progesterone
Glucocorticoid receptor (GR)	Cortisol
Mineralocorticoid receptor (MR)	Aldosterone
Androgen receptor (AR)	Testosterone
Lipid X receptor (LXR)	Cholesterol metabolite(s)
Peroxisome proliferator-activated receptor (PPAR)	Putative fatty acid metabolite

volved in vision under conditions of low illumination. Similar complexes occur in cone cells to give three specific iodopsins with different absorption maxima, resulting in blue, green and red cones. These serve colour vision and vision in bright illumination. When light strikes the retina a series of complex biochemical changes takes place, resulting in the generation of a nerve impulse (Rando, 1994) (Figure 3.7).

Vitamin A, in the form of all-*trans* retinol is delivered by the blood to the retinal pigment epithelium (RPE) where it is either esterified for storage, or isomerized to 11-*cis* retinol which is then oxidised to 11-*cis* retinal. This is transported to the photoreceptor rod or cone cells where it combines with the protein opsin to form rhodopsin or iodopsin, respectively, which are light sensitive. On exposure to light 11-*cis* retinal is isomerized back to all-*trans* retinal and the nerve impulse is generated. On release from the protein all-*trans* retinal is reduced to all-*trans* retinol and is transported back to the RPE to complete the cycle.

Table 3.7. An outline of functions of vitamin A

Vision	Photopic and colour, scotopic
Cellular differentiation, morphogenesis	Gene transcription
Immune response	Non-specific, cell-mediated
Haemopoiesis	Iron metabolism (?)
Growth	Skeletal
Fertility	Male and female

The space between the RPE and the layer of photoreceptor cells is taken up mostly by a large glycoprotein called the interphotoreceptor retinoid-binding protein (IRBP) the function of which is to transport two different molecular species of vitamin A to and from two different cell types, as outlined above. Chen and Noy (1994) have proposed mecha-

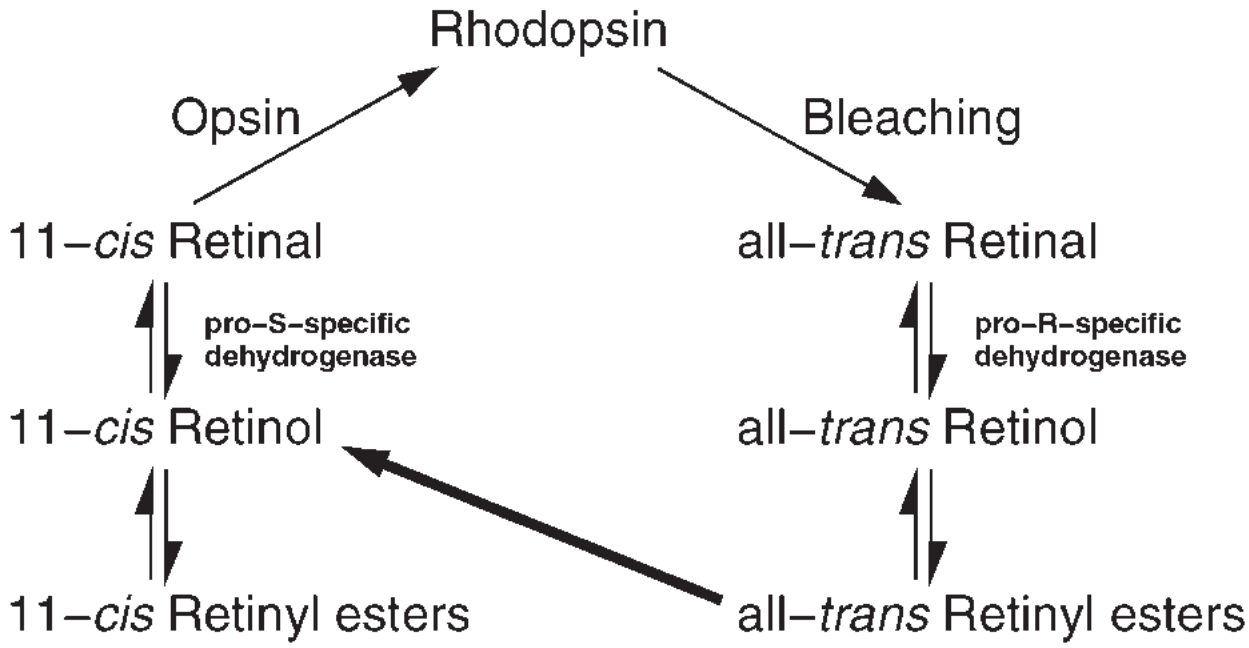


Figure 3.7. Current model of the visual cycle (Rando, 1994).

nisms to account for this process. Another retinoid-binding protein is also unique to the eye. It is cellular retinaldehyde (retinal)-binding protein (CRALBP). It binds closely to 11-*cis* retinal and to 11-*cis* retinol and protects the former from photoisomerization.

In the developing embryo RA is highly concentrated in certain tissues (Dräger, Wagner, McCaffery, 1998). These patterns are generated by the local expression of RA-synthesizing aldehyde dehydrogenase enzymes. The eye, especially vulnerable to vitamin A deficiency, is one of the RA-richest regions in the embryo. In the mature eye the pattern of these enzymes is stable, but the amount of RA synthesized is variable and is dependent on ambient light levels. This results from changing levels of the RA precursor retinal, which is released from illuminated rhodopsin. Thus this is a mechanism whereby light can directly influence gene expression. This phenomenon of light-induced release of retinal from rhodopsin occurs only in vertebrate, but not in invertebrate, photoreceptors. It might be speculated that this may have accelerated the rapid evolution of RA-mediated transcriptional regulation, at the transition from invertebrates to vertebrates. It may also explain the prominent role of RA in the eye. Readers interested in more detail should consult the references in Further Reading.

Cellular differentiation

In vitamin A deficiency keratin-producing cells replace mucus-secreting cells in many epithelial tissues of the body. This is the basis of the pathological process termed xerosis that leads to the drying of the conjunctiva and cornea of the eye (see Chapter 5). The process can be reversed by vitamin A. It has become clear recently that vitamin A, mainly in the form of RA, plays a key, hormone-like, role in cell differentiation throughout the tissues and organs of the body (Figure 3.8). It follows from this that the formation of RA must be precisely regulated. The enzymatic systems responsible are under investigation (Wolf, 1996).

Glycoprotein and glycosaminoglycan synthesis

Glycoproteins are polypeptides with short chains of carbohydrates. Glycosaminoglycans are related compounds that are cell surface molecules. Retinoids have been shown to control the expression of enzymes involved in the synthesis of some of these compounds (Vahlquist, 1994). Impairment of this function by VAD may contribute to lack of mucin secretion and liquefaction of the cornea seen in xerophthalmia (see Chapter 5).

Embryogenesis

Severe vitamin A deficiency on the one hand, and excessive dosing with vitamin A and also RA on the other, result in malformations of the embryo affecting most organs of the body in many vertebrate species. The embryo is only sensitive to teratogenic influences, that is to say those that induce congenital malformations, during a relatively short period after conception. After that period, known as the organogentic period, has passed, usually the first three months of fetal life in man, there is

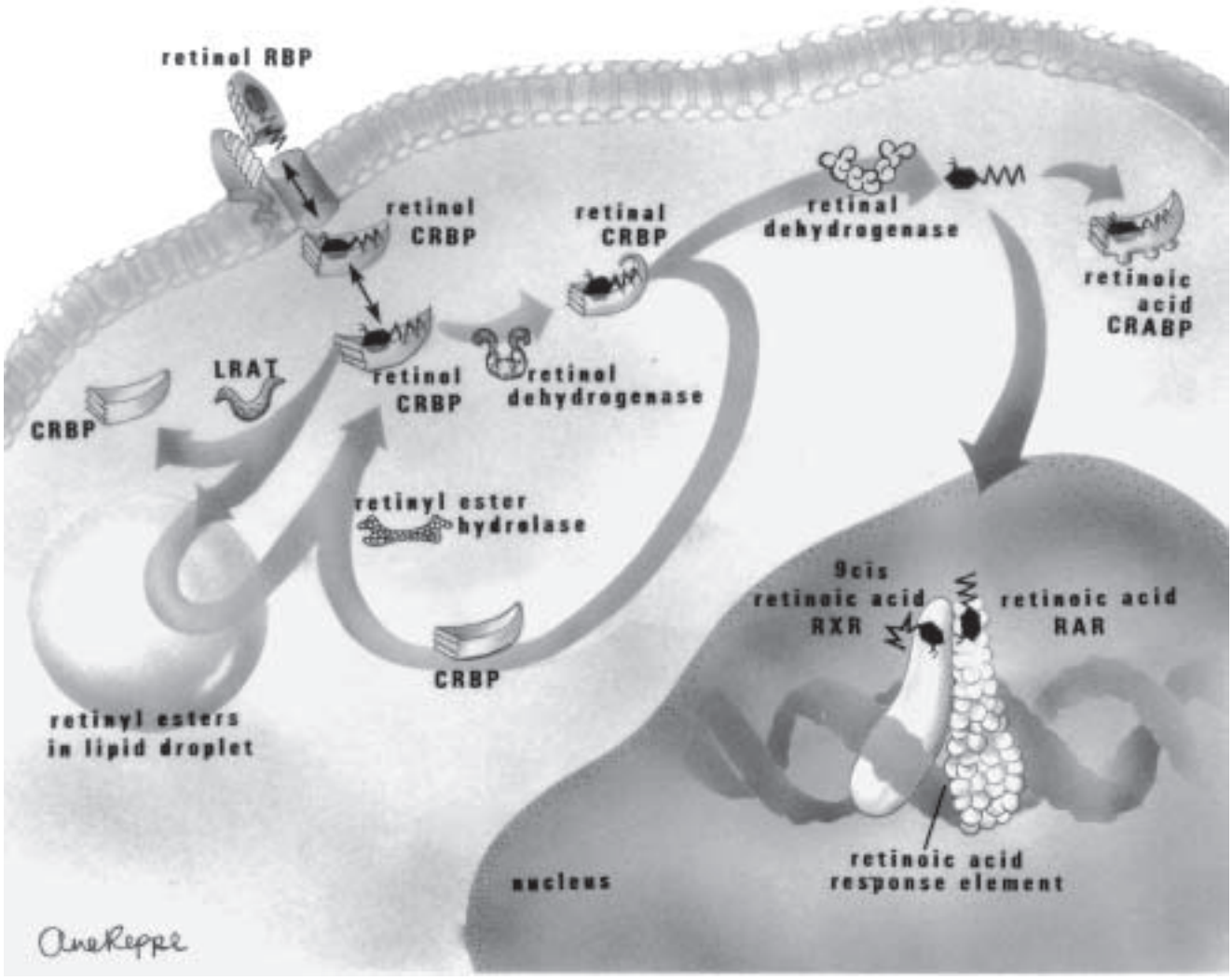


Figure 3.8. Suggested roles of CRBPs and CRABPs. See text for details (Blomhoff,1994).

no longer any risk in this regard to the fetus (Gerster, 1997; Wiegand, Hartmann, Hummler, 1998; Ward, MacGowan, Hornby et al, 2000). To date it has not conclusively been demonstrated that human VAD causes congenital malformations. A number of individual case reports have been published but the association might well be coincidental and not causal. Although some of the synthetic retinoids are clearly teratogenic in man, there is very much less evidence for a similar effect of large doses of retinol (see Chapter 10). Some other aspects of this topic are considered later in relation to the safe use of vitamin A (see Chapters 10 and 11). Many of the effects of vitamin A are now known to be mediated by retinoid-regulated expression of homeobox genes and other pattern-forming genes. RA receptors are closely involved (Ross, 1999).

Immune response

Many of the epithelial tissues are important barriers to infection and VAD impairs this function in a nonspecific way. In addition, vitamin A is known to be involved in maintaining immunocompetence in more specific ways. It helps to maintain the lymphocyte pool. Vitamin A also functions in T-cell-mediated responses. Some aspects of the immune response, such as immunoglobulin production, previously considered unrelated are now known to be affected by retinoids (Semba, 1998). Direct involvement of RA in the immune system has not been definitely established. It has been suggested (Buck, Ritter, Dannecker et al, 1991) that a third oxidized metabolite of retinol, 14-hydroxy-4,14-*retro* retinol is the active molecule in the immune system, but how it acts is not known.

Much of the evidence for the functioning of vitamin A in the immune response comes from evidence in both experimental animals and human subjects who are vitamin A deficient. This subject is consequently pursued further in Chapters 6 and 7.

Reproduction

Until recently it was believed that the male and female reproductive organs shared in common with the eye the fact that they were the only tissues known to be unable to maintain normal function when only retinoic acid is given. They appeared to require retinol for normal spermatogenesis in the male and the prevention of placental necrosis and fetal resorption in the female rat (Thompson, Howell, Pitt, 1964). This was not so in birds (Thompson, Howell, Pitt et al, 1969). However, high levels of CRABP have been found in the testes (Ong, Newcomer, Chytil, 1994) and high doses of RA injected peritoneally support spermatogenesis (Van Pelt, De Rooj, 1991). In addition, dietary RA appears to be taken up by testicular interstitial Leydig cells, as it supports testosterone production (Appling, Chytil, 1991). The mechanisms of vitamin A action in reproduction are only now beginning to be understood.

Haemopoiesis

VAD in man and in experimental animals is consistently associated with an iron deficiency type of anaemia. It has been repeatedly shown that in these circumstances in addition to iron, vitamin A is required for a full haematologic response. The mechanism remains unclear. VAD might interfere with the absorption, transport or storage of iron. On the other hand it might act directly on haemopoiesis, although that seems less likely (Sommer, West 1996, pp 150-162). The demonstration that RA is necessary for erythrocyte differentiation implies that it controls the usage of iron (Pfahl, Chytil, 1996) (see also Chapter 7).

Growth

Retinoic acid is known to play its hormone-like function in the control of growth and development of tissues in the musculo-skeletal

system, just as it does elsewhere. In communities subject to widespread VAD the occurrence of many other adverse factors has made the demonstration of the retarding effect of VAD difficult (Sommer, West, 1996, pp 163-188) (see Chapter 7). One possible mechanism for the influence on growth is the very recent demonstration that both vitamin A and retinoic acid produce rapid release of cyclic AMP (adenosine monophosphate) and human growth hormone secretion (Djakoure, Guibourdeuche, Porquet et al, 1996).

Other functions

Energy balance

Heat production (thermogenesis) by mitochondria in small collections of brown adipose tissue plays a part in the regulation of energy balance and consequently possibly in the control of obesity. In mitochondria, an enzyme not present in white adipose tissue controls the local production of energy as heat. This enzyme is regulated by the sympathetic nervous system, but as has been recently shown (Alvarez, De Andres, Yubero et al, 1995) it is under transcriptional regulation by RA.

Regulation of the dopaminergic system

RA plays a major role in the development of the fetal central nervous system. All-*trans* and 9-*cis* RAs bound to their receptors in the brain and the pituitary gland regulate the expression of the dopamine receptor D2R, which is part of the dopaminergic system. Dopamine is a signalling molecule in the central nervous system. It controls coordination of movement and also the synthesis of pituitary hormones. Parkinson's disease results from a defect in the dopaminergic system. D2R-knockout mice lack these RA receptors and develop neurological damage similar to that in Parkinson's disease (Wolf, 1998).

Gap junctional communication

Gap junctions are narrow, hydrophilic pores connecting the cytosol of two adjacent cells. There is evidence that gap junctions play a role in regulation of morphogenesis, cell differentiation, secretion of hormones, and growth. Effects on gap junctional communication might be involved in carcinogenesis and teratogenesis. RA and its analogues exert their effect by acting as ligands of nuclear receptors RAR or RXR (see page 28 and Stahl and Sies 1998).

Activities of carotenoids

Olson (1999) has classified the many activities of carotenoids into functions, actions, or associations but these divisions are not strict and rigid. In Table 3.8 an attempt is made to bring these together in outline form. The underlying mechanisms of some of these activities of carotenoids have been discussed in Chapter 2.

Table 3.8. Functions and actions of carotenoids

Functions
- Some carotenoids are only known precursors of vitamin A and its derivatives
- Accessory pigments in energy transfer in photosynthesis
- Phototropism in simple and higher plant forms
- Photoprotective role in bacteria and also man
- Plant growth regulation
- Reproduction regulation in fungi

Actions
- Quenching of singlet oxygen
- Colour attractants in flowers for insect pollination
- Colouration of food for mankind

Functions usually denote a more or less clearly defined purpose. Through the conversion of provitamin A carotenoids to vitamin A in the animal body this function is the most evident one. Others, as indicated in Table 3.8, are less well understood in chemical terms. Actions, both biological and chemical, are readily demonstrated but their consequences are not so clear. Finally, associations of carotenoids are mostly known from epidemiological studies and usually have their importance in relation to occurrence of diseases (see Chapter 10).

Human requirements

Along with the requirements for other nutrients those for vitamin A for humans of either sex, of different ages and in various physiological states, such as pregnancy and lactation, are kept under regular review. As mentioned earlier (see Chapter 2) it is customary to express these in terms of Retinol Equivalents (RE)/day to take account of the different activities in the body of retinol and the provitamin carotenoids. Table 3.9 gives Recommended Dietary Intakes (RDIs) for vitamin A from several different sources.

RDIs like those shown in Table 3.9 are designed to prevent deficiency and to provide a safe intake for the large majority of a population (covering about ±2SDs from the mean). They were originally meant to ensure that, if met, deficiency would not occur, although a considerable proportion of a population would consume more than they needed to.

However, evidence has recently emerged for a protective effect against some chronic diseases of increased amounts of antioxidant nutrients, such as the carotenoids (see above and Chapter 10). These possible beneficial effects have not previously been taken into consideration when RDIs have been formulated. The usual advice being given is to increase the intake of fresh fruits and vegetables.

Table 3.9. Recommended Dietary Intakes of vitamin A (μg RE/day). ASG* (Age and sex grouping) refers to data immediately on right only (McLaren, 1994)

ASG*	FAO/WHO[1]	ASG	Olson[2]	ASG	NRC[3]	ASG	UK[4]
0–1	350		375		375		350
		1–1.9	375	1–3	400	1–3	400
1–6	400	2–5.9	400	4–6	500	4–6	500
6–10	400	6–9.9	500	7–10	700	7–10	500
10–12	500	M10–11.9	600	M11–51+	1000	M11–14	600
12–15	600	12–70+	700			15–50+	700
M 15–18+	600						
F 15–18+	500	F10–70+	600	F11–51+	800	F11–50+	600
Pregnancy	600	6–9 mo	+200		800		+100
Lactation	850	0–5.9 mo	+400	1st 6 mo	1300		+350
		6+ mo	+320	2nd 6 mo	1200		

1 FAO/WHO, 1988
2 Olson, 1987
3 National Research Council, 1989
4 Panel on Dietary Reference Values, 1991

ASSESSMENT OF VITAMIN A STATUS

Introduction

Nutritional status, or nutriture, is an important concept that often tends to be misunderstood. It is sometimes thought to be represented by dietary intake, but this is not so. A financial analogy may be helpful. A person may have an income, probably from a number of sources, and also an expenditure on a variety of things. If income exceeds expenditure then that person's financial balance or status is positive (in the black) and if expenditure exceeds income the status is negative (in the red). In this analogy "income" may be replaced by "nutrients in the diet" and "expenditure" by "utilization of nutrients by the body". Then the resulting nutritional status (balance) may be either deficient (negative) or normal (positive). No analogy is perfect and whilst very few indeed would object to having a very high financial status, excess nutrient intake, including that of vitamin A, may be harmful.

Although, as pointed out above, assessment of dietary intake on its own is not equivalent to assessment of nutrient status, yet it is clearly closely related. Consequently the methodology of the assessment of dietary intake of vitamin A is given some attention at the end of this chapter.

It is clear that deviations of nutritional status from the usually accepted normal range (which is arbitrary to a considerable extent) may vary in degree. The terms "mild", "moderate" and "severe" deficiency are often used. Another useful division of status on the deficiency side is the use of the terms "subclinical" and "clinical" deficiency.

Nutritional status concerns some aspect of the state of the body and consequently there are different types of possible test for the assessment of status. Table 4.1 outlines the types of test that have been employed with some examples.

Table 4.1. Types of test of nutritional status

Test	Examples of use
Physical signs	Later stages of any deficiency disease
Physiological function	Dark adaptation
Subclinical structural change	Conjunctival impression cytology
Serum level of nutrient	Serum retinol
Enzyme activity	Erythrocyte transketolase
Urinary excretion	Vitamin C, iodine
Body stores	Relative dose response

Some of the different kinds of test are not applicable in the case of assessment of vitamin A status at present. For example, there are none for testing the activity of enzymes that are dependent for their action on vitamin A, as is the case for some other vitamins. Measurement of urinary excretion of metabolic products of vitamin A metabolism, although theoretically possible, has not been utilized until the present.The use of somatic measurements, such as weight and height, is the mainstay of detecting protein-energy malnutrition under field conditions. Although there is increasing evidence that vitamin A deficiency does have an effect on growth (see Chapter 7) there are many other nutritional factors and infections that act similarly. There is no aspect of retardation of growth that can be linked specifically to deficiency of vitamin A. In fact, to describe a community as "underweight" or "stunted" contributes no understanding of the nature of the underlying causes.

In the field of assessment of vitamin A status the first steps were taken when in 1974 WHO convened an expert group to report on the knowledge at that time of the problem of VAD and xerophthalmia (WHO Expert Group, 1976). A classification of the eye lesions was agreed upon. With the limited experience at that time some of these eye signs were chosen, together with serum retinol, and criteria proposed for the identification of a problem of vitamin A deficiency of public health magnitude.

Several years later the first national point prevalence survey of vitamin A deficiency was carried out in Indonesia (Sommer, 1982). A second WHO Expert Group on the Control of Vitamin A Deficiency and Xerophthalmia (WHO Expert Group, 1982) was largely influenced by this study to make several alterations in the classification and the public health criteria (see Tables 4.2 and 4.3).

Table 4.2. Xerophthalmia classification by ocular signs (WHO Expert Group, 1982)

Night blindness (XN)
Conjunctival xerosis (X1A)
Bitot's spot (X1B)
Corneal xerosis (X2)
Corneal ulceration/keratomalacia <$^1/_3$ of corneal surface (X3A)
Corneal ulceration/keratomalacia ≥$^1/_3$ of corneal surface (X3B)
Corneal scar (XS)
Xerophthalmic fundus (XF)

Table 4.3. Criteria for assessing the public health significance of xerophthalmia and vitamin A deficiency, based on the prevalence among children less than six years old in the community (WHO Expert Group, 1982)

Criteria	Minimum prevalence
Clinical (primary)	
Night blindness (XN)	1.0%
Bitot's spot (X1B)	0.5%
Corneal xerosis and/or ulceration/keratomalacia (X2 + X3A + X3B)	0.01%
Xerophthalmia-related corneal scars (XS)	0.05%
Biochemical (supportive)	
Serum retinol (vitamin A) less than 0.35 μmol/l (10 μg/dL)	5.0%

A more detailed consideration of the nature of the eye lesions and the way in which they may be used in the assessment of VADD is the subject of Chapter 5. More recent proposals for examination of visual functions as a means of assessing subclinical vitamin A deficiency will be discussed later in this chapter.

This raises a point which requires some discussion and explanation relating to the terms subclinical and clinical introduced above. Whilst the division has its uses, there is no clear-cut borderline between the two. Subclinical implies that there is no clinical evidence of disease. The subject has no complaints of ill health and the examiner is unable to elicit any physical signs of disease. Some indicators of vitamin A status span this borderline between subclinical and clinical. This is the case for both impairment of retinal rod function and abnormal bulbar conjunctival histology. Both of these will be discussed in detail later, but it is useful at this point to see how they span the subclinical-clinical divide (see Tables 4.4 and 4.5).

Table 4.4. Increasing impairment of retinal rod function, illustrating the subclinical–clinical divide

Tests of retinal rod function	Abnormal response
Subclinical	
Dark adaptometry	Abnormal final rod threshold
Vision restoration	Delayed response after bleaching
Pupillary contraction	Failure of contraction in low illumination
Clinical	
Night blindness	Subjective impairment of vision in low illumination

Table 4.5. Progressive changes in conjunctiva and cornea, illustrating the subclinical–clinical divide

Test	Abnormal appearance
Subclinical	
Conjunctival impression cytology	In epithelial and goblet cells
Clinical	
Conjunctival xerosis (X1A)	Dryness
Bitot's spot (X1B)	Foamy, cheesy heaping up of keratinized epithelial cells in interpalpebral fissure
Corneal xerosis (X2)	Hazy cornea
Keratomalacia (X3)	Liquefaction of part or all of cornea

These tables show how impairment of function and abnormality of structure, respectively, increase progressively with increasing degree of deficiency.

Although the ocular lesions of xerophthalmia are dealt with in the following chapters, after the subclinical stages of vitamin A deficiency and the tests that have been developed for them, it is important to recognize that, in historical terms, the eye signs were studied first. This is because xerophthalmia, attributable to severe deficiency, was first recognized to be a serious public health problem. It was the recognition that xerophthal-

mia was the most common cause of blindness in young children around the world, as indeed it still is, that justified all the efforts that were expended to control it from the 1960s onwards. When in the 1980s it became evident that subclinical deficiency was associated with a significant increase in young child mortality (see Chapter 6), what might be termed a second wave of concern resulted. This realization provided the impetus for research into reliable methods for the assessment of vitamin A status at the subclinical level.

Indicators for the assessment of vitamin A status at the subclinical level

Over the past decade or so WHO has played a leading role in bringing together the results of research in this field (WHO, 1996). For a detailed account of the technical and logistical aspects of this subject this report and some others cited in Further Reading should be consulted. It is the present purpose to give a balanced overview, to indicate the present state of the art and to suggest how it might develop in the near future. The recommendations of the 1996 report will need to be tried and tested in field studies, just as those of the 1976 report were.

Serum retinol (Pilch, 1987)

The level of retinol in serum is under homeostatic control over a wide range of body stores and reflects these stores only when they are very high or very low. Thus an isolated serum retinol value is not an accurate indicator of vitamin A status. Serum retinol is best used when a frequency distribution can provide useful information about the status of a population and about response to an intervention programme. The disadvantages described for the RDR tests regarding blood sampling and the effect of infections (see Chapter 9) apply equally.

In the past it has usually been considered that a serum retinol level <0.35 µmol/l was "deficient" and <0.70 µmol/l was "low". The WHO 1996 report recommended to retain the "low" value at 0.70 µmol/l and to consider this consistent with the presence of a subclinical deficiency status. It proposed the following cut-off points: 2–10% mild; 10–20% moderate; >20% severe (see Table 4.7, p 48). There has to be a question raised about the point at which the mild subclinical deficiency status has been set; i.e. >2%. In the United States (Pilch, 1987) and in the United Kingdom (Gregory, Collins, Davies et al, 1995) nutrition surveys that included serum retinol determinations on large numbers of preschool age children have been conducted (Figure 4.1). In both instances values for both sexes and all age groups fall well within the present definition of a mild subclinical vitamin A deficiency problem. If true this would suggest that all countries would have some degree of subclinical vitamin A deficiency. In view of the serious consequences of this state in terms of increased risk of morbidity and mortality it would imply the need for universal vitamin A intervention.

Serum retinol-binding protein (RBP)

Several studies (Blaner, Piantedosi, Gamble, 1999; Donnen, Dramaix, Zihindula et al, 1999; Almekinder, Manda, Kumwenda et al, 1999) have recently shown that measurement of serum RBP correlates very closely with serum retinol (see p 7).

The immunodiffusion technique commonly employed is much simpler and cheaper than HPLC usually used for retinol. A simple, portable microtechnique for measuring holo-RBP using a fluorometer has been introduced by Craft (1999).

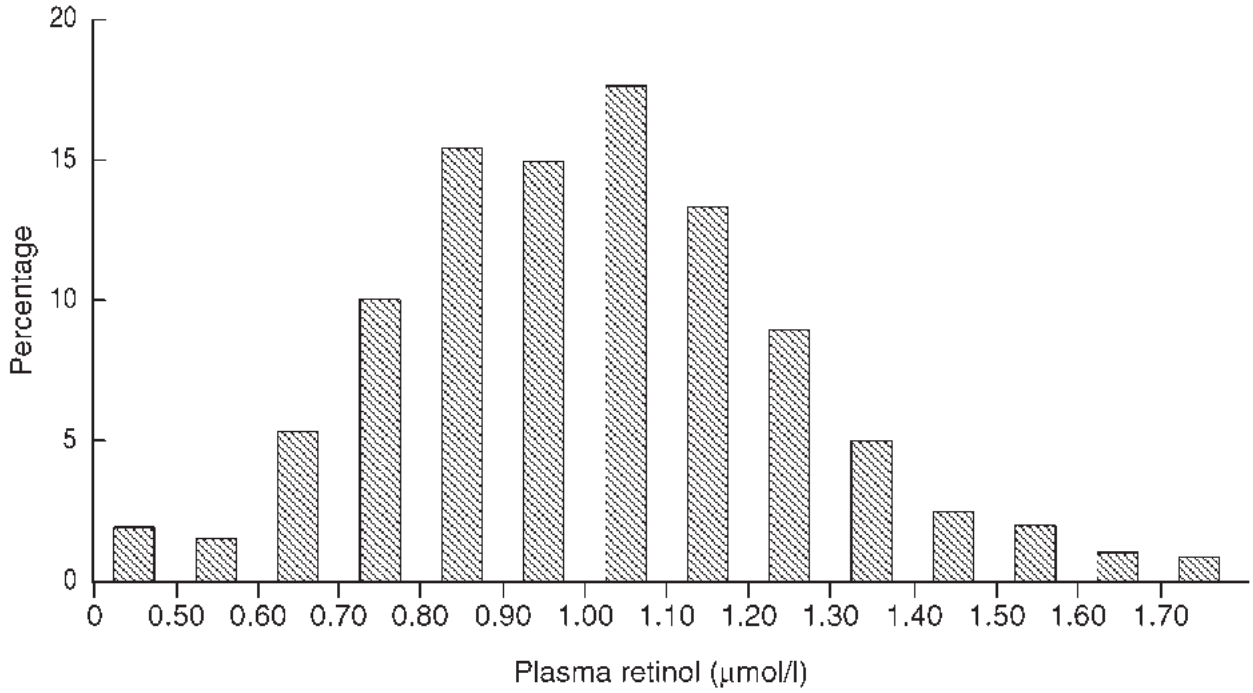


Figure 4.1. Distribution of plasma retinol in children aged 1.5 to 4.5 years (Gregory, Collins, Davies et al, 1995)

RBP/TTR molar ratio

This ratio has been introduced in an attempt to assess vitamin A status in the presence of inflammation (Rosales, Ross, 1998). In the acute phase response (APR) (see also Chapter 9) RBP in plasma falls, as it does in VAD. On the other hand, TTR (transthyretin), to which retinol is also bound in plasma, falls in the APR but is unaffected by VAD. Studies in experimental animals and children with serious infections have indicated that a low molar ratio of RBP/TTR can distinguish VAD in the presence of infection. Recently, further work was carried out in malaria (Rosales, Topping, Smith et al, 1999) and negative results were reported from Congo (Donnen, Dramaix, Zihindula et al, 1999).

RAG hydrolysis test

Retinoyl β-glucuronide (RAG) is a naturally occurring metabolite of vitamin A. It is highly active biologically with little toxicity. It may have therapeutic applications (Barua, 1997). When administered orally to rats with adequate vitamin A status RAG is only slowly hydrolyzed to RA, but in animals with VAD RA appears in the plasma in high concentrations. Recent work (Olson, Barua, Kaul et al, 1999) found that the RA response was proportional to the degree of VAD. This appears to be a promising new test.

Assessment of body reserves

In the past there was considerable advocacy for the concept of relying upon the estimation of vitamin A concentration in the livers of patients at postmortem as a means of characterizing the vitamin A status of a population (WHO Expert Group, 1976). In practice this has proved difficult, except in the context of research. Furthermore, newer indirect and direct assessment methods have come to occupy centre stage.

Indirect assessment of liver stores

In Chapter 3 it was mentioned that apo-RBP accumulates in the liver when retinol is in short supply. Once retinol becomes available holo-RBP is released into the circulation. This phenomenon is the basis of the relative dose-response tests. The magnitude of the retinol released from the liver when a test dose is given is proportional to the degree of prior depletion of the liver.

Relative dose response (RDR)

(Underwood, 1990)

A fasting serum retinol is measured (A0). Vitamin A as 450–1000 mg retinyl ester in oily solution is given orally. Five hours after dosing, another serum retinol is measured (A5).

The RDR is calculated as follows:

RDR = (A5 – A0) x 100/A5

If the result of the calculation is >20% then the test is considered to be positive, i.e. stores are deficient. It has been shown that a result of 20% in the test is approximately equivalent to a liver reserve of 0.07 µmol/g. Two blood samples are required.

Modified relative dose response (MRDR) (Tanumihardjo, Koellner, Olson 1990)

This test employs a metabolite of vitamin A, 3, 4-didehydroretinyl acetate (DR, also known as dehydroretinol, vitamin A_2). DR binds to RBP and appears in the serum after a test dose is given if liver reserves of vitamin A are low. A single oral dose of DR is given and only one blood test is required, 4–6 hours after the dose.

Serum concentrations of retinol (SR) and dehydroretinol (SDR) are measured. The molar ratio is calculated in the following way:

MRDR = SDR/SR

An MRDR-value is considered to be abnormal when the above ratio is >0.06.

Serum 30-day dose response (+S30DR)

(Flores, Campos, Aranjo et al, 1984)

This test is similar to the RDR described above but the second blood sample is taken 30–45 days after the first. It has been used at the population level and has been used for monitoring the effectiveness of intervention programmes for improving vitamin A status.

Advantages

It is known from work in experimental animals that in depletion studies vitamin A levels in the liver fall steadily over a considerable period of time before serum retinol levels begin to fall and even longer before any functional or structural changes begin to occur. These tests appear therefore to be good measures of subclinical VAD.

Disadvantages

Blood samples are required. In many populations this may not be acceptable for cultural reasons and in view of the risks of transmitting HIV, hepatitis etc. The single sample for MRDR raises less of a problem than the two samples required for the RDR.

Retinol-binding protein is among a number of proteins that take part in the acute phase reaction in infection and inflammation. The release of RBP from the liver is repressed by a number of factors that are likely to be widely prevalent in the communities under study. These include acute and chronic infections and infestations and protein-energy malnutrition. This subject is considered in more detail later (see Chapter 9). Other than in research projects the means for estimating se-

rum retinol on a routine basis are not readily available. The compound DR, used in the MRDR test, is costly and is not readily available (WHO, 1996).

Recommendations and comments

The WHO 1996 report proposes cut-off points of prevalence for each of the above three response tests that are similar. These are to be used to indicate respectively "mild", "moderate" and "severe" public health problems. The cut-off points given are: <20%; 20–<30% and ≥30%. It should be noted that according to this any % prevalence will constitute at least a mild problem (possibly >2% has been omitted in error; see page 7, table 2 of the report) (see table 4.7, p 48). The same comment applies to the "mild" category of breast milk retinol (<10%). In the case of "mild" night blindness 0 –1% implies that the presence of a single case will always indicate the presence of a public health problem.

Several recent studies have made comparisons between various methods of assessment of vitamin A status (Rice, Stoltzfus, de Francisco et al, 2000; Apgar, Makdani, Sowell et al, 1996; Wahed, Alvarez, Khaled et, al 1995; de Pee, Yuniar, West et al, 1997).

Deuterated-retinol-dilution technique

This technique using the stable isotope of hydrogen, deuterium, measuring total body vitamin stores indirectly but quantitatively is being used increasingly in research projects (Haskell, Mazumder, Peerson, 1999; Ribaya-Mercado, Mazariegos, Tang et al, 1999; Haskell, Handelman, Peerson et al, 1997). A dose of vitamin A labelled with the stable isotope deuterium is given by mouth and about three weeks allowed for equilibration with the reserves of the body. Blood is sampled at this point and extent of dilution of the labelled tracer relates to the amount of endogenous reserves. Olson (1997) has reviewed the difficulties inherent in this new method but describes isotope-dilution techniques like this as "a wave of the future in human nutrition".

Breast milk vitamin A concentration

It has long been known that the concentration of vitamin A in the breast milk of undernourished mothers is low. The proposal to use this as an indicator of vitamin A status of a community is relatively new and needs to be tested under varying conditions. It has the advantages of being non-invasive, readily acceptable and the sample is easy to collect. It is important to follow standardized methods of collection of the sample, and the method of expression of the concentration of vitamin A should be agreed upon. Results to date suggest that in vitamin A-sufficient populations average breast milk concentrations range from 1.75 to 2.45 μmol/L. In a vitamin A-deficient population average values are below 1.4 μmol/L. The borderline of deficiency in a population is suggested to be <1.05 μmol/L. Prevalence rates proposed are mild <10%; moderate 10–25%; severe ≥25% (see Table 4.7, p 48).

In one study in Indonesia (Stoltzfus, Hakimi, Miller et al, 1993) there was a close correlation between breast milk concentrations below 1.05 μmol/L and the prevalence of positive RDR tests in infants 6 months of age. More recently in Bangladesh a comparison of serum retinol, MRDR and breast milk found that overall the most responsive indicator to post-partum maternal vitamin A supplementation was the measurement of breast milk vitamin A per gram of fat in casual breast milk samples (Rice, Stoltzfus, de Francisco et al, 2000).

Histological indicator [Conjunctival Impression Cytology (CIC)]

In Chapter 3 attention was drawn to the major role played by vitamin A in cellular differentiation. The abnormal changes that occur when vitamin A is deficient have been documented best for the conjunctiva and to a lesser extent for the cornea. Basically there is a drying process, xerosis, which may ultimately in the conjunctiva lead to the development of a Bitot's spot (X1B) (see Chapter 5). The earlier change that is visible with the naked eye, called conjunctival xerosis (X1A), is much less distinctive (see Chapter 5). This in turn is preceded by lesser degrees of change that are at the subclinical level.

With the introduction of the technique of CIC (Wittpenn, Tseng, Sommer, 1986) and a modification termed Impression Cytology with Transfer (ICT) (Luzeau, Carlier, Ellrodt et al, 1988) it has become possible to study these very early changes under a microscope. Details of the techniques that have been advocated are given in a training manual (Wittpenn, West, Keenum et al, 1988), in several original papers and also in the WHO 1996 report (WHO, 1996). In essence, a normal impression of conjunctival cells when stained will show one or more sheets of small regular epithelial cells and numerous mucin-secreting goblet cells. In VAD the epithelial cells become flattened, enlarged and reduced in number. The goblet cells are markedly reduced in number or absent (see Figures 4.2–4.7).

Table 4.6 shows the relationship between the prevalence of abnormal CIC and vitamin A status assessed by some other indicators. Unfortunately when the results of CIC have been compared with those of biochemical tests there has been little correspondence (Tanumihardjo, Permaesih, Dahro et al, 1994; Makdani, Sowell, Nelson et al, 1996).

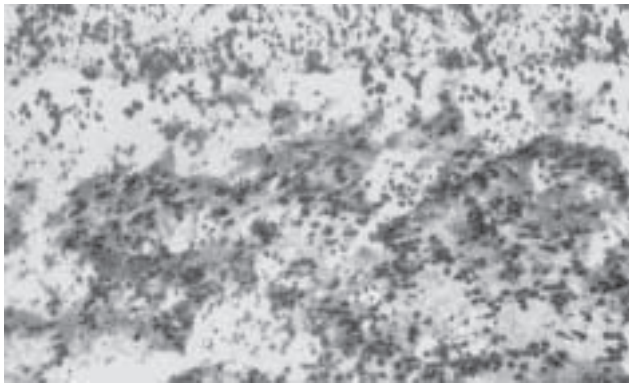
Limitations

Difficulties have arisen over the significance of some of the appearances. While there is no doubt about those described above for clearly normal and clearly abnormal impressions, the vast majority of impressions will not be so distinct. Standardization of existing interpretation schemes is required if the technique is to come into widespread use (WHO, 1996). The test is well accepted after the age of about 3 years, but has posed problems in younger children. The presence of acute conjunctivitis and trachoma (Lietman, Dhital, Dean, 1998) interferes with CIC.

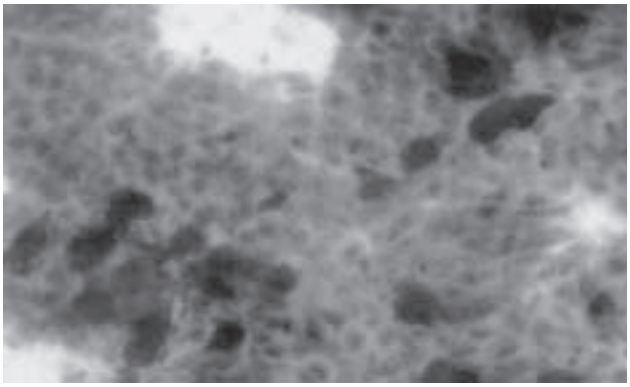
More recent experience suggests that Conjunctival Impression Cytology with Transfer (CICT) identical with ICT above is the preferable technique (Chowdhury, Kumar, Ganguly et al, 1996). These investigators reported that healing after vitamin A dosing takes from about 70 to 110 days to be completed (Chowdhury, Kumar, Ganguly, 1997). This may seem to be a long time for a minimal lesion such as early xerosis and keratinization of the conjunctiva, especially in comparison with healing of the more severe clinical lesions (see p 64). In this regard it should be remembered that microscopic techniques are likely to be more precise than clinical observations. CIC is being used increasingly in clinical ophthalmology and new refinements are constantly being introduced (Thiel, Bossart, Bernauer, 1997).

Impaired dark adaptation

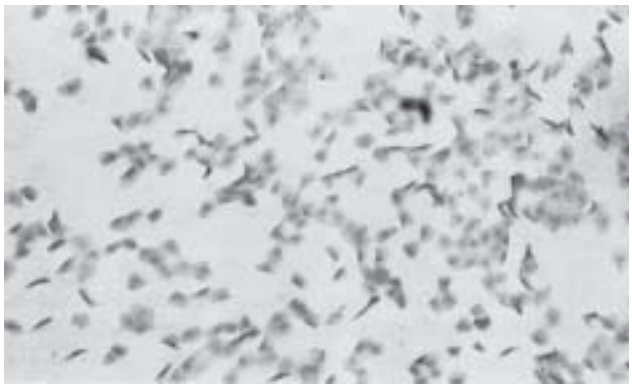
It will be recalled that the rod cells of the retina contain vitamin A in the form of 11-*cis* retinal bound to opsin to form rhodopsin (see Chapter 3). These cells are photosensitive under conditions of low levels of illumination (night or scotopic vision). Table 4.4 above showed the various stages of increasing deficiency at which different kinds of testing may be applied.



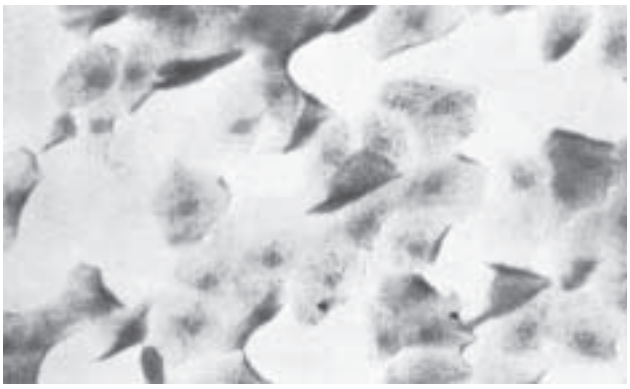
4.2. Normal conjunctival impression with abundant goblet cells, sheets of small epithelial cells, and mucin spots [periodic-acid Schiff (PAS) and Harris' haematoxylin, x 160].



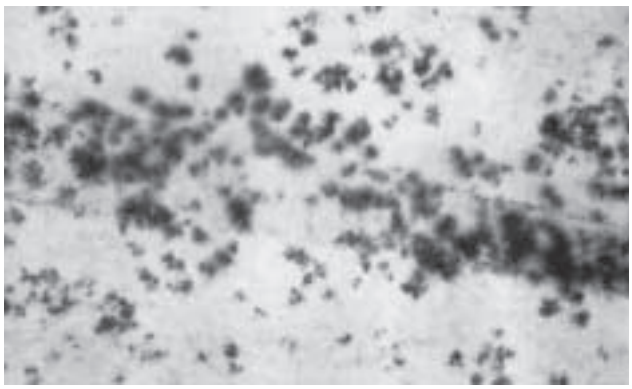
4.3. Higher power of normal conjunctiva, showing contrast between PAS-positive goblet cells and epithelial cells (PAS and Harris' haematoxylin, x 400).



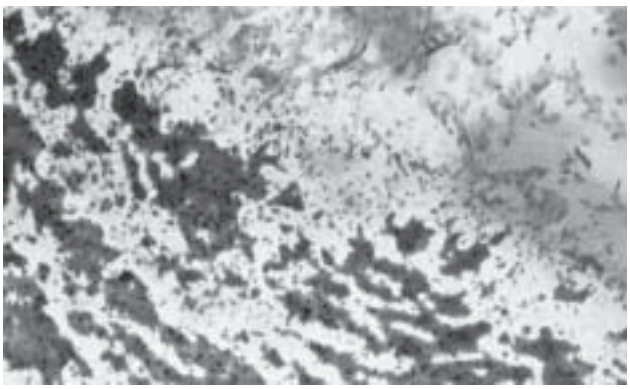
4.4. Abnormal conjunctival impression with complete loss of goblet cells and mucin spots, along with appearance of enlarged epithelial cells (x 100).



4.5. Higher power of abnormal, enlarged conjunctival cells (PAS and Harris' haematoxylin, x 400).



4.6. PAS-positive mucin spots representing "impressions" of goblet cells on conjunctival surface (PAS and Harris' haematoxylin, x 400).



4.7. Impression cytology from normal child showing transition from abundant normal epithelium (lower left) to abnormal epithelium (upper right). Specimen was graded as normal (x 100).

Figure 4.2–4.7 Conjunctival impression cytology (Wittpenn, Tseng, Sommer, 1986).

Table 4.6. Abnormal conjunctival impression cytology and vitamin A status (Natadisastra, Wittpenn, Muhilal et al, 1988)

Vltamin A status	Patient characteristics	Number	Abnormal CIC (%)
Definite deficiency	• XN(+) plus X1B(+) responding to vitamin A • Serum retinol <20 μg/dL	14	93
Probable deficiency	• Bilateral X1B(+) responding to vitamin A	22	82
Probable deficiency	• XN(+) responding to vitamin A • serum retinol <20 μg/dL	15	67
Possible deficiency	• Unilateral X1B(+) [XN(–)] responding to vitamin A	8	50
Possible deficiency	• Normal exam • Serum retinol <20 μg/dL	26	46
Borderline deficiency	• XN(+) plus retinol ≥20 μg/dL or normal exam and retinol 20–25 μg/dL	43	14
"Normal"	• Normal exam • Serum retinol >25 μg/dL	18	6

Night blindness

This is the most advanced stage of deficiency that can be related to rod dysfunction. In a subject able to cooperate this is the subjective sensation of inability to see adequately in poor illumination, such as at dusk. It is sometimes erroneously termed hemeralopia (Greek *hemera*, day) or nyctalopia (Greek *nyct*, night; *alaos*, obscure). Dusk blindness would be a better term.

The dark adaptometer is a standard piece of ophthalmology department equipment for examination of night vision. Some versions are available for use under field conditions. These may be used for investigating the problem of night blindness in cooperative subjects such as school children and pregnant and lactating women, among whom a high prevalence has been reported in some countries (Sommer, West, 1996, p 338).

In young children, the major vulnerable group of VADD, direct observation and an interview have usually been used. A standardized interview of the child's guardians has been developed (Sommer, Hussaini, Muhilal et al, 1982) and use is made of the fact that frequently in endemic areas terms occur in the local language. These often liken poor vision at dusk to "chicken eyes" (chickens have no rod cells in their retina) or Bitot's spots to "fish scales". If the child is old enough to walk, it may be observed to stumble in a room at dusk, or it may fail to grasp a proffered toy or sweet (see also Chapter 5).

Vision restoration time (VRT)

The ability of the bleached eye to recognize a letter under low levels of illumination has been measured in school-age children in Thailand (Udomkesmalee, Dhanamitta, Sirisinha et al, 1992). From this study it ap-

peared that zinc was a more important determinant of VRT than vitamin A (see also Chapter 7). The test is applicable only to older children with some degree of literacy.

Pupillary threshold

This test is based on the fact that the weakest threshold of light visible in the dark-adapted state is approximately of the same intensity as that needed to cause pupillary contraction. The pupils of night blind subjects fail to react or constrict normally in low illumination. Children as young as one year old have been studied successfully (Congdon, Sommer, Severns et al, 1995). Further testing in children with VAD in India (Sanchez, Congdon, Sommer et al, 1997) and of pregnant women in Nepal (Congdon, Dreyfuss, Christian et al, 1999) has been distinctly promising. The test has proved readily acceptable, is associated closely with serum retinol, and responds to vitamin A supplementation. It also has the advantages of being non-invasive, applicable to young children and sensitive at the subclinical stage of VAD.

Table 4.7 indicates how some of these biological indicators may be used to grade different levels of subclinical VAD (WHO, 1996).

Assessment of dietary intake of vitamin A

As mentioned above, this is strictly speaking not a measure of vitamin A status but can provide useful and complementary information not available otherwise. It has the advantages of not being invasive, expensive or complicated, large numbers of subjects can be readily targetted and a profile of a population obtained. Knowledge of available and utilized sources of vitamin A can be collected that may prove to be of value in subsequent dietary interventions. Two organizations, International Vitamin A Consultation Group (IVACG) and Helen Keller International (HKI) have been largely instrumental in promoting the development of assessment methods and field studies.

IVACG (1989) introduced a simplified dietary assessment (SDA) as a method to identify and monitor groups at risk of suboptimal vitamin A intake. Intake information is obtained by past 24-hour dietary recall and by history of the usual pattern of consumption of vitamin A-rich foods during the previous month. Food quantities were estimated. Some experience with this method has found it to be difficult, time-consuming, or poorly correlated with measures of vitamin A status. The semi-quantitative 24-VASQ (twenty four hours vitamin A semiquantitative) method is partly based on the IVACG method. It estimates vitamin A intake using 24 h recall data, uses individual ingredients instead of dishes, and from four sources – vegetables, fruits, animal foods and fortified foods. It has been used successfully in Indonesia, Bangladesh and Nepal (de Pee, Bloem, Kiess et al, 1999; de Pee, Bloem, Halati, 1999).

The Helen Keller International Food Frequency Method (HKI FFM) was introduced in 1993 (Rosen, Haselow et al, 1993). This method studies the diet of children 1–6 years of age. A score is assigned to each child based on the number of rich sources (> 100 RE/100 g vitamin A content), both animal and plant, consumed during the previous week, with amounts ignored.

Animal milk was ignored in the original method, but subsequently it has been shown that in some areas this can provide 5–18% of the “safe” RDI and should be considered. Experience with five communities in each the Philipinnes, Tanzania and Guatemala, using serum retinol <20 μg/dL as deficient, found correct classification as deficient in 73.3%, but a high false-positive rate of 42.9% (Sloan, Rosen, Paz, 1997).

Table 4.7. Biological indicators of subclinical vitamin A deficiency in children 6–71 months of age (WHO, 1996)

Indicator (cut-off)	Prevalence below cut-offs to define a public health problem and its level of importance		
	Mild	Moderate	Severe
Functional			
Night blindness (present at 24–71 months)	>0–<1%	≥1–<5%	≥5%
Biochemical			
Serum retinol (≤0.70 μmol/L)	≥2–<10%	≥10–<20%	≥20%
Breast milk retinol (≤1.05 μmol/L or ≤8 μg/g milk fat)	<10%	≥10–<25%	≥25%
RDR (≥20%)	<20%	≥20–<30%	≥30%
MRDR (ratio ≥0.06)	<20%	≥20–<30%	≥30%
+S30DR (≥20%)	<20%	≥20–<30%	≥30%
Histological			
CIC/ICT (abnormal at 24–71 months of age)	<20%	≥20–<40%	≥40%

Unfortunately, the uncertainty that now exists about the bioavailability and bioconversion factors for provitamin A carotenoids (see Chapter 2) overshadows these and all methods of assessment of dietary vitamin A intake. Until these matters are resolved results of field work will have to be interpreted with caution.

Ecological indicators

The WHO 1996 report introduced the concept of the use of what it termed “Ecological and related indicators associated with risk of VAD”. It recognized that these indicators could not be used alone to replace biological indicators (those described above and specifically related to VAD) or to define whether a population has a VAD problem of public health significance. It goes on to describe and to define these indicators. Table 4.8 shows these indicators and how they may be used in VAD surveillance.

Table 4.8. Ecological indicators of areas/populations at risk of VAD: Nutrition- and diet-related indicators* (WHO, 1996)

Indicator	Suggested prevalence
Breast-feeding pattern	
<6 months of age	<50% receiving breast milk
≥6–18 months of age	<75% receiving vitamin A-containing foods in addition to breast milk, 3 times/week
Nutritional status (< –2SD from WHO/NCHS reference for children <5 years of age)	
Stunting	≥30%
Wasting	≥10%
Low birth weight (<2500 g)	≥15%
Food availability	
Market	DGLV[1] unavailable ≥6 months/year
Household	<75% households consume vitamin A-rich foods 3 times/week
Dietary patterns	
6–71 months old children, pregnant/lactating women	<75% consume vitamin A-rich foods at least 3 times/week
Semi-quantitative/qualitative food frequency	Foods of high vitamin A content eaten <3 times/week by ≥75% vulnerable groups

* There are also illness-related indicators not reproduced here

[1] Dark Green Leafy Vegetables

The WHO 1996 report proposes that a public health problem exists when the criteria set in Table 4.7 are met in the case of at least two of the biological indicators, or when one biological indicator is supported by at least four of the ecological indicators in Table 4.8 (two of which are nutrition- and diet-related).

The 1996 WHO report suggests a tentative ranking of the biological indicators discussed here for usefulness in various activities (see Table 4.9). This is bound to be very subjective. It is disappointing to see how low CIC/ICT ranks, as, on *a priori* grounds, it might be considered one of the most promising tests of all.

In conclusion it has to be said that now there is available a plethora of methods of assessment of vitamin A status at the subclinical level. Most have been developed only in the last two decades at most. Each tends to have its advocates and this makes decisions about choice of method very difficult. This is expecially so while discrepancies continue to be reported between the results with the various tests.

Table 4.9. Relative ranking on a population base of some biological indicators useful for various surveillance purposes (WHO, 1996)

Indicator	Risk assessment	Targeting programmes	Evaluating effectiveness
Night blindness	+++	+++	+++
Breast milk retinol (lactating mothers and breast-fed infants)	++	+++	++
Serum retinol	++	+	++
RDR/MRDR	+++	+++	+++
CIC/ICT	+	--	--
+S30DR	--	--	+++

XEROPHTHALMIA

Definitions

The term xerophthalmia literally means “dry eye”. However, dryness or xerosis, which also affects other parts of the body (see Chapter 3), is only part of the abnormal process undergone by the eye in vitamin A deficiency. Xerosis is confined to the epithelial structures of the eye, the conjunctiva and the cornea. Only the conjunctiva covering the globe, known as the bulbar conjunctiva, and not that lining the eyelids or the palpebral conjunctiva, is affected by xerosis. In addition, the cornea undergoes other changes, known as keratomalacia, as will be described later. After recovery from acute vitamin A deficiency that affects more than just the most superficial layer of the cornea, scars of varying extent and depth remain (XS).

In vitamin A deficiency the retina is also affected. The rhodopsin system in the rod cells of the retina is much more sensitive to deficiency than is the iodopsin system in the cone cells. As a result, rod function is impaired early on, resulting when sufficiently marked in impairment of night vision. Impairment of cone function, i.e. day vision and colour vision, is rarely seen clinically. There have been a few reports of structural damage to the rod cells in the retina.These have been called xerophthalmic fundus (XF).

All of these eye changes are included in the term xerophthalmia. In other words, xerophthalmia is synonymous with all of the clinical signs and symptoms that affect the eye in vitamin A deficiency. The various ocular appearances in xerophthalmia were classified by WHO in 1976 (WHO Expert Group, 1976) and modified in 1982 (WHO Expert Group, 1982). Criteria of a public health problem were also developed. These tables were referred to previously (see Chapter 4, Tables 4.2 and 4.3) but for convenience they are reproduced here (see Tables 5.1 and 5.2).

To a considerable extent the ocular signs in Table 5.1 have been listed in an increasing order of severity. This means that retinal function tends to be impaired before xerosis affects first the conjunctiva and then the cornea. Liquefaction of the cornea, of increas-

Table 5.1. Xerophthalmia classification by ocular signs (same as Table 4.2)

Night blindness (XN)
Conjunctival xerosis (X1A)
Bitot’s spot (X1B)
Corneal xerosis (X2)
Corneal ulceration/keratomalacia $<1/3$ of corneal surface (X3A)
Corneal ulceration/keratomalacia $\geq 1/3$ of corneal surface (X3B)
Corneal scar (XS)
Xerophthalmic fundus (XF)

Table 5.2. Criteria for assessing the public health significance of xerophthalmia and vitamin A deficiency, based on the prevalence among children less than six years old in the community (same as Table 4.3)

Criteria	Minimum prevalence
Clinical (primary)	
Night blindness (XN)	1.0%
Bitot's spot (X1B)	0.5%
Corneal xerosis and/or ulceration/keratomalacia (X2 + X3A + X3B)	0.01%
Xerophthalmia-related corneal scars (XS)	0.05%
Biochemical (supportive)	
Serum retinol (vitamin A) less than 0.35 μmol/l (10 μg/dL)	5.0%

ing severity, is a very late stage normally. Corneal scars are not part of the active deficiency process.They may be considered to be stigmata of previous deficiency, providing there is circumstantial evidence suggestive of vitamin A deficiency as their cause. These characteristics are discussed later. The xerophthalmic fundus is a rarity and does not fit neatly into the classification (see later).

Background

There are two other aspects of the subject of the definition of xerophthalmia that need to be made quite clear. Firstly, there are other causes of xerophthalmia than dietary vitamin A deficiency, the epidemic disease that primarily affects young children in developing countries and that is our main concern in this Manual. These cases arise sporadically and are caused by various defects in the utilization of vitamin A within the body. Most of these are quite uncommon. They are instances of secondary or endogenous VAD and are considered later in Chapter 10.

The other aspect of the subject that needs to be pointed out is that some of the eye changes may be caused by diseases that are not related to vitamin A deficiency. For example, night blindness is a symptom of several rare degenerative disorders of the retina. It is also a main feature of a not uncommon blinding disease called retinitis pigmentosa. For the most part these diseases do not respond to treatment with vitamin A (see Chapter 10).

Dryness of the conjunctiva and cornea is also a feature of several diffuse connective tissue disorders, one of which is Sjogren's syndrome. The xerosis results from atrophy of the secretory epithelium of the lacrimal glands and does not respond to vitamin A.

There are several facts that shed light on the way in which xerophthalmia has tended to be neglected over the years. For reasons that are not clear xerophthalmia was usually not included among the classical vitamin deficiency diseases when these were first identified in the 19th and early 20th century. Beriberi, scurvy, pellagra and rickets were usually considered together and xerophthalmia was rarely included (Funk, 1912). Even though fat-soluble A was the first vitamin identified (McCollum, Davis, 1915) and was therefore given the first letter in the alphabet, xerophthalmia was not studied in detail at the time. Night blindness and Bitot's spots tended to be described in isolation from the blinding changes of corneal xerophthalmia. It may be that scientists and general physicians did not feel competent to examine the eye and deal with eye disease. It is of interest that the only

vitamin deficiency to have reached epidemic proportions and to persist at these levels today is xerophthalmia. This historical aspect of VADD has been discussed recently (McLaren, 1999).

Corneal xerophthalmia is usually associated with a severe degree of vitamin A deficiency which is often accompanied by severe generalized malnutrition (PEM) and serious infections (McLaren, Shirajian, Tchalian et al, 1965). Death is the most likely outcome at this late stage, even in adequately treated patients. In this way death "removes" the problem of long-term care of the blinded survivors. Follow-up over a period of one year after the diagnosis of corneal xerophthalmia has shown that only about 40 % survive (ten Doesschate, 1968). Of these survivors about 25 % remain totally blind and 50–60 % are partially blind. It is, consequently, uncommon to find a high proportion of inmates of blind schools in developing countries having xerophthalmia as the cause of their blindness. Even those that do recover from acute severe vitamin A deficiency when young are likely to fare badly in the community. In such circumstances there is little possibility of their receiving an education. Without special training they will be unable to do any work and their survival constitutes a continuing burden on the family and the community at large.

The close association between vitamin A deficiency and susceptibility to infections was noted in both experimental animals and in children many years ago (Semba, 1999; Sommer, West, 1996, pp 62-98). It was only in the past twenty years or so that this phenomenon was explored systematically and scientifically at the community level. This research has resulted in, among other things, an appreciation of the public health importance of subclinical vitamin A deficiency in the context of the increased susceptibility of young children to suffer from various infections and also their diminished chance of survival. These matters are the topic of Chapter 6. This new knowledge has given a tremendous impetus to the drive to control and eventually eradicate vitamin A deficiency of all degrees. This important and fully justified shift of emphasis in recent times must not, however, be allowed to draw attention away from the fact that large numbers of young children still suffer visual impairment as the result of consuming a diet deficient in vitamin A and its precursors.

It is interesting to note that this revolution in the thinking about the significance of vitamin A deficiency in man is part of a general pattern also seen in other micronutrient deficiency diseases. This is particularly true of the two elements iron and iodine, which are now very commonly associated with vitamin A and termed Micronutrient Disorders (WHO, 1992). Iron deficiency was for long considered to be of little more public health significance than an important cause of anaemia. Now we know that work capacity may be seriously affected and in the young mental development and ability to profit from schooling may be impaired. In the case of iodine the term Iodine Deficiency Disorders (IDD) has been coined. It is now known that endemic colloid goitre is not the most significant consequence for public health of iodine deficiency. Development of the brain in fetal and early postnatal life may be retarded and relatively minor degrees are much more common than clinical cretinism. Other examples of expansion in recent years of the significance of micronutrient deficiency are provided by vitamins D and K and the element zinc.

Night blindness (X1N)

It will be recalled that the subject of retinal dysfunction was considered previously in connection with indicators of VAD (see Chapter 4). As shown in Table 4.3 night blindness is the most extreme form of retinal dysfunction; sufficiently severe to cause subjective impair-

ment of vision at night. As an indicator of VADD it has both advantages and disadvantages. On the positive side, tests are noninvasive and some of them may be applied by investigators with no specialized ophthalmological training. In this category are questionnaires and observation of the performance of young children under conditions of low illumination.

On the negative side, the objective testing of night vision requires sophisticated and expensive equipment, operated by skilled ophthalmological staff. The subjects need to be of sufficient age and education to cooperate fully in the testing. This applies to dark adaptometry, rod scotometry, and to some extent to electroretinography, which is less sensitive.

The most promising of the new tests of dark adaptation is measurement of the pupillary threshold (see Chapter 4). This has been pioneered by one group and is still undergoing field trials. Some of its advantages and disadvantages were discussed previously. In field studies the most practical method of detecting night blindness at the present time is by interview, and WHO has recommended a scheme for this purpose (see Table 5.3).

This scheme may increase the specificity and reduce the error of classification solely on the basis of familiarity with the term. The use of focus group discussions in different situations may be useful in identifying local terms for night blindness and the specificity with which they are useful for identifying VAD.

One study in Bangladesh (Hussain, Kvale, Odland, 1995) compared over a hundred children aged 2–15 years and reported to be night blind by their parents with a similar number of matched controls. Both groups received an eye examination, test of scotopic vision by a luxometer – a simple form of dark adaptometer – and serum retinol level. Although there

Table 5.3. Scheme for the classification of night blindness by interview (WHO, 1996)

1) Does your child have any problem seeing in the daytime?
2) Does your child have any problem seeing at nighttime?
3) If (2) = yes, is this problem different from other children in your community? (Note: this question is particularly appropriate where VAD is not very prevalent.)
4) Does your child have night blindness (use local term that describes the symptom)?

was fairly close correlation between the two methods of diagnosing night blindness, the parents' report appeared to be less sensitive.

The occurrence of names for night blindness in local languages suggests that this rather distinctive phenomenon is occurring with some regularity in a community. The commonest terms used are "night eyes" and "chicken eyes" (chicken have no rods and are therefore night blind). Night blindness is such a distinctive symptom that it has given rise to some interesting stories about its occurrence. For instance, many years ago it occurred in fishermen in Newfoundland in Canada. They learned to bind up one eye before they went out in the bright glare on the ocean. The retina of the exposed eye would become bleached and vitamin A deficiency prevented regeneration of rhodopsin. Fishing could continue by unbinding the other eye.

Little night blindness is detected in very young children because the effects of deficiency only become evident when the child tries to move around at dusk. From about 2 years onward rates of night blindness tend to rise, because of increased

activity. Evidence has accumulated in recent years to show that school age children and pregnant and lactating women (Khan, Haque, Khan, 1984; Katz, Khatry, West et al, 1995) are also vulnerable groups in which the measurement of rod vision may be a useful means of assessing vitamin A status in a community. These groups can cooperate in dark adaptometry and this is the method of choice.

Clinical dark adaptometers, designed for use in hospitals, are expensive and sensitive pieces of equipment. There are simpler and more robust forms of equipment that may be readily transported for use in field studies. Vitamin A deficiency results in delay in rod adaptation to conditions of illumination of low intensity, followed by reduction in threshold sensitivity. Figure 5.1 shows the curve that is obtained on testing a normal subject. Figure

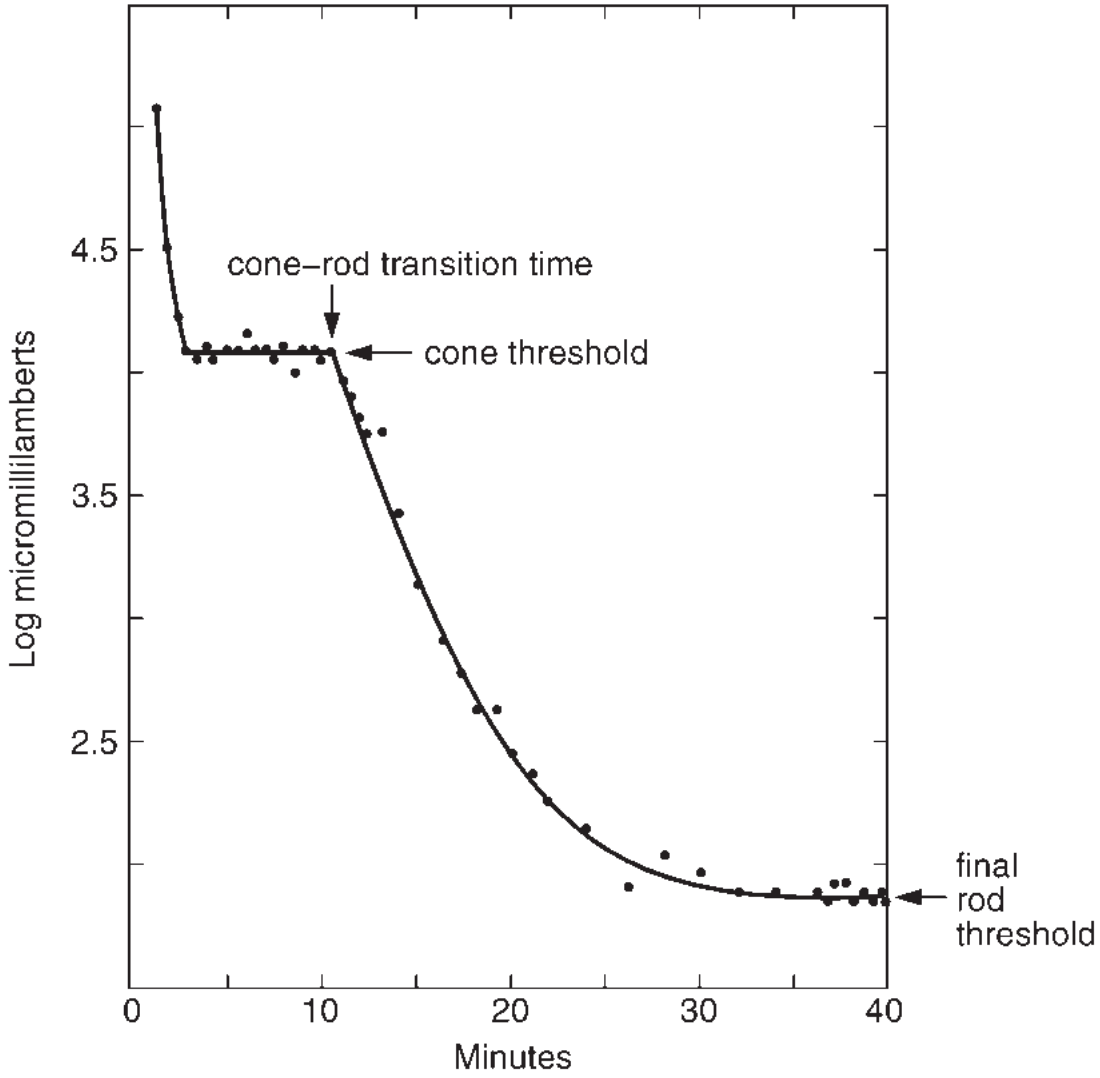


Figure 5.1. Normal curve of dark adaptation showing cone-rod transition time, cone threshold, and final rod threshold (Hume, Krebs, 1949). Log millilamberts are units of intensity of illumination.

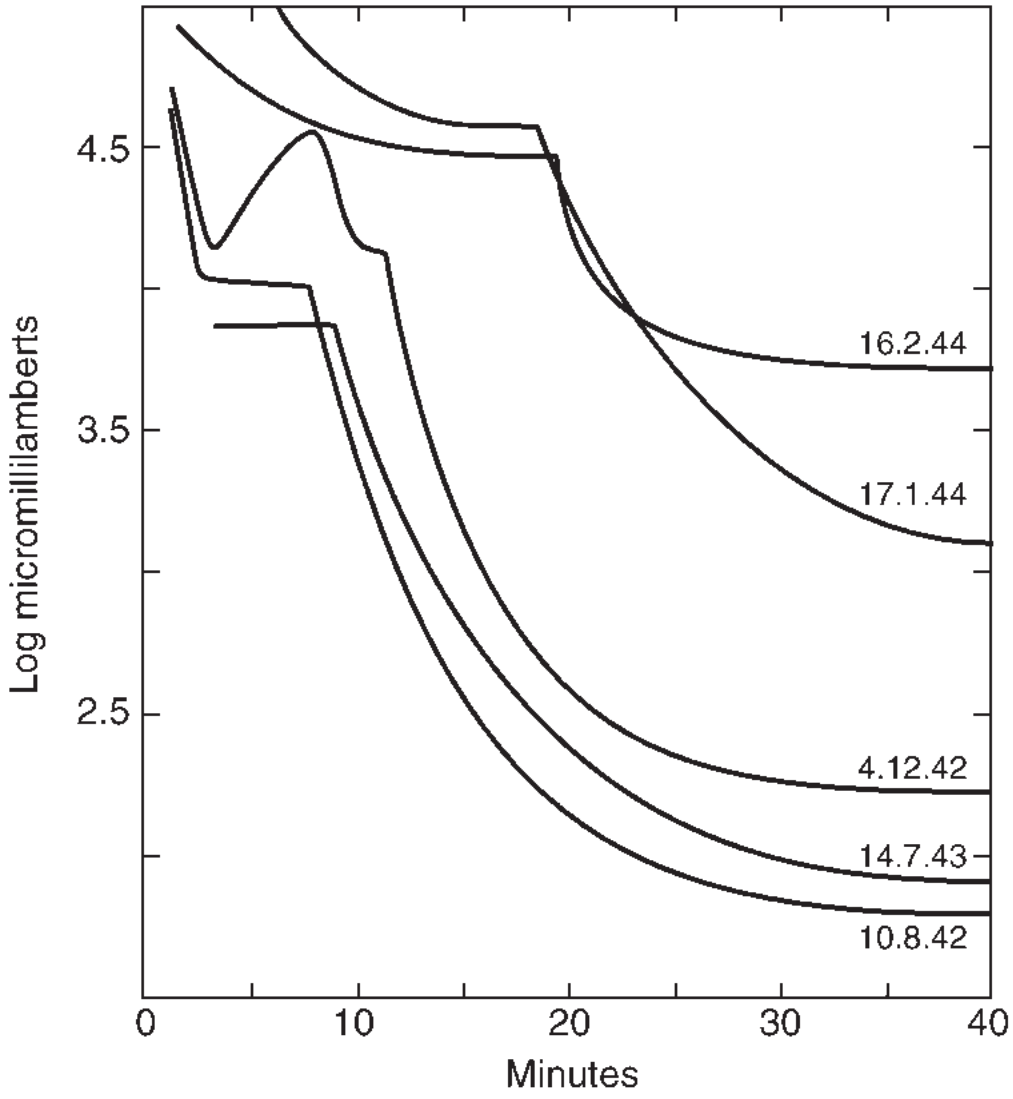


Figure 5.2. Curves of dark adaptation for one deprived subject at different stages of depletion (Hume, Krebs, 1949). Log millilamberts are units of intensity of illumination, numbers on right are dates of tests.

5.2 illustrates how test curves rose progressively in a volunteer subject receiving a vitamin A-deficient diet.

In practice it is usually sufficient to allow both examiner and a group of subjects for testing to undergo dark adaptation for about 30 minutes in a darkened room. At this point maximum rod adaptation possible will have occurred and the tests are carried out at the final rod threshold (see Figure 5.1).

Night blindness is frequently the most prevalent form of xerophthalmia, as might be expected. Although serum retinol of >20 μg/dL has customarily been considered to be “normal” it was found that about 20% of children with night blindness and 10% with night blindness and Bitot’s spot had higher levels of serum retinol (see Table 5.4). As might be expected, impaired dark adaptation could be detected with serum retinol between 20 and 30 μg/dL.

Conjunctival xerosis (X1A)

The term conjunctival xerosis could apply to any stage of xerotic change in the conjunctiva (see Figure 5.3). This would range from abnormal impression cytology, through dryness of the conjunctiva, to keratinization and heaping up of material as in Bitot's spot (X1B, see below). CIC is subclinical and was dealt with previously (see Chapter 4). In the Xerophthalmia Classification (see Table 5.1) X1A distinguishes dryness from Bitot's spot. Before this scheme was agreed upon a great deal of attention was given to various conjunctival appearances, including thickening, wrinkling and pigmentation. It is now recognized that these changes and the degree to which they occur are not related directly to vitamin A deficiency. If the classification (see Table 5.1) and the criteria (Table 5.2) are compared it will be evident that X1A is not one of those appearances chosen for use in surveys. This is because it has repeatedly been found that, while advanced instances of X1A can be readily identified, minor changes are subject to great inter- and intra-observer variation. This makes X1A an unreliable indicator of VAD. Even so it is often, wrongly, used in surveys, making interpretation of results very difficult. To obtain reproducible and comparable results it is of great importance that WHO guidelines be followed in matters like this (WHO Expert Group, 1982).

Both experimental and clinical evidence suggests that the process of xerosis, which also affects other epithelial tissues than the conjunctiva and cornea, is primarily due to changes in the proteins of the tissue itself. Loss of tears also occurs but is a secondary phenomenon, tending to make existing xerosis worse. Loss of goblet cells and lack of secretion of mucin, is an integral, but secondary part of the process of xerosis in the conjunctiva. Local eye infections are frequent, making the clinical condition worse, but are not aetiologically involved.

Table 5.4. Association between xerophthalmia status and serum retinol (μg/dL) (from Sommer, West, 1996)

Clinical status	Deficient (<10)	Low (10–20)	Adequate (>20)	Mean	Number of cases
XN (+), X1B (-)	27%	55%	18%	13.9	174
XN (-), X1B (+)	31%	57%	12%	13.4	51
XN (+), X1B (+)	38%	53%	9%	12.1	79
Neighbourhood controls	–	–	–	17.7	282
Random sample	8%	37%	55%	20.0	268

XN = night blindness; X1B = Bitot's spot; controls = neighbourhood peers of same age and sex; random sample = peers from throughout the six villages studied

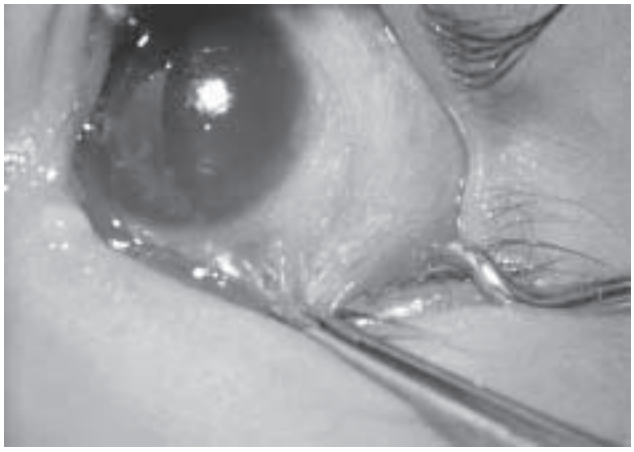


Figure 5.3. Conjunctival xerosis (X1A). Marked conjunctival thickening and wrinkling, with infiltration and haziness of the cornea (X2). Plasma vitamin A, 3 µg/dL.

Bitot's spot (X1B)

As was mentioned earlier, Bitot's spot is the final part of the process of xerosis affecting the bulbar conjunctiva. The typical Bitot's spot (see Figures 5.4–5.6) occurs in the exposed part of the conjunctiva, the interpalpebral fissure.

The temporal aspect is usually first affected and consequently most Bitot's spots are found there. The nasal aspect is affected later and only in extensive involvement do the inferior and then finally the superior quadrant undergo change. A Bitot's spot consists of a heaping up of desquamated, keratinized epithelial cells which form a slightly raised area that may be readily wiped away. This leaves an uneven, eroded base in the superficial epithelium on which more abnormal cells may accumulate over a few days. The transient nature of Bitot's spots creates a problem over their use in surveys. A subject may "remove" a spot by vigorously rubbing their own eyelids.

Bitot's spots vary considerably in size and shape. The areas of conjunctiva affected may be multiple, but more usually there is a single spot to an eye. Some single spots are ovoid, others linear, and occasionally they take a roughly triangular form with the base close to the limbus. None of these characteristics has any special clinical significance (McLaren, 1962).

Broadly speaking the appearance of Bitot's spots has been likened either to 1) foam or 2) cheese (see Figures 5.4 and 5.5). Gas-forming bacteria may be responsible for the first appearance. There is no known significance to this difference in appearance.

Undoubtedly the most significant way in which Bitot's spots may be classified is as to whether they are 1) responsive to vitamin A treatment, or 2) unresponsive (Djunaedi, Sommer, Pandji et al, 1988). There are several characteristics that may be of assistance in any attempt to identify the nature of any Bitot's spot (see Table 5.5).

It is usually not possible to determine the cause of an unresponsive Bitot's spot. Some appear to be stigmata of earlier deficiency and may therefore in this sense resemble corneal scars (XS) that are considered to relate to earlier deficiency (see later). For others there is no evidence of either earlier or present deficiency and these may result from local trauma of some kind. This might be due to a

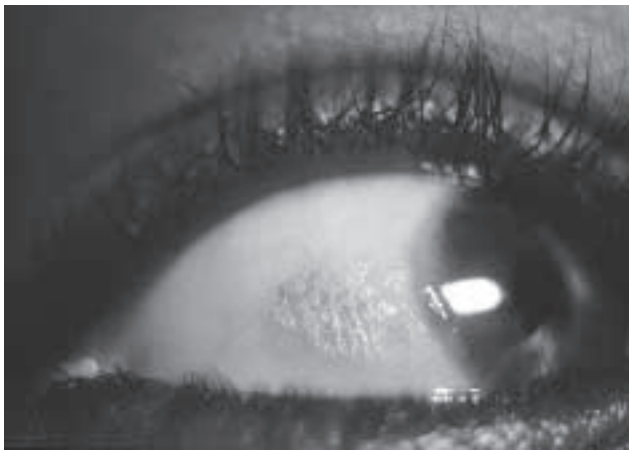


Figure 5.4. Bitot's spot (X1B) on temporal aspect of bulbar conjunctiva in inter-palpebral fissure. Bubbles of foam are clearly visible.

combination of environmental factors among which might be included ultra-violet exposure at high altitude, smoke-filled huts, or chronic eye infections, especially trachoma. This hypothesis is supported by the fact that Bitot's spots form most readily on the most exposed part of the conjunctiva. Further evidence for a role of exposure in Bitot's spot formation are reports of their presence on unusual aspects of the conjunctiva as a result of distortions of the eyelids (McLaren, 1980) (see Figure 5.6).

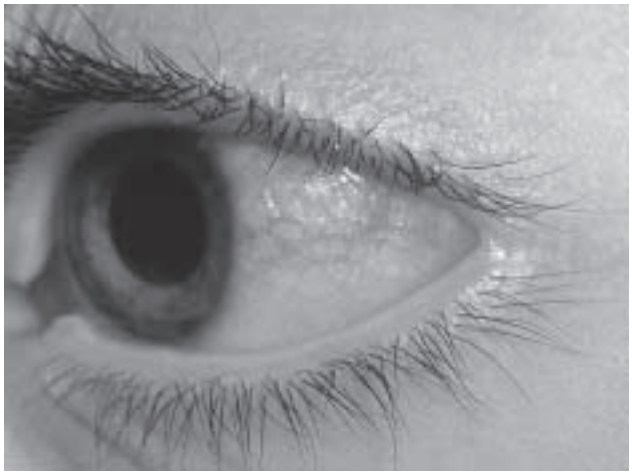


Figure 5.5. Bitot's spot without foam and of a "cheesy" appearance. The nature of the material is not known to have any significance.

Corneal xerosis (X2)

Many instances of conjunctival xerosis are accompanied by a superficial punctate keratopathy (SPK), which can be seen when examined with the slit-lamp microscope (Sommer, Emran, Tamba, 1979). This suggests that the process of xerosis tends to spread from the conjunctiva to later involve the cornea. Clinically evident corneal xerosis (X2) in which the cornea has a distinct hazy appearance (see Figure 5.7) tends to last for a matter of only a day or two before advancing to deformation of the cornea; known as keratomalacia (see below).

Table 5.5. Characteristics of vitamin A-responsive and unresponsive Bitot's spots (X1B)

Responsive

- Subject usually a child of less than 6 years of age
- With the maximum vitamin A dosage, response usually evident within one week
- Usually accompanied by generalized conjunctival xerosis and night blindness
- Males more commonly affected than females

Unresponsive

- Commonly occur in children over 6 years and in otherwise healthy adults
- Usually a small, single spot
- No accompanying evidence of VAD
- Largely responsible for the apparent increase of X1B prevalence with increasing age

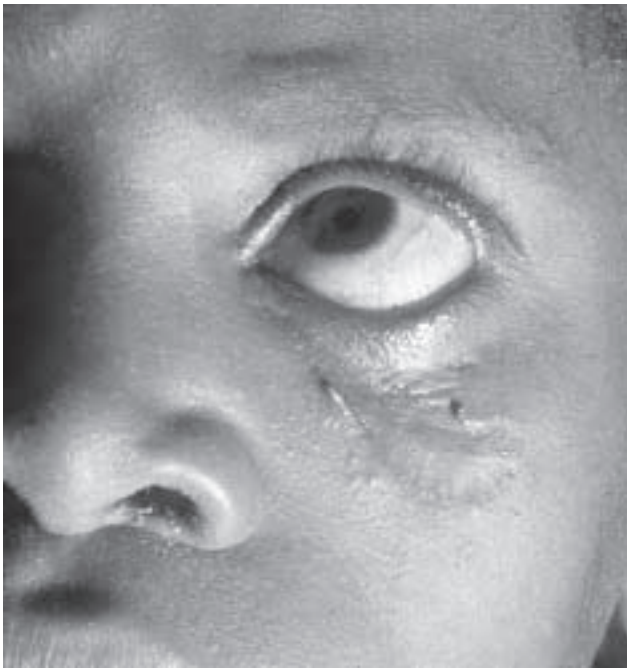


Figure 5.6. A single Bitot's spot associated with abnormal exposure of the conjunctiva due to ectropion associated with scar on cheek.

Up to the stage of corneal xerosis (X2) prompt treatment with large doses of vitamin A can result in full preservation of sight without any residual impairment. It is of paramount importance that all stages of xerophthalmia should receive the maximum treatment with vitamin A (see Chapter 11). In contrast to the lengthy full response to vitamin A therapy of abnormal conjunctival cytology mentioned earlier (see p 44), clinical improvement has been reported to take place in 70% of corneal lesions (X2/X3) within four days, in 95% within one week and the vast majority were entirely healed within two weeks (Sommer, West, 1996, p 285). Histological and clinical healing are both subjective and clearly not strictly comparable.

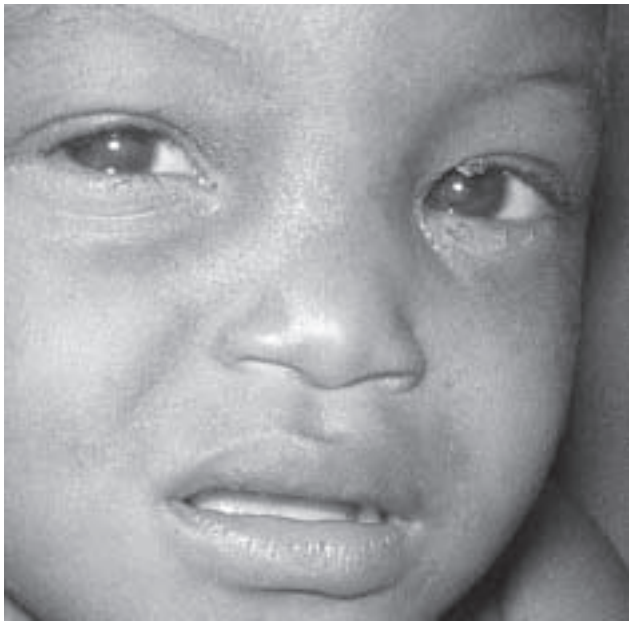


Figure 5.7. Bilateral haziness of the cornea (X2) in a young child identified in a survey.

Keratomalacia (X3A, X3B)

In the 1982 WHO Xerophthalmia Classification keratomalacia has been divided into two stages according to the extent of the involvement of the cornea (see Table 5.1). Keratomalacia is characterized by softening of the corneal substance in addition to increasing xerosis of the epithelium (see Figures 5.8 and 5.9).

Corneal softening is due to a unique pathological process termed colliquative necrosis. The stroma becomes oedematous. It is suspected that activation of collagenases and other enzymes may be responsible but the precise pathogenesis is not known.

Many years ago (Pillat, 1929) cases of keratomalacia in adults in China were also described as having similar colliquative changes in the skin. This was termed “dermomalacia”. Figure 5.9 shows one eye and the surrounding skin of a five-month-old Palestinian refugee child who died shortly after admission to hospital in Amman, Jordan.There is advanced keratomalacia with almost the entire cornea affected and the central area about to prolapse. In addition, the skin has a markedly burnished appearance which also affected much of the skin of the head and neck. The skin change is very reminiscent of that described earlier as dermomalacia. As a result of this process in the cornea there will always be some degree of residual damage and deformity.

In corneal ulceration there is usually only one ulcer per eye. The typical ulcer is inferonasal in position, about 0–2 mm in diameter and 0.25 – 0.5 mm in thickness. In about 20% of cases both eyes are affected and the characteristics tend to be similar. Hypopyon (collection of sterile pus in the anterior chamber) is common and infection is frequent.

Corneal scar (XS) – vitamin A-related

Scarring of the cornea may result from a wide variety of diseases affecting the eye. Visual impairment is inevitable; its degree depends on the location and the density of the scar. Damage that is confined to the cornea may

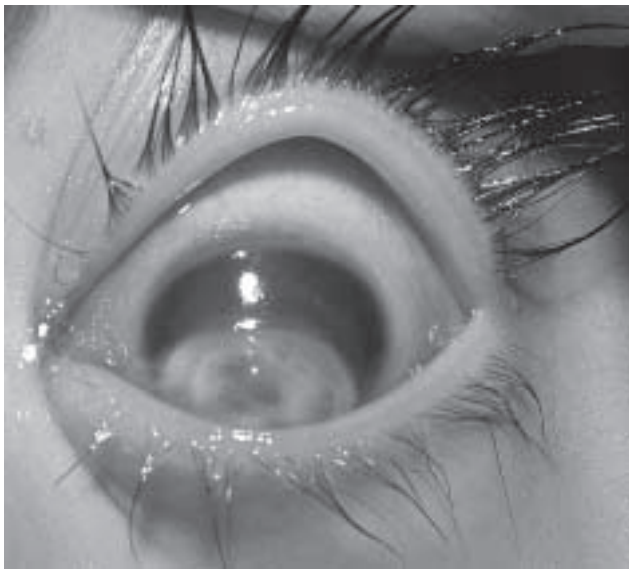


Figure 5.8. Colliquative necrosis (keratomalacia) affecting the greater part of the cornea (X3B).The relative sparing of the superior aspect is typical. Plasma vitamin A, 4 µg/dL.

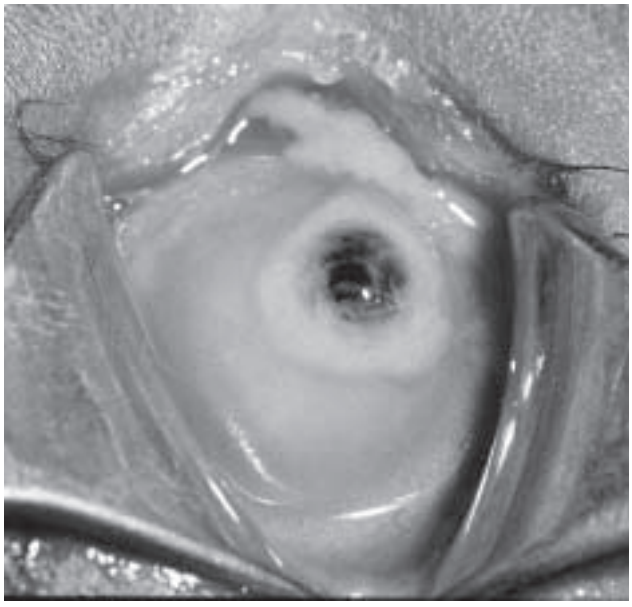


Figure 5.9. Entire cornea is undergoing liquefaction (X3B). Skin surrounding the eye shows hyperkeratinization suggestive of “dermomalacia” (see text).

be overcome by surgery. This is not possible when internal structures are also involved, usually as a result of accompanying infection. Prevention is clearly infinitely preferable. Careful and detailed history taking and general physical examination, in addition to the eye examination, are of particular importance in this instance (see Figure 5.10). If, as a result of these, there is any residual doubt about the likely aetiology of a corneal scar, then it should not be attributed to vitamin A deficiency. Table 5.6 gives the information to be elicited in order to come to a diagnosis.

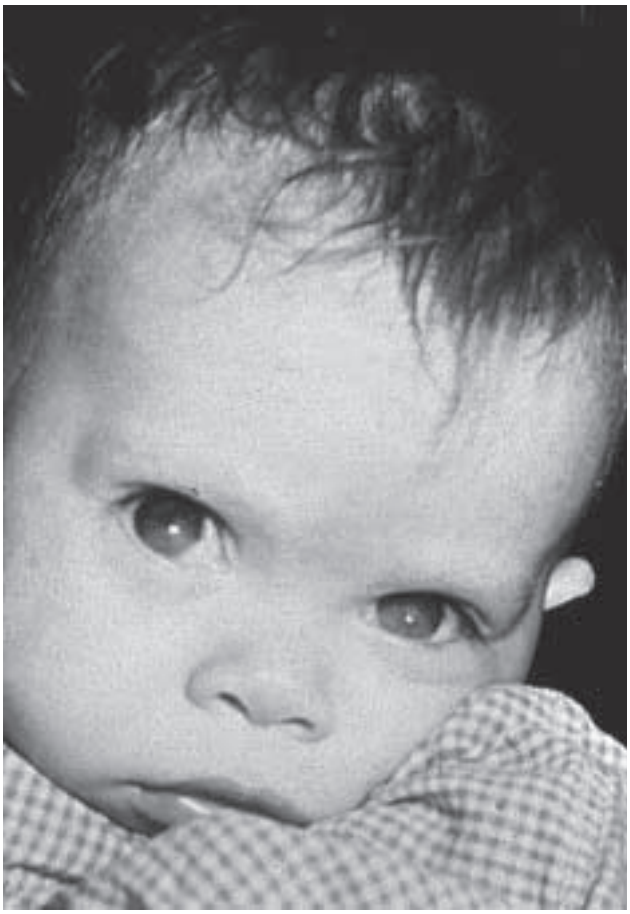


Figure 5.10. Bilateral corneal scars (leucomata XS) in an anaemic and generally malnourished infant. The inferior situation of the scars is typical.

Table 5.6. Distinguishing characteristics of vitamin A-related corneal scars (XS)

Eye examination
- Location: typically nasal and inferior on the cornea if only a small part involved
- Often bilateral, not necessarily equal in extent

History
- Onset between about 2 months and 5 years
- Accompanied at that time by severe PEM, measles, severe diarrhoea, respiratory infection
- Absence of trauma, or prolonged purulent discharge

Xerophthalmic fundus (XF)

This rare condition has been described mainly in school age children or young adults in south east Asia (Teng-Khoen-Hing, 1959; Sommer, Sugana, Djunaedi et al, 1978). It appears to result from prolonged deficiency of vitamin A in which impairment of rod function is succeeded by structural damage to the retina. Recently a report from Malawi associated the whitening of the retina described in children with cerebral malaria with low vitamin A status and abnormal CIC (Lewallen, Taylor, Molyneux et al, 1998). Retinal changes and signs of raised intracranial pressure responsive to vitamin A were reported in a case of secondary VAD in the United States (Panozzo, Babighian, Bonora, 1998).

Remarks

In conclusion, there are several general points that should be noted. Signs of xerophthalmia are usually present in both eyes, but not necessarily to the same degree. Keratomalacia may proceed very rapidly, noticeably within a matter of some hours rather than days. This is especially true in very young children. In this age group keratomalacia may present without any evidence of xerosis in the conjunctiva or cornea. It follows that a diagnosis of vitamin A deficiency may be made in the absence of xerotic changes. In corneal involvement accompanying infection is probably the rule. Frequently this obscures the classical picture of changes due to xerophthalmia. Unless this is remembered the result may be underdiagnosis of xerophthalmia.

MORTALITY AND MORBIDITY

Introduction

It can be instructive, on occasion, to look back in time and speculate about the reasons why events in history took the path they did. In Chapter 5 it was pointed out that recognition of xerophthalmia as a vitamin deficiency disease lagged behind that of several other diseases of nutritional aetiology. In the present context it is a cause of surprise that widespread acceptance of the significant impact of vitamin A on mortality and morbidity has come only in recent years (Semba, 1999). Not long after the discovery of fat-soluble A the vitamin was dubbed “the anti-infective vitamin” (Green, Mellanby, 1928). This was on the basis of the susceptibility of experimental animals fed on diets deficient in the vitamin to infections resulting in death and of the response of humans with infections to vitamin A.

Sommer and West (1996, pp 19-61) have recently reviewed hospital-based reports of children with xerophthalmia and severe PEM in whom mortality was significantly increased by the presence of eye signs accompanying general malnutrition. In some of these studies there was a four-fold (McLaren, Shirajian, Tchalian et al, 1965) or even greater mortality in children severely vitamin A deficient as compared with those equally generally malnourished but with normal eyes. The implications of these findings were not pursued further at that time. In explanation it should be pointed out that epidemiological and statistical methodologies had not advanced sufficiently in those times and there was still great reticence on the part of governmental and other agencies to recognize the magnitude and severity of VAD.

Mortality associated with non-corneal xerophthalmia

The significance of vitamin A deficiency in child survival in the community was first suggested by a study of Sommer, Hussaini, Tarwotjo et al (1983) that followed a point-prevalence survey of vitamin A deficiency in Indonesia (see Chapter 8). The results, presented in Table 6.1, show a significantly increased relative risk of death in those groups with evidence of xerophthalmia. The greatest risk is among those with both X1B and XN. It should be noted that only those with the milder, non-corneal signs of xerophthalmia were included.

It is probable that the mechanism of the greater mortality in those children who had clinical VAD was in some way related to an impairment in their immune response to an accompanying infectious disease. Whilst it is relatively easy to ensure that all deaths are recorded in a study like this, it is much more difficult to obtain accurate information as to the cause or causes of death. In addition, in the circumstances that prevail in field studies it is usually not possible to make a definitive diagnosis of any accompanying infections. These matters are further discussed later

Table 6.1. Mortality in relation to xerophthalmia (vitamin A) status (Sommer, Hussaini, Tarwotjo et al, 1983)

Ocular status	Child intervals*	Deaths	Mortality (per 1000)	Relative risk
Normal	19,889	108	5.4	1.0
XN (+), X1B (-)	547	8	14.6	2.7
XN (-), X1B (+)	269	6	35.5	6.6
XN (+), X1B (+)	215	10	46.5	8.6

* Examined at six three-monthly intervals

when the relationship between vitamin A deficiency and morbidity is considered.

Vitamin A intervention and mortality in young children

The first of these trials was carried out in Aceh, Indonesia (Sommer, Tarwotjo, Djunaedi et al, 1986). A large dose of vitamin A (200,000 IU) was given every 6 months. The initial report found a 34% reduction in mortality in the treated group. Alternative analyses suggested at least 40% to >50% reduction (Tarwotjo, Sommer, West et al, 1987; West, Pokhrel, Katz et al, 1991). Figure 6.1 shows some of the results from this study.

Over a period of several years further field trials were carried out in other locations. A summary of these major community mortality prevention trials is given in Table 6.2.

As an indication of the magnitude of trials of this nature it should be noted that more than 150,000 preschool age children took part in the eight trials described in outline above. All or some of them have formed the basis of several meta-analyses that have been made. The results of all the meta-analyses are in general agreement that improvement in the vitamin A status of deficient populations reduces significantly the overall preschool-age child mortality. Figure 6.2 is a summary of the results of one of these meta-analyses of the eight major mortality intervention trials (Beaton, Martorell, Aronson et al, 1993). The overall reduction of mortality from this was 23%.

These results are remarkably consistent in view of the fact that the communities studied were very heterogeneous with regard to many factors such as culture, ecology, disease patterns, and details of the interventions carried out differed considerably.

It should also be noted that there are several reasons why the reported reductions in mortality may have underestimated the actual reductions. Some deaths may have been included that occurred before the vitamin A intervention had had a chance to act. In most studies children with xerophthalmia were identified, treated and excluded from the main study. In the absence of the intervention these children would be expected to be among the most vulnerable. Periodic large-dose vitamin A supplementation may not be the optimal means of improving vitamin A status in the

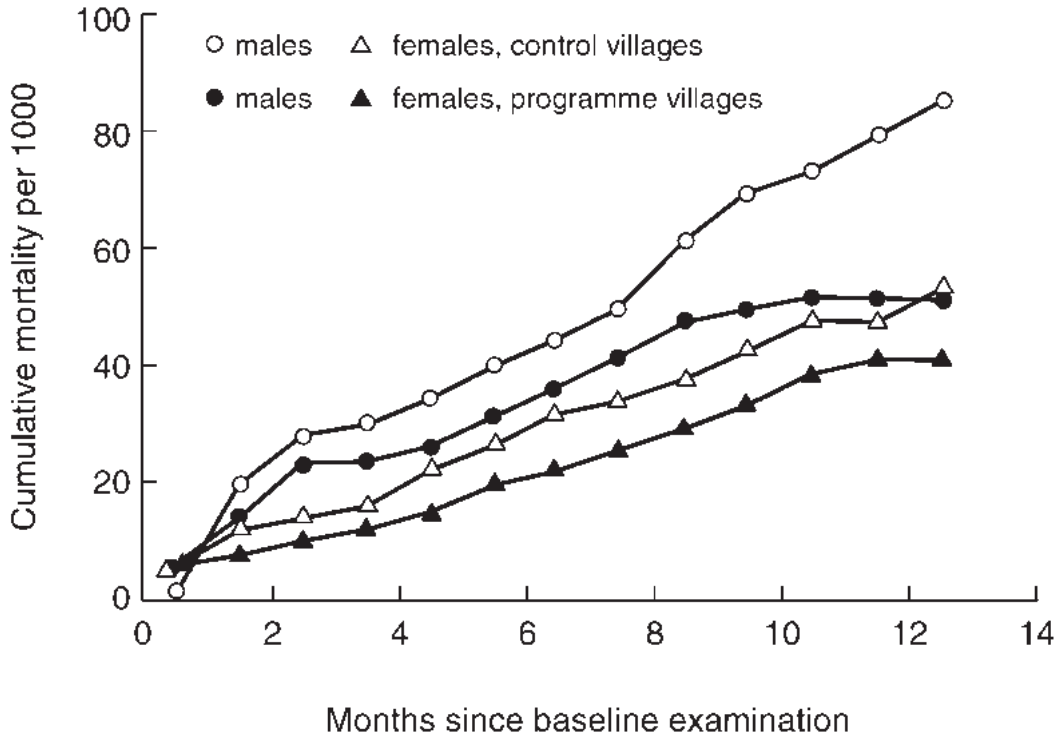


Figure 6.1. Cumulative mortality, by sex, of preschool-age children in Aceh, Indonesia, from Sommer, Tarwotjo, Djunaedi et al,1986.

long term. Consistent with this may be the highest reductions in mortality being reported from Bogor, Indonesia, and Tamil Nadu, India (see Table 6.2), where vitamin A was provided in a more physiological way. Finally, each of the eight studies reported what is termed an "intention-to-treat" analysis. In this it is assumed that a full intervention was received by each subject supposed to do so. It has been found (Tarwotjo, Sommer, West et al, 1987) that in most health interventions of this kind those who fail to participate, termed "noncompliers", are usually those most in need of the intervention. Their mortality has been found to be greater than that of the control group (see Figure 6.3).

Knowledge of the level of noncompliance in any study is essential for assessing impact. It is also a reminder that when any measure is adopted for routine application there will always be those who for various reasons fail to participate. Furthermore, they are likely to be the most at risk.

In a reassessment of most of these studies Sommer and West (1996, pp 34-37) found that in children aged 6 months and older the reduction in mortality tended to increase in magnitude with age. The majority of deaths occurred in children < 2 years of age. These authors also discuss the evidence for the effect on mortality of vitamin A dosing in children under the age of 6 months. Under the age of 3 months 100,000 IU appeared to have a detrimental effect, but 50,000 IU was beneficial. In infants over the age of 3 months the larger dose was effective. 300,000 IU in a single maternal dose has been shown to raise maternal stores, vitamin A levels in breast milk, serum retinol of breast-fed children, and to reduce mortality in infants by about 30% (de Francisco, Yasui, Chakraborty, 1994).

Table 6.2. Major community mortality prevention trials (Sommer, West, 1996, p 28)

Study	Country	Vitamin A supplement	Reported mortality reduction	Primary reference
Aceh	Indonesia	Large dose every 6 mo	34%	Sommer et al,1986
Bogor	Indonesia	Vitamin A-fortified MSG	45%	Muhilal et al,1988
NNIPS	Nepal	Large dose every 4 mo	30%	West et al,1991
Jumla	Nepal	One large-dose follow-up at 5 mo	29%	Daulaire et al,1992
Tamil Nadu	India	Weekly RDA[1]	54%	Rahmathullah et al,1990
Hyderabad	India	Large dose every 6 mo	6% (ns*)	Vijayaraghavan et al,1990
Khartoum	Sudan	Large dose every 6 mo	+6% (ns*)	Herrera et al,1992
VAST	Ghana	Large dose every 4 mo	19%	Ghana VAST Study Team, 1993

* Not statistically significant
[1] Once a week a dose was given, providing the RDA

Cause-specific mortality

This was studied in four of the eight interventions subject to meta-analysis and the results in Table 6.3 indicate that vitamin A intervention tends to make more of an impact on measles and diarrhoea than it does on respiratory infections in this regard.

It should be noted that this seems to be consistent with the results of studies that have attempted to investigate the impact of vitamin A intervention on several aspects of the evolution of various infectious diseases (see section on morbidity later).

Contribution of vitamin A deficiency to child mortality

While subclinical vitamin A deficiency is much less fatal than overt xerophthalmia, it is very much more widespread (see Chapter 8). Beaton, Martorell, Aronson et al, 1993 (see Figure 6.4), were unable to find any association in the field trials that have been cited above between relative risk (RR) of mortality and prevalence of xerophthalmia.

This may have been due in part to differences in the reporting of xerophthalmia in the

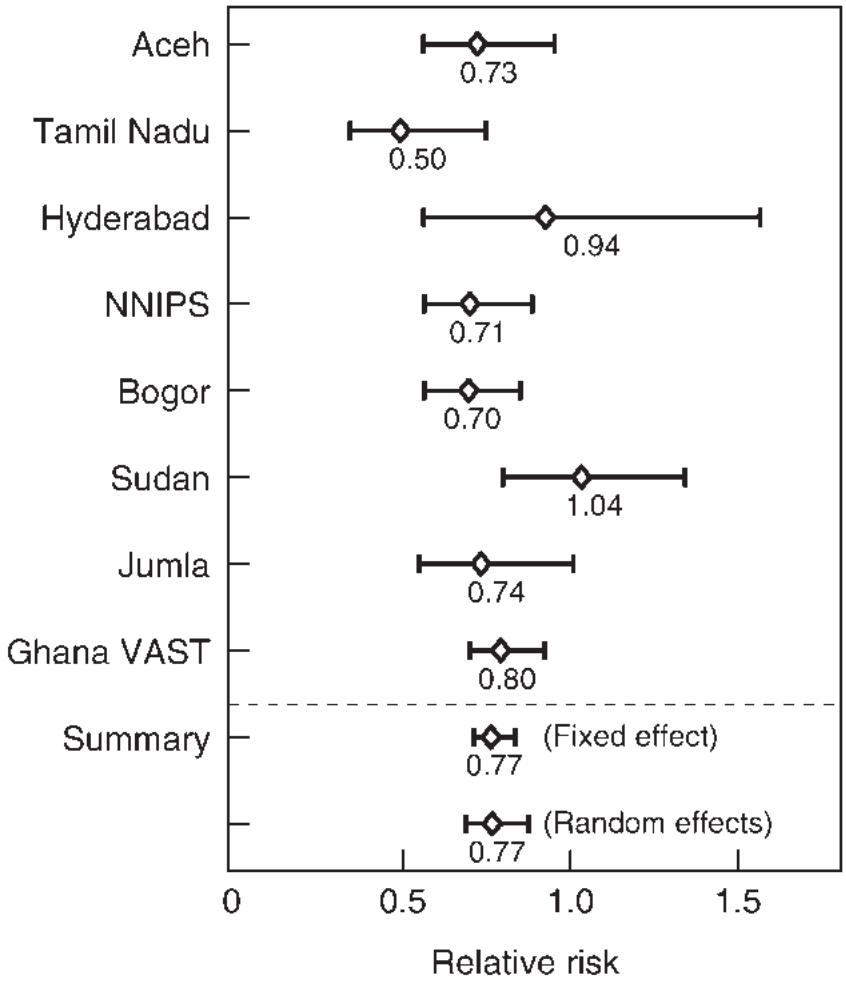


Figure 6.2. Meta-analysis of the eight major community mortality intervention trials. For details including explanation of fixed effect and random effects, see reference (Beaton, Martorell, Aronson et al, 1993).

different studies (McLaren, 1991), but it probably indicates that xerophthalmia does not make a major contribution to mortality within a community. The largest contribution is most likely to be attributable to subclinical vitamin A deficiency.

In Nepal a sustained reduction in child mortality with vitamin A was shown for the first time (Pokhrel, Khatry, West et al, 1994). Where deficiency is endemic vitamin A supplementation can achieve a rapid reduction in early childhood mortality and a lower level of mortality can be sustained as long as capsule coverage is adequate (>85%).

In Malawi 377 HIV-negative women had serum retinol mesured in the second and third trimester of pregnancy. Their infants were observed from delivery until 12 months of age. Mothers whose infants died had serum retinol levels lower than mothers of the survivors. Infants born to mothers whose serum retinols were in the lowest quartile had a three-fold greater mortality risk than those of mothers with serum retinols in the higher quartiles (Semba, Miotti, Chiphangwi et al, 1998).

It has been estimated (Humphrey, West, Sommer, 1992) that worldwide vitamin A deficiency may be responsible for as many as

Table 6.3. Cause-specific mortality, vitamin A supplementation community prevention trials (Sommer, West, 1996, p 41)

	Symptoms/Diseases					
	Measles		Diarrhoea		Respiratory	
Study[c]	Vitamin A	Control	Vitamin A	Control	Vitamin A	Control
Tamil Nadu						
Deaths (n)	7	12	16	33	2	3
RR[a]	0.58		0.48		0.67	
NNIPS						
Deaths (n)	3	12	39	62	36	27
RR	0.24		0.61		1.29/1.00[b]	
Jumla						
Deaths (n)	3	4	94	129	18	17
RR	0.67		0.65		0.95	
Ghana VAST						
Deaths (n)	61	72	69	111	47	45
RR	0.82		0.66		1.00	

[a] RR (Relative Risk): Cause-specific mortality rate of vitamin A group divided by rate in control group
[b] Originally published results RR=1.29; reanalysis as an associated cause that recognizes other underlying causes RR=1.00
[c] For details see Table 6.2

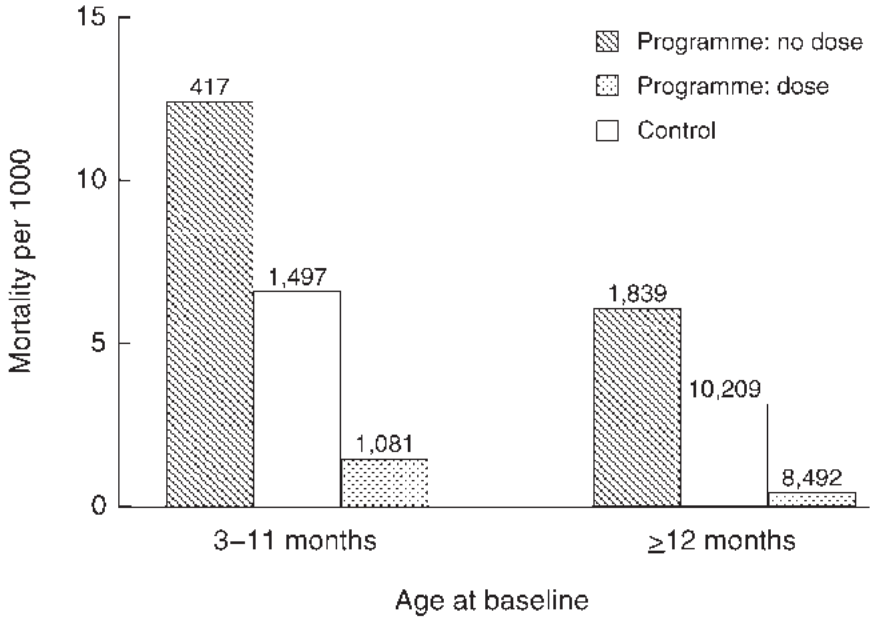


Figure 6.3. Mortality in the Aceh trial among children in treatment villages who did and did not receive their intended vitamin A dose, compared with children in control villages. Mortality was higher among children who were assigned to receive vitamin A but did not get it than for any other group, including children in control villages (Tarwotjo, Sommer, West et al, 1987).

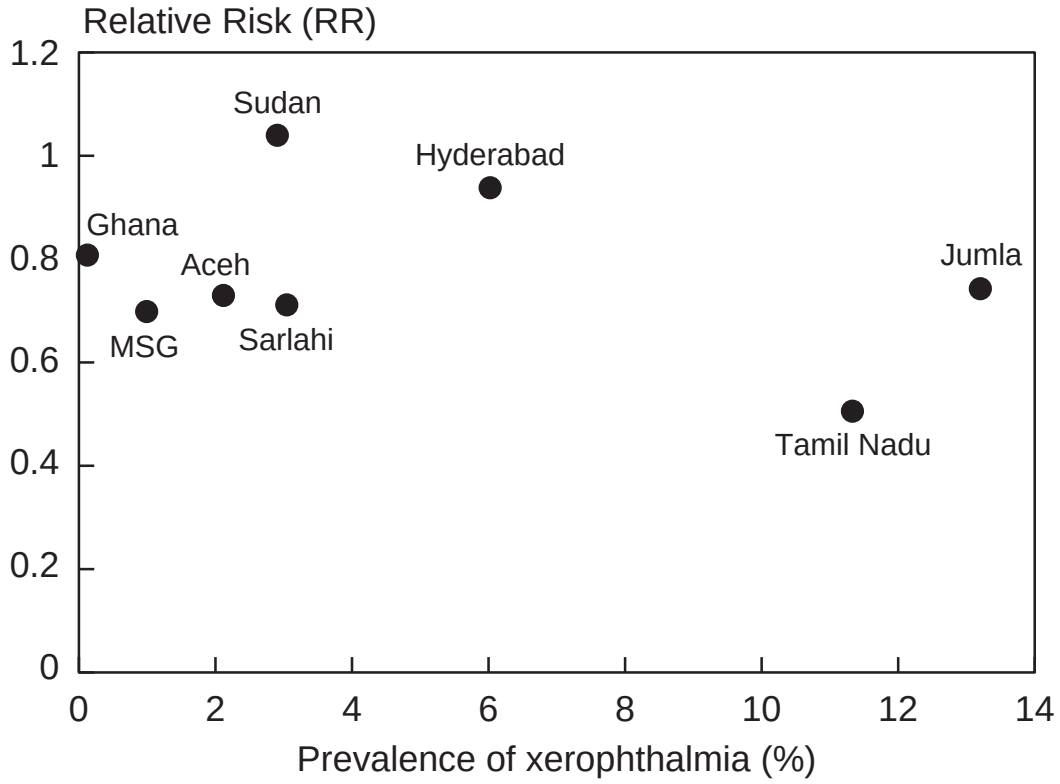


Figure 6.4. Relative risk of mortality and prevalence of xerophthalmia. No relationship could be established (Beaton, Martorell, Aronson et al, 1993).

1.3 to 2.5 million deaths annually. In addition, PEM is considered to be a contributory cause of infant and child deaths in nearly 50% (Puffer, Serrano, 1973). Recently much attention has been given to the importance in infections of other micronutrient deficiencies, especially of zinc (see Chapter 7).

Measles mortality and vitamin A treatment

Measles occupies a unique position among common childhood infectious diseases and vitamin A deficiency. Of these diseases, it is in measles alone that the infective agent, in this case a virus, invades the eye. In serious cases it causes a damaging measles keratoconjunctivitis. Even immunization against measles has been shown to result in symptomless, minimal invasion of the cornea which may take months to disappear. This eye pathology may predispose to corneal liquefaction in the presence of vitamin A deficiency.

Measles generally takes a more serious form in the generally undernourished child, resulting in more frequent and more serious complications and a much higher death rate than in the well-nourished. This was shown in the community prevention trials referred to already (see Table 6.3). Table 6.4 shows that in hospital the majority of deaths occur in children under the age of 2 years.

Dramatic reduction in mortality rates in hospitalized cases of measles has been repeatedly shown in those where treatment included vitamin A supplementation (see Figure 6.5).

Table 6.4. Measles mortality – Cape Town Trial (Hussey, Klein, 1990)

Age (months)	Vitamin A			Controls			Relative Risk Control: Vitamin A
	Children	Deaths	Mortality (%)	Children	Deaths	Mortality (%)	
<6	4	0	–	3	1	33.3	33 : 0
6–12	53	1	1.9	64	7	10.9	5.7 : 1
13–23	19	1	5.3	18	1	5.6	1.1 : 1
0–23	76	2	2.6	85	9	10.6	4.1 : 1
≥24	16	0	–	12	1	8.3	8.3 : 0
Total	92	2	2.2	97	10	10.3	4.7 : 1

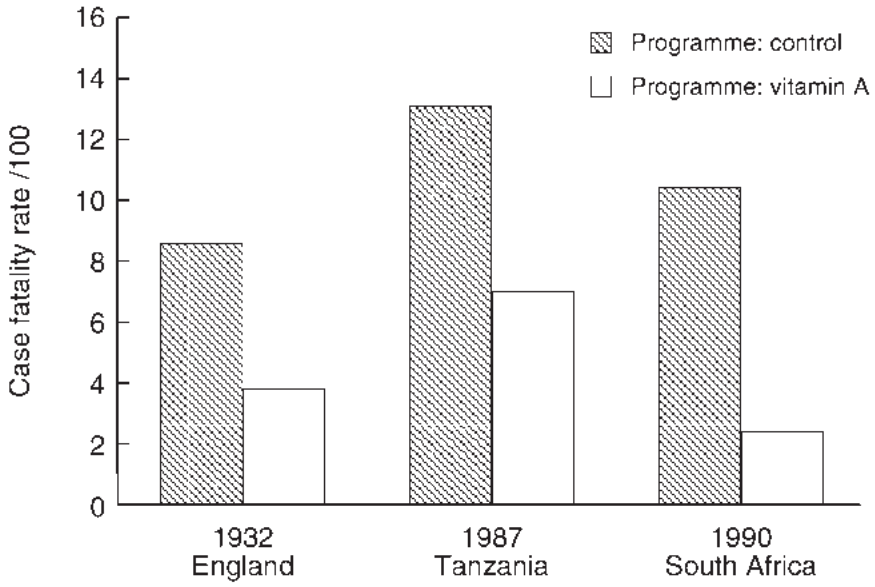


Figure 6.5. Measles case-fatality rates among hospitalized patients randomized to receive high-dose vitamin A (cod liver oil in the London trial) compared with those of their controls. Vitamin A supplementation reduced mortality by 50% in all three trials (Sommer, West, 1996, p 53).

Vitamin A supplementation and maternal mortality

The preliminary results of the first trial of this kind caused a considerable stir when they were reported at the XVIII IVACG Meeting in Cairo, 22–26 September 1997 (West, Khatry, Katz et al, 1997). The definitive paper was published nearly 18 months later (West, Katz, Khatry et al, 1999) together with an editorial (Olsen, 1999). An area of Nepal was chosen for the study where VAD is known to be common in pregnant women as well as in young children. Well over 40,000 women participated and were observed over $3^1/_2$ years. Approximately equal numbers in three matched groups received vitamin A (7,000 μg retinol equivalents on a weekly basis), β-carotene (42 mg or 7,000 RE on a weekly basis), or a placebo). Maternal deaths from any cause during pregnancy or within 12 weeks of delivery were the end point. The preventive effects, expressed as relative risks were 0.60 (40% reduction) for vitamin A and 0.51 (49% reduction) for β-carotene (see Table 6.5). It is suggested that the antioxidant effect of β-carotene might have had some relationship to the higher reduction rate rather than that of vitamin A. There was no reduction in fetal or infant mortality through until 6 months of age nor any impact on neonatal birth weight.

It is understood that similar trials are being conducted in other areas but results are not yet available. A preliminary report at the XIX IVACG Meeting in March 1999 (Hakimi, Dibley, Suryono et al, 1999) on a trial with vitamin A and zinc supplements on maternal postpartum infections showed that there was significant benefit in groups receiving vitamin A but zinc gave no improvement. There is no doubt that if the results from Nepal are confirmed from elsewhere there could be a significant extension of the public health importance of vitamin A intervention into the area of maternal health where the mortality rate in developing countries far exceeds that in the developed countries.

Vitamin A supplementation and infectious morbidity

Mortality and morbidity are related but clearly quite distinct. All disease states carry some element of morbidity. In a medical context the word relates in an unspecific way to illness.

Table 6.5. Impact of supplementation on mortality related to pregnancy up to 12 weeks post partum (West, Katz, Khatry et al, 1999)

	Placebo	Vitamin A	β-carotene	Vitamin A or β-carotene
Number of pregnancies*	7,241	7,747	7,201	14,948
Number of deaths	51	33	26	59
Mortality (per 100,000 pregnancies)	704	426	361	395
Relative risk (95% CI)	1.0	0.60 (0.37–0.97)	0.51 (0.30–0.86)	0.56 (0.37–0.84)
p value		<0.04	<0.01	<0.005

* Includes 157 pregnancies that were lost to follow up (43, 70, and 44 in the placebo, vitamin A, and β-carotene groups, respectively)

Some instances of morbidity may result in death, but that aspect is normally dealt with separately. Fatal diseases have, until recently, been given more attention than the much more numerous non-fatal ones. This may be partly because the latter have been considered to be of only secondary importance. It may also be because they are much more difficult to document. A fatality is a discrete, one-off event; a morbidity or disability is quite vague. Morbidity may be acute or chronic, mild or severe.

The relationship of morbidity due to VAD and infection is complicated by several further points. There is an apparent synergistic relationship between impaired vitamin A status and infections; each seems to increase the risk of the other (see also Chapter 9). Other nutritional deficiencies, especially PEM of mild degree, are often also present and this makes interpretation of results difficult. Episodes of infectious diseases vary greatly from case to case and may also differ to a considerable extent from one location to another. For many reasons their recognition in different studies may be based upon very different criteria. Finally, although the phenomenon has been noted for many years, it is only relatively recently that research workers have given due regard to the fact that the acute-phase reaction associated with infections also causes a depression of serum retinol. This casts doubt on the value of serum retinol as an indicator of vitamin A status under such circumstances. This subject will be considered in more detail in Chapter 9.

Vitamin A deficiency has been induced in experimental animals for many decades. The uniform experience has been that this nutritional deficiency, more than any other, is associated from a relatively early stage with the development of infections, especially those affecting epithelial tissues. Most prominent have been those of the upper and lower respiratory tracts, parts of the gut, and the genitourinary tract (Moore, 1957). These infections often became apparent early on in the deficiency, long before the changes of xerophthalmia occurred (McLaren, 1959).

Diarrhoeal diseases

This is probably a less-well defined group of diseases than the others to be considered here. There is a variety of bacterial and viral organisms that have been isolated from patients with diarrhoea. Isolation does not necessarily mean that an organism was responsible for the diarrhoea. In addition, even with full laboratory facilities, in some instances no organisms have been isolated. Furthermore, the difficulties of defining what constitutes a case are probably greater for diarrhoea than for any other infection or group of infections.

Cross-sectional studies of impaired vitamin A status and various infections have been carried out in many developing countries (Sommer, West, 1996, pp 62-98). The strongest association in most was with diarrhoea, especially when it was persistent, chronic, or severe. From these studies the relationship is not clear. Which came first?

Intervention trials cast some light on this last point. Vitamin A supplementation appears not to have an effect on mild diarrhoea (usually defined as 3 or 4 stools/day). As was noted earlier, it does reduce diarrhoea-related deaths. As expected then, it also reduces the incidence of severe diarrhoea and the benefit is proportional to the number of stools/day (see Table 6.5).

Respiratory diseases

This term usually applies to diseases affecting the lower respiratory tract; in general this means one form or another of bronchitis or pneumonia. Fever, cough, rhonchi or rales (sounds detected by stethoscope) are customarily required to be present for the diagnosis to be made.

Table 6.6. Diarrhoeal episodes by frequency of movements (Brazil) (Barreto, Santos, Assis et al, 1994)

Number of loose movements	Relative Risk (Vitamin A group)
3	0.92
4	0.90*
5	0.80**
6	0.77**

* $p < 0.05$, ** $p < 0.01$

Animal studies, clinical and autopsy studies in children, and observational studies in human populations all show an association between vitamin A deficiency and respiratory infections. Respiratory disease prevalence increased in a linear fashion with increasing severity of xerophthalmia in a large hospital study (see Figure 6.6).

However, meta-analysis from mortality and morbidity intervention trials showed in general a lack of any impact of vitamin A supplementation on acute lower respiratory tract infections (ALRIs). There was some indication that there might be reduction in the severity, if not the duration, of respiratory symptoms. It has been pointed out that increase in cough in the supplemented group might be a favourable response (Herrera, Fawzi, Nestel et al, 1996). Strength of the cough reflex could be beneficial in removing infective material. These conclusions have been supported in general by subsequent studies from other

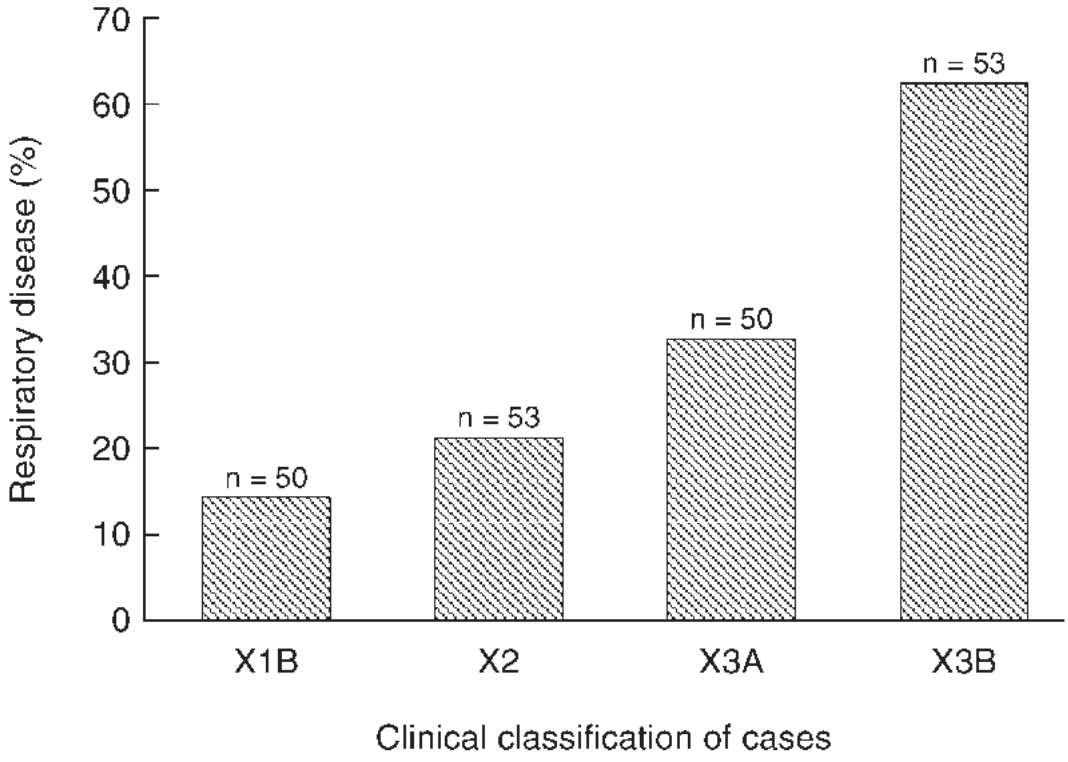


Figure 6.6. The prevalence of respiratory disease among Indonesian children presenting to the Cicendo Eye Hospital increased with the severity of their xerophthalmia ($p < 0.01$ for linear trend) (Sommer, 1982).

countries. Treatment with vitamin A of respiratory syncitial virus (RSV) infection also had no significant benefit (Dowell, Papic, Bresee et al, 1996).

Measles

The evidence for the association between vitamin A deficiency and measles, in its various aspects (see Chapters 6 and 9), is stronger than for any other infectious disease. Diagnosis probably has a firmer basis, as a single organism is responsible and the clinical picture is usually typical. Measles is the only specific disease for which there is firm evidence that vitamin A status influences morbidity and mortality (Sommer, West, 1996, pp 62-98).

Hospital-based studies in South Africa demonstrated that both the complications (see Table 6.7) and outcome (see Table 6.8) in measles are significantly improved by vitamin A supplementation.

Outcome during the prolonged recovery after discharge home is also markedly improved by a further dose of vitamin A (see Table 6.9).

Both measles and vitamin A deficiency are known to impair immune response and this combination helps to explain the serious nature of the disease in most developing countries. With the widespread take-up of measles immunization as part of the WHO Expanded Programme on Immunization (EPI) there is already evidence of a marked fall in corneal blindness associated with measles (Foster, Yorston, 1992). The combination of vitamin A supplementation with measles immunization is discussed later (see Chapter 11).

Recent work suggests that a single dose of 200,000 IU (210 μmol) retinol does not enhance the immune response (Rosales, Kjolhede, 1994) nor is it effective in reducing measles complications (Rosales, Kjolhede,

Tables 6.7. Measles complications – Cape Town vitamin A controlled treatment trial (Hussey, Klein, 1990)

Complication	Vitamin A N=92	Controls N=97	Relative Risk Vitamin A : Controls
Pneumonia ≥10 days	12	29	0.44 (0.24, 0.80)
Diarrhoea ≤10 days	8	21	0.40 (0.19, 0.86)
Post-measles croup	13	27	0.51 (0.28, 0.92)
Requiring airway	3	9	0.35 (0.10, 1.26)
Herpes stomatitis	2	9	0.23 (0.05, 1.06)
Intensive care	4	11	0.38 (0.13, 1.16)
Hospital days	10.54	15.24	$p < 0.004$

Table 6.8. Hospital outcome – Durban measles vitamin A controlled treatment trial (Coutsoudis, Broughton, Coovadia, 1991; Coutsoudis, Kiepiela, Coovadia et al, 1992)

Outcome	Vitamin A (N = 29)	Placebo (N = 31)
Duration (days) – pneumonia	3.8 ± 0.4	5.7 ± 0.8
Duration (days) – diarrhoea	3.2 ± 0.7	4.5 ± 0.4
Duration (days) – fever	3.6 ± 0.3	4.2 ± 0.5
Clinical recovery in <8 days	28 (96%)	20 (65%)
IMS on day 8*	0.24 ± 0.15	1.37 ± 0.40

* Integrated Morbidity Score

Table 6.9. Post-hospital outcome – Durban measles vitamin A controlled treatment trial (Coutsoudis, Broughton, Coovadia, 1991; Coutsoudis, Kiepiela, Coovadia et al, 1992)

	6 Weeks		6 Months	
	Vitamin A (N = 24)	Placebo (N = 24)	Vitamin A (N = 20)	Placebo (N = 16)
Weight gain (kg)	1.29 ± 0.17	0.90 ± 0.14	2.89 ± 0.23	2.37 ± 0.20
Diarrhoea episodes	6	12	3	6
Score/episode – diarrhoea	2.17 ± 0.31	2.25 ± 0.25	1.67 ± 0.67	2.17 ± 0.31
URI* episodes	7	9	3	8
Score/episode – URI	1.71 ± 0.28	2.66 ± 0.17	2.00 ± 0.58	2.37 ± 0.18
Pneumonia episodes	5	6	0	3
Score/episode – pneumonia	4.40 ± 0.98	6.67 ± 0.67	–	6.67 ± 0.67
Chest x-ray score ≥3	2	6	0	3
IMS	2.21 ± 0.45	5.74 ± 1.17	0.60 ± 0.22	4.12 ± 1.13

* Upper Respiratory Infections

Goodman, 1996) and doubling the dose may be required. Where access to healthcare and immuninization is good and where VAD is mild to moderate vitamin A supplementation may have no effect on general morbidity (Ramakrishnan, Latham, Abel et al, 1995). High-dose treatment (one dose of 200,000 IU vitamin A) did not improve morbidity in hospitalized, malnourished children in Congo, but daily low dosage (5,000 IU) reduced the incidence of diarrhoeal disease but had no effect on acute lower respiratory infections (Donnen, Dramaix, Brasseur et al, 1998). In one study in Indonesia (Humphrey, Agoestina, Wu et al, 1996) neonatal vitamin A supplementation reduced infant mortality and the prevalence of severe respiratory infection among young infants.

HIV/AIDS

Evidence is accumulating that vitamin A status in HIV infection is of considerable importance. Serum retinol levels are depressed and in proportion to the severity of infection (Beach, Mantero-Atienza, Shor-Posner et al, 1992). Mortality in AIDS patients is higher in those with lower serum retinol levels (Tang, Graham, Kirby et al, 1993). Vitamin A supplementation of a group of HIV-positive injection drug users had no significant effect on HIV load or CD4 lymphocyte count (Semba, Caiaffa, Graham et al, 1995).

It was at first reported that mothers with HIV infection have an increased chance of passing on the infection to their offspring if their serum retinol is low (Semba, Miotti, Chiphangwi et al, 1994). However, this has not been subsequently confirmed by three recently completed controlled trials of vitamin A supplementation of HIV-positive pregnant women. No significant differences were found in mother-to-child-transmission between control and supplemented groups (Humphrey, 2000; Blaner, Gamble, Burger et al, 1997). One study was unable to show any beneficial effects of vitamin A supplementation on pregnancy outcomes and T-cell counts in HIV-infected women, although these occurred with multivitamins (Fawzi, Msamanga, Spiegelman et al, 1998). Maternal vitamin A deficiency during HIV infection is reported to predispose to growth failure (Semba, Miotti, Chiphangwi et al, 1997) and to increased infant mortality (Semba, Miotti, Chiphangwi et al, 1995). Vitamin A supplementation of HIV-infected infants significantly reduced morbidity (Coutsoudis, Bobat, Coovadia et al, 1994) and reduced mortality among both HIV-infected and non-infected malnourished children (Fawzi, Mbise, Hertzmark et al, 1999).

An interesting study was carried out in Mombasa, Kenya (Mostad, Overbaugh, DeVange et al, 1997), on factors that affect HIV-1 shedding in cervical and vaginal secretions. Both hormonal contraceptive use and vitamin A deficiency were associated with increased risk of vaginal shedding. After adjustment for CD4 count severe VAD moderate VAD and low normal vitamin A status were associated with 12.9, 8.0, and 4.9-fold increased risk of vaginal shedding, respectively. The public health implications of these results in the control of HIV/AIDS spread are evident.

Malaria

In an early study no relation was found between vitamin A status and malarial infection (Binka, Ross, Morris et al, 1995). A later study in Papua New Guinea (Shankar, Genton, Semba et al, 1999) found a substantially lower incidence of clinical episodes of falciparum malaria (p=0.001), parasite density (p=0.09), and prevalence of spleen enlargement (p=0.01) in children attending health facilities. No such effects were found in vivax infection. The role of iron and zinc supplementation in malaria are considered in Chapter 7.

Other infections

Urinary tract

Bacteriuria was more than fourfold greater in children with xerophthalmia than in those without and the prevalence increased with the severity of xerophthalmia (Brown, Gaffar, Alamgir, 1979).

Otitis media

Children with abnormal CIC had a significantly greater risk of middle ear infection in a study in Truk (Lloyd-Puryear, Humphrey, West et al, 1989).

Meningococcal disease

Meningococcal disease is a major cause of child morbidity and mortality in sub-Saharan Africa. A study in Rwanda (Semba, Bulterys, Munyeshuli et al, 1996) found a case fatality rate of 20%, most patients had low serum retinol levels, and mean CD4 lymphocyte percentage was higher and mean CD8 lymphocyte percentage lower in children with meningitis compared with reference populations. Vitamin A intervention appears not to have been carried out yet.

Remarks

Despite a great deal of research there is still much we do not yet know about the precise contribution that VAD makes to morbidity in infectious diseases. This will probably remain so until we are in possession of more accurate methods of defining the various manifestations of morbidity and until some existing discrepancies have been resolved. These latter have been discussed by Kirkwood (1996). Epidemiological studies, described here, have indicated that vitamin A supplementation reduces child mortality and severe morbidity and that most of the reduction is due to the effect on diarrhoea and measles. These studies have not shown a similar effect on acute lower respiratory tract infections (ALRIs). No explanation exists at present for this difference, especially in view of the evidence that supplementation with zinc benefits both diarrhoeal and respiratory infections (see Chapter 7). Observational studies and animal experiments tend to show a beneficial effect of supplementation on lung disease. Moreover, in clinical trials in measles much of the impact of vitamin A on mortality and morbidity in measles has been related to reduction in incidence and severity of associated pneumonia. Notwithstanding, all are agreed that undernourished children treated for pulmonary infections should be given supplemental vitamin A to improve their status.

OTHER EFFECTS OF VITAMIN A DEFICIENCY

Introduction

In the first edition of the Manual it was suggested that much of the evidence for the importance of VAD in this chapter was based on work in experimental animals and lacked firm evidence as far as human disease was concerned. In recent years there has been a steady increase in our knowledge of the role of vitamin A, especially in the form of retinoic acid (see Chapter 3) in many systems of the body. We are on ever increasingly firm ground to use the term vitamin A deficiency disorders (VADD) to cover all possible consequences of the lack of dietary intake of vitamin A.

Another development that has gained increasing momentum in recent years is the concept that VAD should not be considered in isolation, but recognised to occur together with other nutrient deficiencies. This is, of course, by no means a new concept. It has long been accepted that almost always dietary deficiencies are complex, involving many nutrients to varying degree. In practical terms this has usually come down to a small group of nutrients. In the case of vitamin A its interactions with two essential elements, iron and zinc, have been highlighted in particular. These interactions as they apply to our understanding of VADD are dealt with in this chapter. At the same time this broadening concept has begun to have an influence on thinking in relation to the control of VADD (see Chapter 11).

Growth

The first clearly evident response of a young animal to dietary restriction of any kind is a decrease in the velocity of growth. Young animals, and children, have relatively higher nutritional requirements partly because they need nutrients for growth as well as maintenance. Vitamin A is no exception (see Chapter 3) and in fact is among the nutrients to a deficiency of which the growing organism appears to be most sensitive.

The situation in the young child is infinitely more complicated than that in animals. It cannot be so clearly defined nor can specific restrictions be applied as in the case of experimental animals. Furthermore, there are frequently accompanying infections and these influence nutrient status in several ways. Appetite is usually impaired, pyrexia tends to increase the demand for nutrients, and absorption may be impaired by gut infestations and infections.

Another difficulty is the multiplicity of ways in which growth is customarily assessed. Skeletal measurements, such as height and head circumference, during undernutrition undergo decrease in the velocity of their rate of increase. There is no absolute loss. Height/age is usually employed to indicate “stunting”.

On the other hand, measurements of soft tissues like muscle and fat may involve absolute loss of tissue. Body weight, skinfold thickness and muscle circumference measurements are of this type. Weight/height indicates "wasting", a more acute state of growth retardation than "stunting". Some of these measurements are subject to quite large observer errors. While body weight can be measured with considerable accuracy, there has to be some doubt about its interpretation, especially in children. Body composition is known to vary considerably in malnutrition; fat and muscle decrease but water increases proportionately. Consequently weight may not accurately reflect healthy body mass. The relationship between anthropometric status and mortality was discussed in Chapter 6.

Children with corneal xerophthalmia have consistently been observed to be stunted. This has perhaps best been documented in

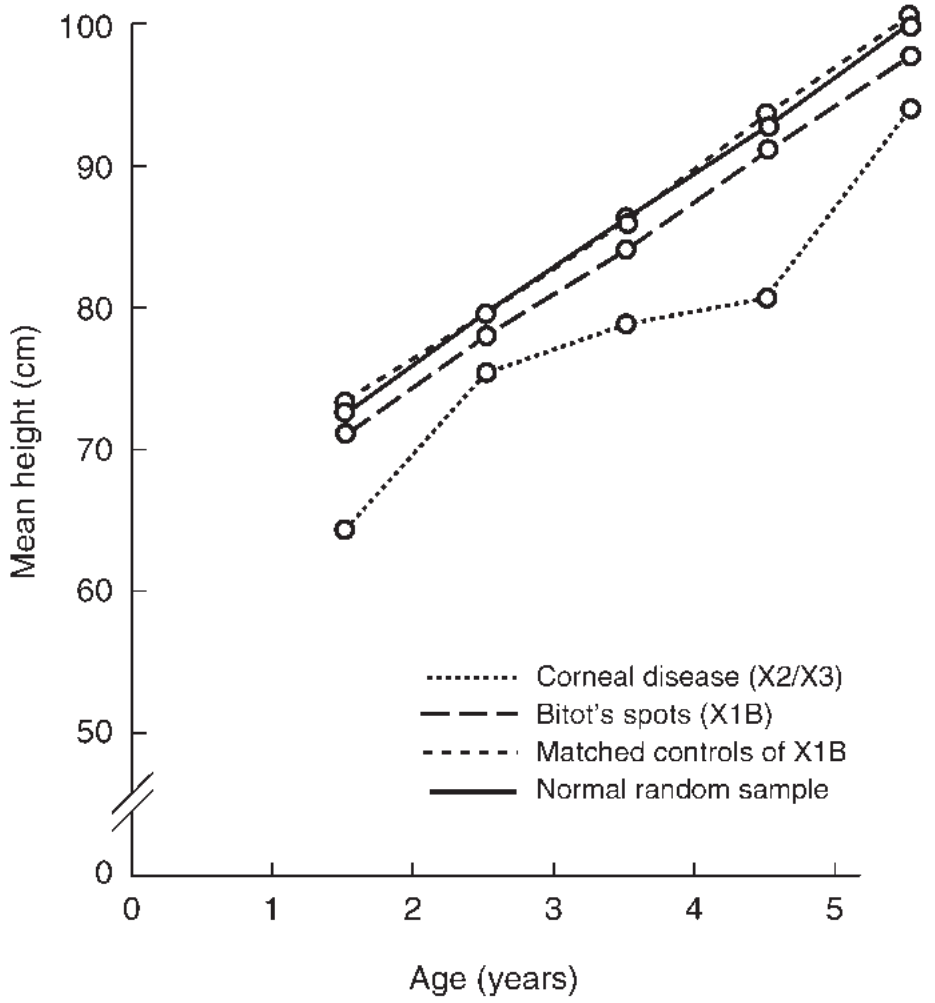


Figure 7.1. Children with corneal disease (X2/X3) were shorter than children who had Bitot's spots (X1B) ($p < 0.01$); children with X1B were shorter than their matched controls ($p < 0.01$). There were no significant differences in height between randomly sampled children without xerophthalmia (normal random sample) and matched controls (Sommer, 1982).

the country-wide survey in Indonesia carried out in 1978–79 (Sommer, 1982) (see Figure 7.1). The degree of vitamin A deficiency was proportional to the degree of retardation of growth. There is also evidence for frequent wasting in xerophthalmic children. In this more acutely deficient state mortality may make interpretation difficult.

It appears that periodic large-dose vitamin A supplementation has a significant impact on growth in children with xerophthalmia, but not on those with evidence of subclinical deficiency. However, three studies in which preformed vitamin A was consumed in adequate amounts regularly showed slight improvement in linear growth (Sommer, West, 1996, pp 163-188).

Several studies of the effect of vitamin A supplementation on growth have failed to demonstrate any effect (Ramakrishnan, Latham, Abel, 1995a; Kirkwood, Ross, Arthur et al, 1996; Fawzi, Herrera, Willett et al, 1997). It may be pointed out that "absence of evidence is not evidence of absence". This general principle in interpretation of results should be applied to the negative results found in these studies. A group in India (Bahl, Bhandari, Taneja et al, 1997) observed improved weight gain with vitamin A supplementation only in the summer season when subclinical vitamin A deficiency peaks. Studies in Nepal (West, Le Clerq, Shrestha et al, 1997) and Indonesia (Hadi, Stoltzfus, Dibley et al, 2000) both reported improved growth with supplemental vitamin A. The effect was seen most in children with the lowest serum retinol levels and in the long term affected mainly height. Soft tissue growth improvement tended to occur earlier on.

A possible mechanism for an effect of vitamin A on growth is provided by a study carried out in a group of short prepubertal children (Evain-Brion, Porquet, Thérond et al, 1994). Fasting plasma retinol correlated with nocturnal growth hormone (GH) secretion but not with stimulated GH secretion. Dietary intake of vitamin A was lower in children with low nocturnal GH secretion, and supplementation with vitamin A for 3 months increased their nocturnal GH secretion.

There is one aspect of growth and development that does not appear to have been addressed in relation to a possible effect of vitamin A deficiency. That is brain growth and intellectual development.

Immune response

Interest in the possibility of vitamin A being involved in the function of the immune system derived first from the association of vitamin A deficiency with infectious diseases. More recently it has been shown experimentally that retinoids can stimulate immune responses.

There are two distinct responses to exposure to antigens; humoral and cellular (cell-mediated) immunity. Humoral immunity results from antibody production mediated by B-lymphocytes, which is often T-lymphocyte-dependent. Cellular immunity is mediated by T-lymphocytes. There are two main effector mechanisms: cytolytic T-lymphocyte (CTL) responses, and delayed-type hypersensitivity (DTH) responses.

There are also natural killer (NK) cells, which are part of the innate or nonspecific immune system. Phagocytic cells also belong to this system.

It has been proposed (Ross, 1996) that there are broadly speaking two hypotheses to explain the protective action of vitamin A against infection (see Table 7.1).

The epithelial cell linings of organs and tissues are generally considered to have a defensive function. They have been considered

Table 7.1. Two contrasting hypotheses for the protective role of vitamin A against infection (after Ross, 1996).

Epithelial barrier hypothesis	Immunologic response hypothesis
The basic reaction is OFFENSIVE	The basic reaction is DEFENSIVE
This protects against invasion by infection	This enhances the body's defence against pathogens
Structural integrity is of greatest importance	Functional integrity is most important, as is cell differentiation
Consequently resistance to establishment of infection will be reduced by VAD	Consequently resistance to proliferation of infection will be reduced by VAD
Major effect of vitamin A intervention will be decreased incidence of infection	Major effect of vitamin A intervention will be decreased duration and/or severity of infection

to provide a first line of defence in resistence to infection taking place. Ross argues that this is a misconception and that they primarily have an offensive role. On the other hand the immunologic response is a defensive response against infection once it has taken place.

The available evidence as to which might be closer to reality from studies of human populations is strictly limited (see Chapter 6). It would be expected that offensive protection would reduce the incidence of infections. A defensive mechanism would be likely to reduce the duration or the severity of an infection. As was shown in Chapter 6, the few studies that addressed these points tended to suggest that the impact of vitamin A supplementation has been on duration and severity. This supports the immunologic response hypothesis.

Most of the evidence from both animal and human studies suggests that the various aspects of humoral immunity are little affected by vitamin A deficiency. Cell-mediated immunity, however, has been shown to be markedly impaired. The production and maturation of lymphocytes are reduced by lack of vitamin A. In a study in Indonesia it was found that the ratio of T-cells bearing CD4+ and CD8+ antigens was lower in the peripheral blood lymphocytes of xerophthalmic children compared with non-xerophthalmic controls (Semba, Muhilal, Ward et al, 1993). After vitamin A supplementation the proportion of

Table 7.2 Main immune response elements that may be regulated by retinoids (after Semba, 1998).

Keratinization
Mucin production
Haematopoiesis (influences many types of cell)
Apoptosis (programmed cell death)
Function of neutrophil, natural killer, monocyte/macrophage, various lymphocyte and other cells
Immunoglobulin production
Production of numerous interleukins

CD4+ to CD8+ T-cells and the percentage of naive CD4+ T-lymphocytes increased.

The precise mechanisms whereby vitamin A plays a part in maintaining normal function of the immune response are not yet fully understood. The active form at the cellular level appears to be retinoic acid, but it is possible that other metabolites of retinol may also be active.

Semba (1998) has recently reviewed the role of vitamin A and related retinoids in immune function. Table 7.2 is a simplified version of the table in his paper.

Interaction of iron and vitamin A

It has long been known that vitamin A deficiency and iron deficiency tend to exist together in population groups. This might be expected to occur because natural dietaries when deficient usually involve more than a single nutrient. Controlled trials have been carried out in several countries in which vitamin A supplementation has brought about a significant increase in haemoglobin level (Sommer, West, 1996, pp 150-162). The best results were obtained when daily ingestion of vitamin A in amounts close to recommended levels of intake was used.

There is some evidence that administration of iron to treat anaemia may exacerbate coexisting infection. Many pathogenic bacteria require iron and might proliferate as they compete for it. In one study (Northrop-Clewes, Paracha, McLoone et al, 1996) an increase in dietary intake of vitamin A under these circumstances appeared to counteract any adverse effects of iron. The authors suggest that the beneficial effects of vitamin A on iron status may be related to reduced levels of infection.

Fortification of sugar with vitamin A was used in Guatemala to study the effect on iron status over a prolonged period (Sommer, West, 1996, pp 150-162). Table 7.3 shows the mean changes sampled at 6-monthly intervals over a 2-year period. It was concluded that there was initially enhanced iron mobilization leading to lower iron stores. This probably triggered increased efficiency of iron absorption which would gradually restore iron stores. These in turn would make available for haemopoiesis iron at an enhanced level over that available before sugar fortification began. It was shown recently (Garcia-Casal, Leets, Layrisse, 2000) that β-carotene improved the absorption of iron in vitro, and also of non-haem iron from rice, wheat and maize in humans (Garcia-Casal, Layrisse, Solano et al, 1998). It was suggested that they form a complex with iron, keeping it soluble in the intestional lumen and preventing the inhibitory effect of phytates and polyphenols on iron absorption.

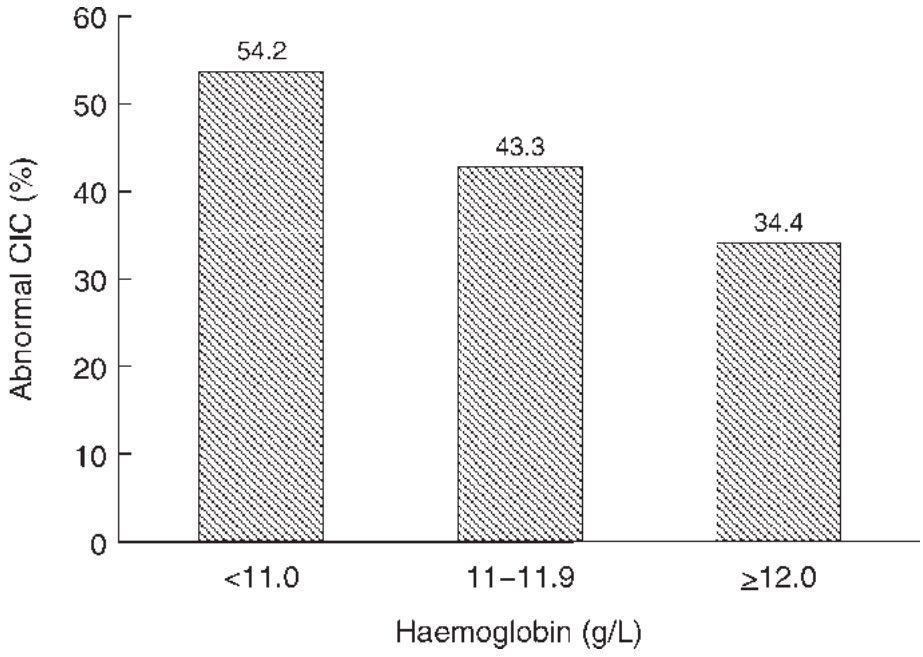


Figure 7.2. Relationship between haemoglobin level and prevalence of vitamin A deficiency assessed by conjunctival impression cytology (CIC) in children 3–6 years of age in Truk, Micronesia (Lloyd-Puryear, Mahoney, Humphrey et al, 1991).

Table 7.3. Change in serum retinol and iron status in preschool children following sugar fortification with vitamin A in Guatemala (Mejia, Arroyave, 1982)

	Duration of study (months)			
	6	12	18	24
No. of subjects analized	77	75	46	51
Retinol (µg/dL)	+5.1*	+5.2*	+3.6*	+2.5
Serum iron (µg/dL)	+4.5*	-3.6	+13.1*	+ 9.1*
Total iron-binding capacity (µg/dL)	+18.3*	-8.8	-7.8	-13.6
Transferrin saturation (%)	+0.6	-1.0	+3.8*	+ 2.2*
Serum ferritin (ng/ml)	-3.3*	+2.1	+5.5*	+ 4.9*

* $p \leq 0.05$

The prevalence of anaemia associated with vitamin A deficiency has not been estimated. It is likely to be quite widespread. Iron deficiency affects hundreds of millions of the world's population. Especially vulnerable groups are young children and women in the reproductive age period. These groups are also at risk of vitamin A deficiency. One recent study in the Pacific region (Lloyd-Puryear, Humphrey, West, 1989) showed that the prevalence of subclinical vitamin A deficiency, as evidenced by abnormal CIC, was inversely proportional to the haemoglobin level (see Figure 7.2).

The mechanism of vitamin A-related anaemia is unclear. It has consistently been shown that vitamin A deficiency restricts the release of iron from the depots, resulting in evidence of iron overload and, in the absence of increased absorption of iron, eventually in anaemia. Although infection also causes iron overload, it can occur in the absence of infection.

Serum iron tends to be low and vitamin A supplementation causes increase in serum iron and in % transferrin saturation (Sommer, West, 1996, pp 150-162). Recent animal work (Rosales, Jang, Pinero et al, 2000) showed that a fall in haemoglobin due to feeding low-iron diets was accompanied by a lowering of plasma retinol but an increase in hepatic retinyl esters. Low iron status seems to interfere with hepatic release of vitamin A.

In conclusion, despite the need for more detailed understanding of vitamin A–iron interrelationships, the prevention of vitamin A deficiency should be considered along with iron supplementation in the control of nutritional anaemia (IVACG, 1998).

Interaction of zinc and vitamin A

This relationship has been known for some time but has only recently begun to be explored in the context of VADD. Many enzymes are zinc-dependent and among them is retinol dehydrogenase involved in rod function. As a result it has been found that some cases of night blindness that are resistant to vitamin A may respond to zinc. Zinc deficiency may interfere with the synthesis of retinol-binding protein.

A number of recent studies have demonstrated that supplementation with zinc has a beneficial effect in diarrhoeal and some other infectious diseases (Bhutta, Black, Brown et al, 1999; Shankar, 1999) very similar to that of vitamin A. One preliminary report (IVACG, 1999) found a significant increase in mortality in a group of children receiving a large dose of zinc. These trials are at an early stage and safety issues and problems with assessment of zinc status have still to be resolved. Combined therapy with vitamin A and zinc does not appear to have been reported until the present.

This area is tending to become complicated by the use of supplementation with varying combinations of the three micronutrients vitamin A, iron and zinc. Lymphocyte responsiveness has been enhanced with vitamin A and zinc (Kramer, Udomkesmalee, Dhanamitta et al, 1993). Growth in height and weight was improved by vitamin A but not by zinc (Smith, Makdani, Hegar et al, 1999). An interesting report claimed a better haematologic response in anaemic women when vitamin A and zinc were added to iron than when treatment was with iron alone or vitamin A and iron (Kolsteren, Rahman, Hildebrand et al, 1999).Recently it was shown that supplementation with iron and

zinc improved serum retinol levels (Muñoz, Rosado, López et al, 2000). It seems likely that there will be many more studies like these in the near future.

Normal and diseased skin

This subject has been extensively reviewed by Vahlquist (1994). Retinol and several other retinoids have been detected in the skin, mainly in the epidermis. Some years ago, when clinical signs and symptoms were relied upon for the diagnosis of nutritional deficiency disease, hyperkeratosis of the skin around hair follicles was frequently attributed to vitamin A deficiency. This change did not correlate with xerophthalmia or serum retinol. At present the general view is that the skin does not undergo any characteristic clinical change in vitamin A deficiency.

In recent years numerous retinoids have been synthesized for use in the treatment of a variety of dermatological disorders (see Chapter 10).

Reproductive systems

Studies in experimental animals have shown that vitamin A is necessary for the proper functioning of both male and female reproductive organs. The subject has been reviewed by Eskild and Hansson (1994). In the male spermatogenesis is impaired and the functions of the Sertoli and Leydig cells are interfered with.

There is growing evidence that vitamin A, in some form, is required for every stage in the reproductive process in the female. This is in addition to the implication of both deficiency and excess of vitamin A during the organogenetic period in the production of malformations of the fetus discussed elsewhere (see Chapters 3, 10 and 11).

Other systems

Vitamin A deficiency in cattle has a marked effect on the modelling of bone. Most notably this has led to compression of the optic nerve in the optic foramen, leading to blindness.

Experimentally keratinizing metaplasia has been described in the cochlea in the inner ear.

GLOBAL OCCURRENCE

Introduction

The magnitude of the problem of VADD throughout the world, within a nation, and in certain regions of a nation, is clearly of paramount importance for the implementation of measures aimed at controlling the problem. Identification of VADD in the first place and monitoring the progress of control measures require data-collecting systems to be in place and operating.

Global occurrence

Up to the 1950s there were reports of endemic xerophthalmia in India, Indonesia and several other countries but little more was known. The World Health Organization sponsored a global survey in the early 1960s in which three consultants visited about 50 countries where VADD was suspected to be a public health problem (Oomen, McLaren, Escapini, 1964). This revealed the widespread nature and serious magnitude of the problem, especially in much of South and East Asia, and parts of Africa and Latin America. Estimates of the worldwide magnitude of the problem were also made (McLaren, 1962;Sommer, 1982).

In recent years the Nutrition Unit of the World Health Organization in Geneva, Switzerland, with its worldwide contacts, has taken on the role of collecting and disseminating data. The data file MDIS (Micronutrient Deficiency Information System) of the Nutrition Unit of the WHO is continuously updated.

This process culminated in the publishing in 1995 of a monograph, *Global Prevalence of Vitamin A Deficiency* (WHO, 1995), which is the most complete database in the field to date. The results of surveys reported from each country, where data are available, are presented in two disaggregated tables: 1) for prevalence of ocular signs and symptoms, and 2) for serum retinol levels. On the basis of these data, and making allowance for partial coverage of a country by multiplication factors, summary tables and maps by WHO region were constructed of the prevalence of vitamin A deficiency (by category: clinical, severe subclinical, and moderate subclinical). Figures 8.1 to 8.7 show the global and regional maps updated by October 2000 from MDIS.

A classification is also presented in tabular form of estimates that have been made from the country data of the category occupied by each country. As mentioned, WHO keeps the database under regular review and from time to time publishes revised lists of countries and their vitamin A status, by WHO region. Table 8.1 is updated by October 2000 from MDIS.

In 1994 the global estimate of the number of children aged 0–4 years clinically affected

GLOBAL OCCURRENCE

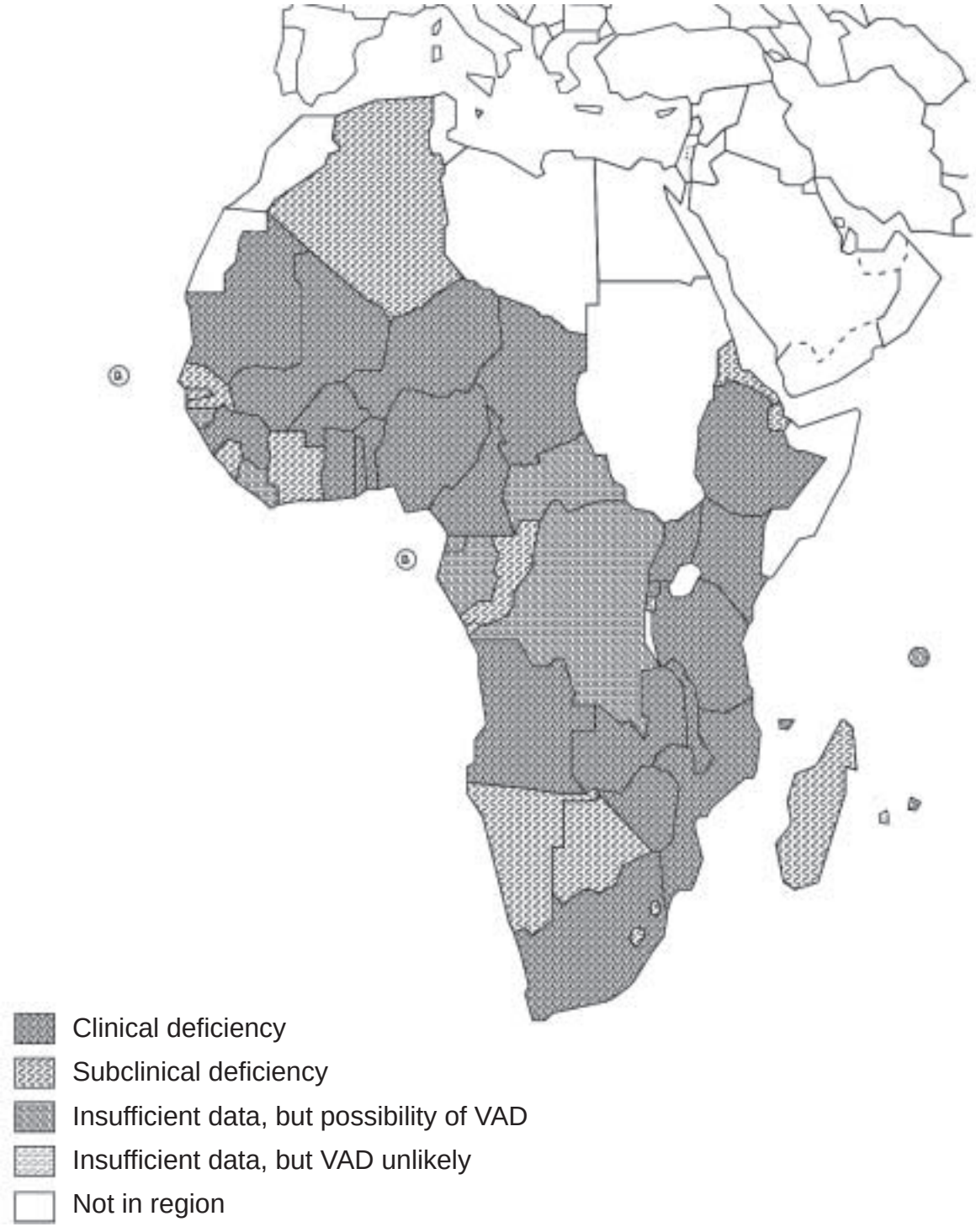


Figure 8.2. Prevalence of vitamin A deficiency in the **African Region**, based on Figure 8.1 (from information available to WHO by the year 2000). The designations do not necessarily imply VAD severity is uniformly distributed throughout each country.

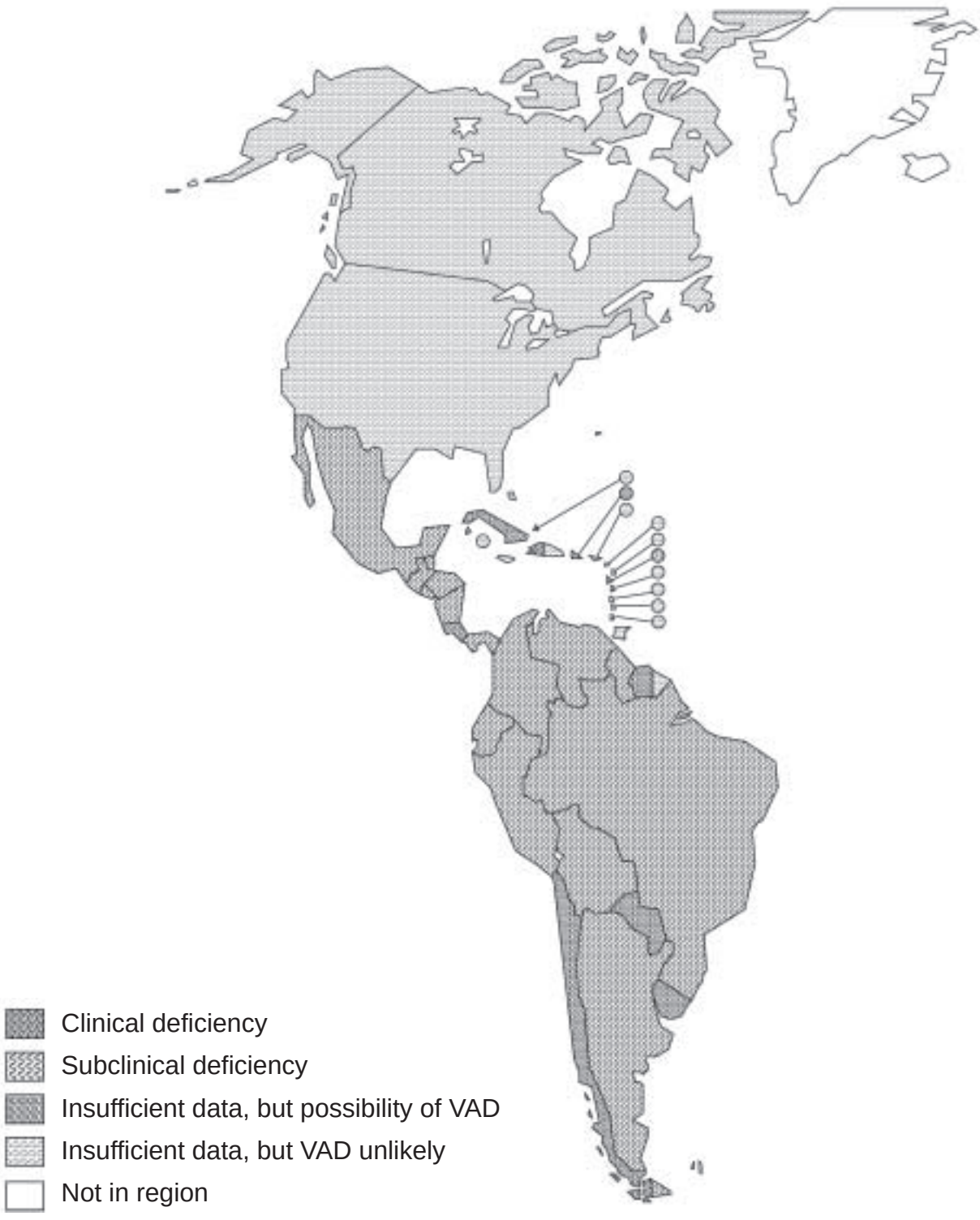


Figure 8.3. Prevalence of vitamin A deficiency in the **Region of the Americas**, based on Figure 8.1 (from information available to WHO by the year 2000). The designations do not necessarily imply VAD severity is uniformly distributed throughout each country.

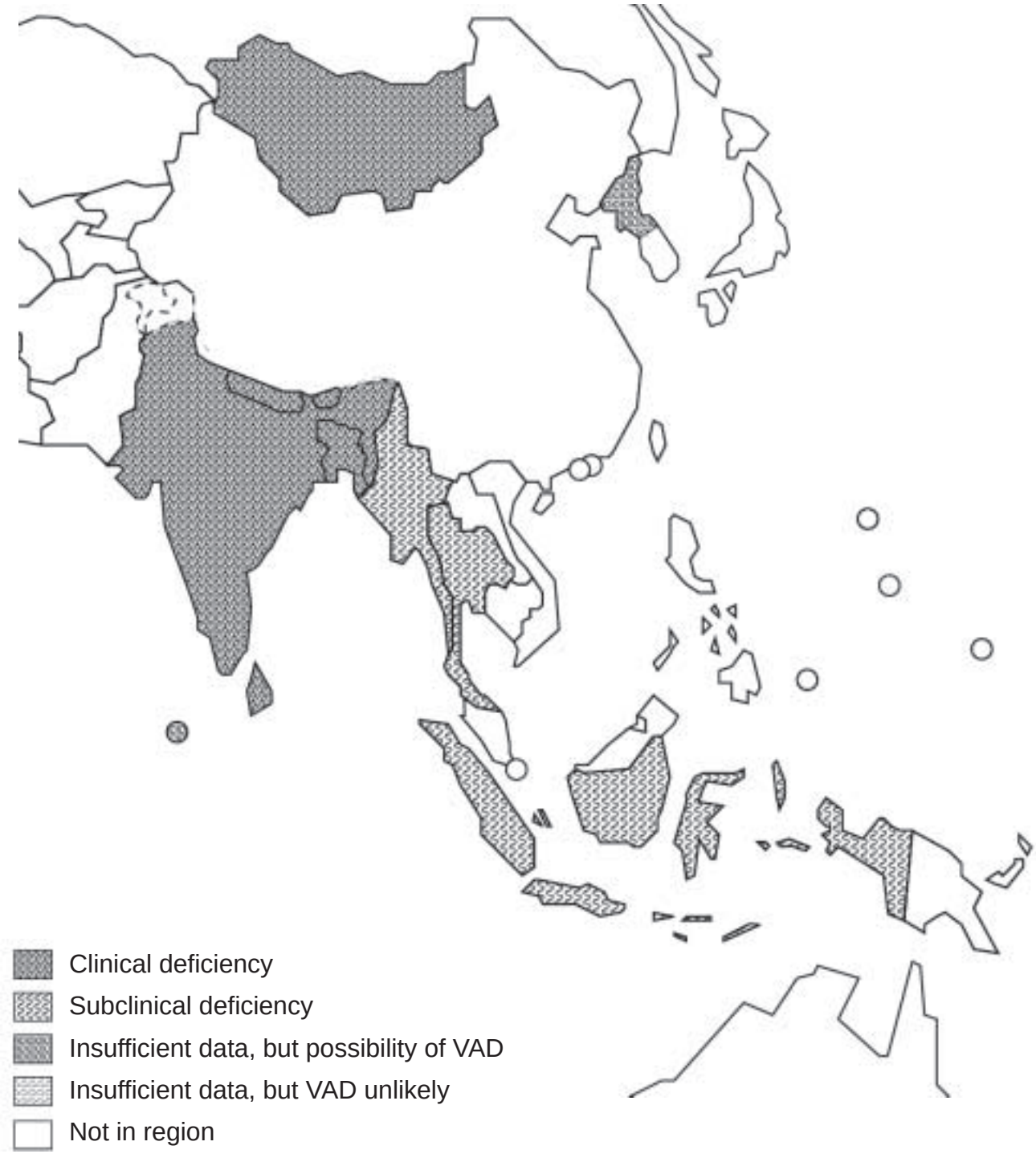


Figure 8.4. Prevalence of vitamin A deficiency in the **South-East Asian Region**, based on Figure 8.1 (from information available to WHO by the year 2000). The designations do not necessarily imply VAD severity is uniformly distributed throughout each country.

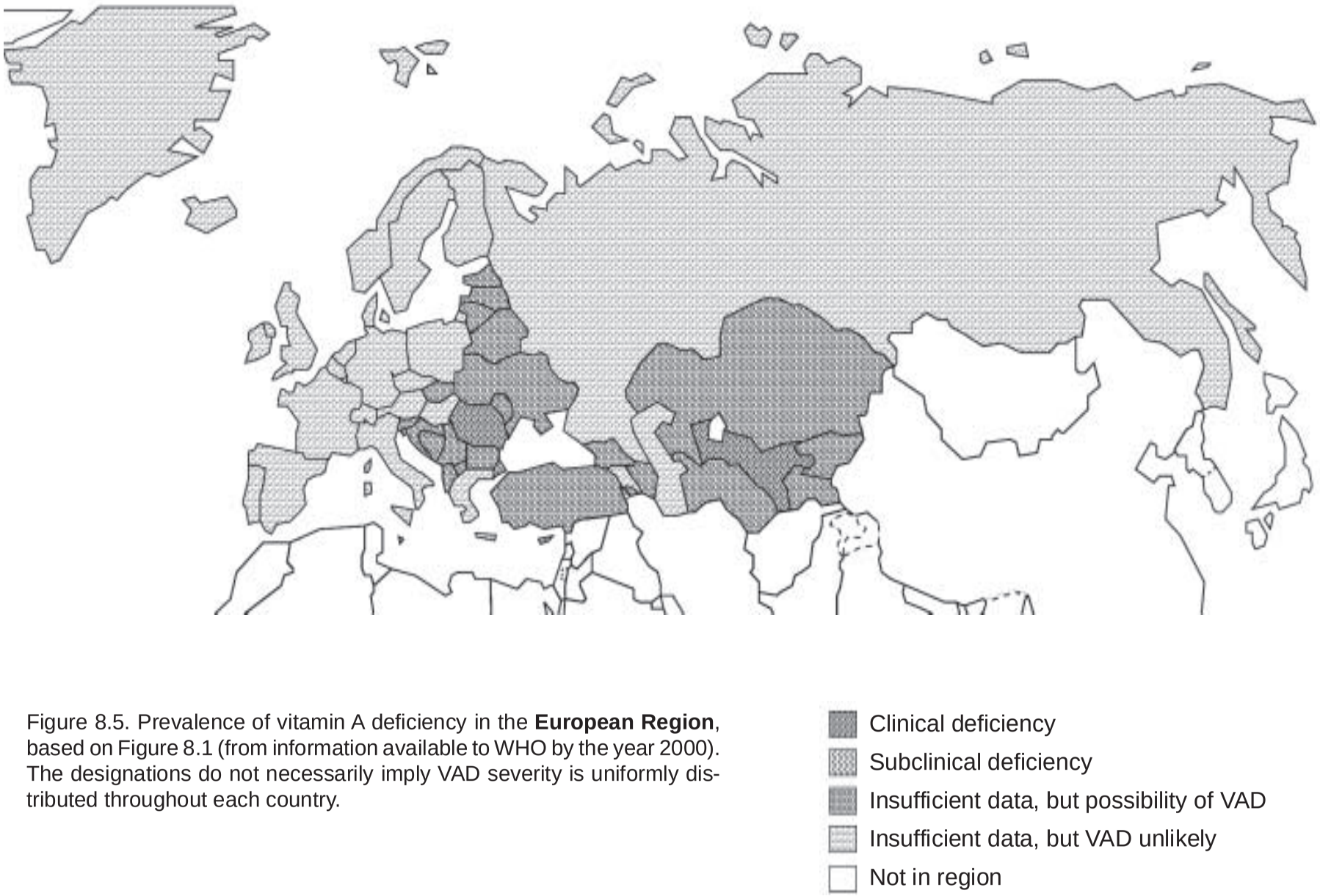


Figure 8.5. Prevalence of vitamin A deficiency in the **European Region**, based on Figure 8.1 (from information available to WHO by the year 2000). The designations do not necessarily imply VAD severity is uniformly distributed throughout each country.

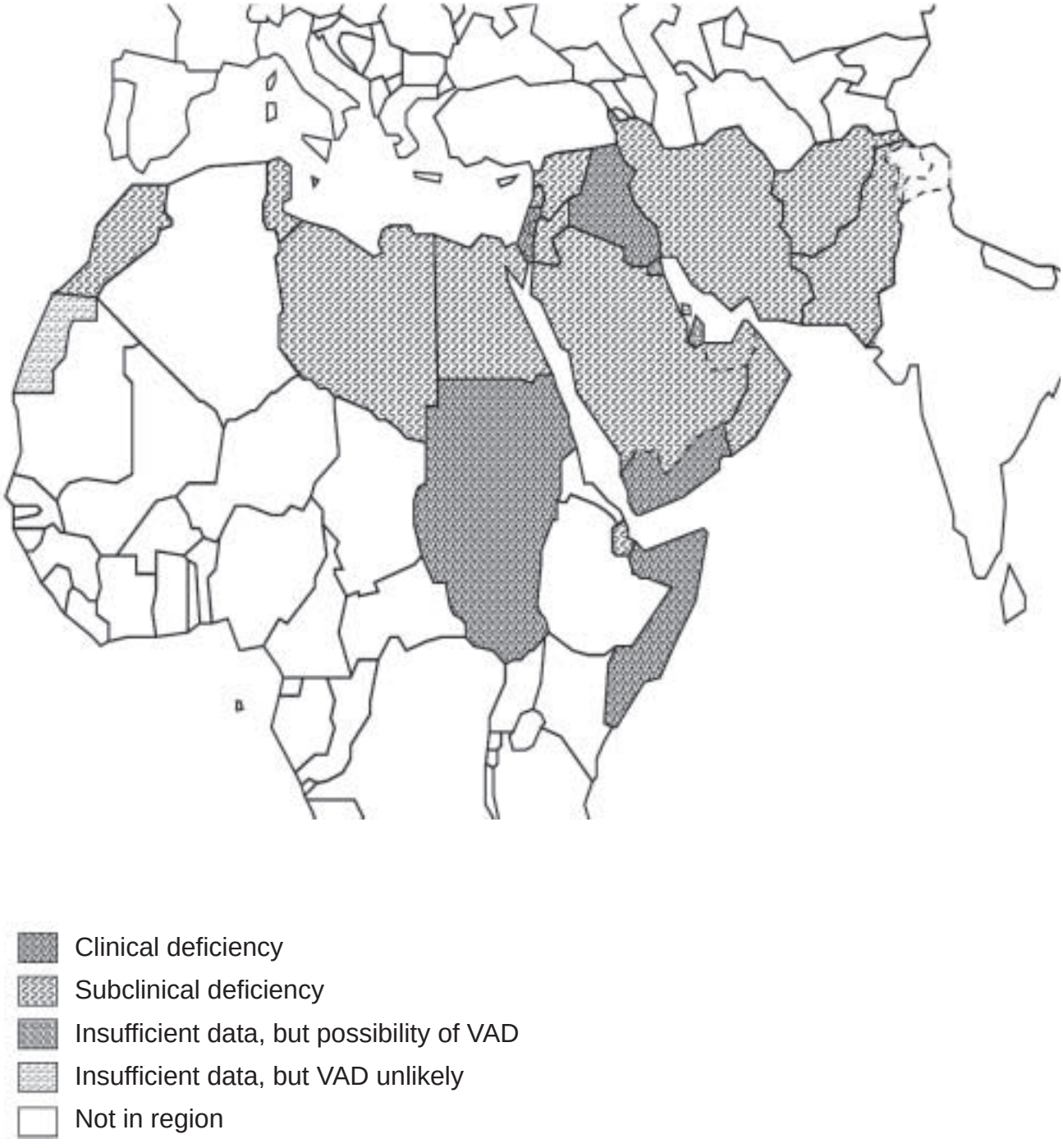


Figure 8.6. Prevalence of vitamin A deficiency in the **Eastern Mediterranean Region**, based on Figure 8.1 (from information available to WHO by the year 2000). The designations do not necessarily imply VAD severity is uniformly distributed throughout each country.

Figure 8.7. Prevalence of vitamin A deficiency in the **Western Pacific Region**, based on Figure 8.1 (from information available to WHO by the year 2000). The designations do not necessarily imply VAD severity is uniformly distributed throughout each country.

Table 8.1. Public health imortance of vitamin A deficiency, from information available to WHO as of October 2000

WHO Region	Clinical deficiency	Subclinical deficiency	Insufficient data, but possibility of VAD	Insufficient data, but VAD unlikely
Africa	Angola Benin Burkina Faso Cameroon Chad Comoros Ethiopia Ghana Guinea Kenya Malawi Mali Mauritania Mozambique Niger Nigeria Rwanda South Africa Togo Uganda United Republic of Tanzania Zambia Zimbabwe	Algeria Botswana Burundi Cape Verde Congo Côte d'Ivoire Eritrea Gambia Lesotho Madagascar Namibia Senegal Sierra Leone Swaziland	Cent. African Republic Equatorial Guinea Gabon Guinea-Bissau Liberia Republic of Congo São Tomé and Príncipe	Mauritius Seychelles
Americas		Argentina Belize Bolivia Brazil Colombia Costa Rica Dominican Republic Ecuador El Salvador Guatemala Guyana Honduras Mexico Nicaragua Panama Peru Venezuela	Chile Cuba Haiti Paraguay Suriname Uruguay	Antigua and Barbuda Bahamas Barbados Canada Dominica Grenada Jamaica St. Kitts and Nevis St. Lucia St.Vincent and the Grenadines Trinidad and Tobago United States of America
South East Asia	Bangladesh Bhutan India Nepal Sri Lanka	Indonesia Myanmar Thailand	Democratic People's Rep. of Korea Maldives	

(Continued Table 8.1)

WHO Region	Clinical deficiency	Subclinical deficiency	Insufficient data, but possibility of VAD	Insufficient data, but VAD unlikely
Europe	Israel Romania Uzbekistan		Albania Azerbaijan Belarus Bosnia and Herzegovina Bulgaria Croatia Estonia Georgia Kazakastan Kyrgyzstan Latvia Lithuania Malta Republic of Moldova Slovakia Slovenia Tajikistan The former Rep. of Yugoslav Republic of Macedonia Turkey Turkmenistan Ukraine Yugoslavia	Armenia Austria Belgium Czech Republic Denmark Hungary Finland France Germany Greece Iceland Ireland Italy Luxembourg Malta Monaco Netherlands Norway Poland Portugal Russian Federation San Marino Spain Sweden Switzerland United Kingdom

WHO Region	Clinical deficiency	Subclinical deficiency	Insufficient data, but possibility of VAD	Insufficient data, but VAD unlikely
Eastern Mediterranean	Iraq Somalia Sudan Yemen	Afghanistan Djibouti Egypt Islamic Republic of Iran Jordan Lebanon Libyan Arab Jamahiriya Morocco Oman Pakistan Saudi Arabia Syrian Arab Rep. Tunisia United Arab Emirates	Kuwait	Cyprus Qatar

WHO Region	Clinical deficiency	Subclinical deficiency	Insufficient data, but possibility of VAD	Insufficient data, but VAD unlikely
Western Pacific	Cambodia Kiribati Lao People's Dem.Rep. Mongolia Marshall Islands Federated States of Micronesia Philippines Solomon Islands	Commonwealth of Northern Mariana Islands Papua New Guinea Viet Nam	China Malaysia Palau	Australia Brunei Darussalam Cook Islands Fiji French Polynesia Guam Japan Rep. of Korea Nauru New Zealand Niue Samoa Tonga Tokelau Tuvalu Singapore Vanuatu

was 2.8 million, and that for severely and moderately subclinically affected children was 251 million. The report acknowledges a number of shortcomings and limitations in the data. These were supplied to WHO by the various country authorities and they constitute a very heterogeneous collection. Many data sets show weaknesses in survey design, subject selection, data aggregation, and in information reporting. With the exception of data from the few large research vitamin A intervention trials reviewed previously (see Chapter 6), the number of subjects in the majority of studies is far too small to meet the minimum sample size specified by WHO (1996).

Many of the studies used either clinical signs or serum retinol, but not both as recommended. This makes categorization difficult. Corneal scar related to vitamin A deficiency (XS) is an indicator of deficiency in the past and is thus unsuitable for inclusion in prevalence data. Xerophthalmia was reported as "clinical vitamin A deficiency", making no differentiation between blinding (corneal) and non-blinding (non-corneal) xerophthalmia. It would have been useful to have an estimate of the number of cases of blindness being produced annually. This could be compared with those of other causes of blindness.

Methodology

It is evident that careful attention should be given to the methods used for data collection on the prevalence of VADD. In a country or area where there has been no previous study it is advisable to employ a preliminary assessment, along the lines of that advised by WHO (WHO Expert Group, 1982). Table 8.2 indicates the kind of background data that should be sought in the first instance.

Table 8.2. Preliminary assessment of vitamin A status of a population (after Sommer, 1995)

1. Interviews, by structured questionnaire, with central and provincial public health officials, clinicians, nutritionists, community health workers, directors and staff of hospitals, feeding and rehabilitation centres, and schools for the blind.

2. Chart reviews at hospitals, clinics, institutions where children suspected to suffer from corneal destruction, or where coded charts do not exist, records from malnutrition, infectious diseases, paediatric and ophthalmic services.

3. Search for clinically active cases among children in high-risk situations – hospitals, clinics, impoverished communities.

4. Search for old, healed disease in schools for the blind etc.

5. Collect existing data on child rearing, including breast and other feeding and hygiene practices.

This can be done in a simple way that is economical of personnel and resources. It will be observed that most of these data relate to the detection of a problem of xerophthalmia. If the health concern is at the subclinical level of VAD then an assessment may be made using the socioeconomic and ecological indicators recommended by WHO (1996). Only if the results are highly suggestive of the presence of a significant problem should a full point prevalence survey be embarked upon. This is much more expensive and time-consuming and should be carried out meticulously if the results are to be relied upon (Sommer, 1995).

Country situations

There is still a surprisingly large number of countries from which no data are available and where the occurrence of a problem might be suspected (see Table 8.1). The largest block of such countries is that of the former Soviet Union with millions of children at risk.

The situation is, of course, fluid and there may be improvement or deterioration over quite a short period of time. For example, there is good evidence that the situation has improved considerably in three of the major countries where VADD was highly endemic only two decades or so ago (see Figure 8.8).

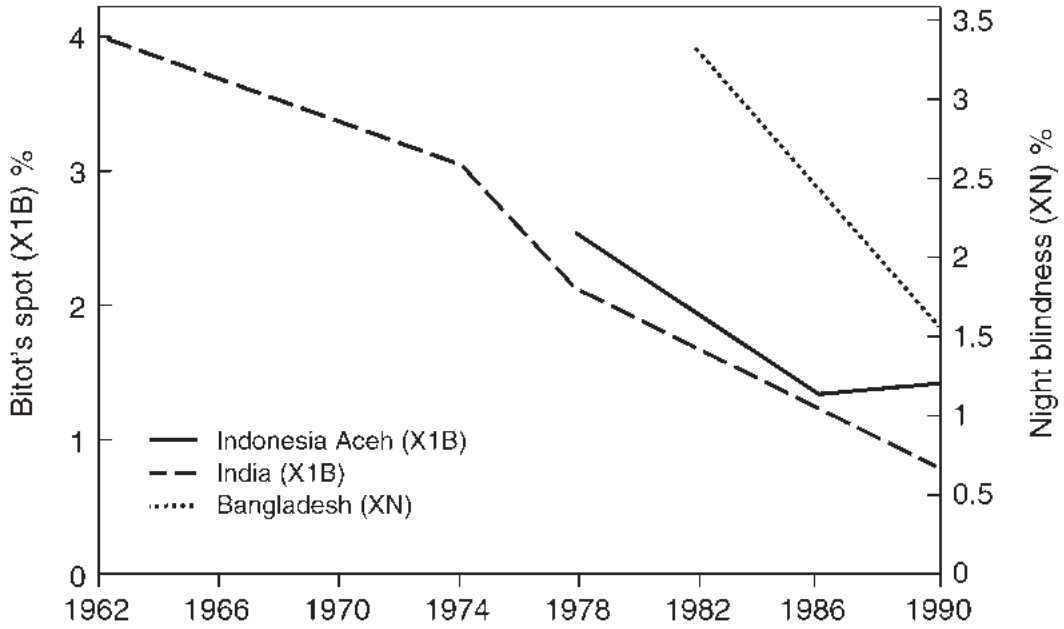


Figure 8.8. Trends in xerophthalmia in children up to six years (ACC/SCN Consultative Group, 1994).

In the Philippines in 1993 a National Nutrition Survey carried out by the Food and Nutrition Research Institute showed that clinical VAD prevalence among six-month- to six-year-old children was 0.4% for XN and 0.2% for X1B, and for the total population prevalence of XN was 0.8% and of X1B 0.3%, indicating that VAD is no longer a clinical public health problem (as shown in Figure 8.7 and Table 8.1). However, serum retinol <0.35 μmol/L among six-month- to six-year-old children occurs in more than 5% in 11 out of 15 regions. Low serum retinol (<0.70 μmol/L) occurs in 24.9%. These results suggest that at present the Philippines should be categorized to have a severe subclinical VAD public health problem (F. Solon, personal communication).

Xerophthalmia was extremely common in Vietnam until after the cessation of hostilities. In recent years, highly efficient capsule distribution nationwide has resulted in a pronounced decrease (Khoi, Khan, Thang et al, 1996). Sometimes improvement is more likely to be attributable to general economic and social development, rather than just to specific vitamin A interventions.

On the other hand, VADD has emerged in several countries in the Pacific region, as a result of rapid changes in lifestyle and the abandonment of traditional dietary and other practices that were protective (Lloyd-Puryear, Humphrey, West et al, 1989; Schaumberg, O'Connor, Semba, 1996).

In other countries a public health problem has long been suspected but data were not available. This seems to be the situation in Laos (Malyavin, Bouphany, Arouny et al, 1996) and is true for Afghanistan.

EPIDEMIOLOGY

Introduction

Epidemiology may be defined as the study of the distribution and determinants of disease in a population. In simple terms various parts of the Manual may be considered to have dealt in turn with the answers to different questions about VADD. For example the first four chapters described *how* VADD occurs. Chapters 5–7 stated *what* VADD causes. Chapter 8 dealt with *where* VADD occurs and *how much* there is. The present chapter sets out to give some answers to the question "*why* does VADD occur?". A clear understanding of these matters is vital before appropriate measures can be taken for the control of VADD. It is logical that this chapter on epidemiology should precede Chapter 11 on "*what* can be done?".

Those factors that are closely associated with the occurrence of a disease are known as risk factors. This association may be demonstrated by the application of statistical methods. However, there is no statistical way of proving that the association is causal; i.e. that the factor causes the disease. A logical relationship between the factor and the disease lends support to the association being causal, but does not provide proof.

Consequently, epidemiology can provide useful indications as to how a disease arises. Vulnerable groups can be identified towards whom interventions may be targeted. Defective or, on the other hand, protective aspects of diet and other aspects of lifestyle may be revealed and as a result this knowledge may be used in planning interventions.

It is important to recognize that epidemiological factors may vary considerably from place to place. Interventions need to be appropriate for local conditions. For example, in some circumstances it may be found that the weaning diet lacks rich sources of provitamin A in the form of dark green leaves or yellow fruits. Ways should be sought to ensure that these are incorporated into the young child's diet if at all possible. On the other hand, it may be found that dietary intake of carotenoids is adequate but VADD is nevertheless a problem. Further investigation may reveal that there is heavy round-worm infestation in the community and it is likely that much of the ingested carotene is not being absorbed. Deworming and measures to improve sanitation should have priority in this case.

There is no special order in which the risk factors are considered here, but in general those of greater importance are taken first.

Age

Evidence from every source agrees that preschool age children form the most vulnerable group (Sommer, 1982). This applies to corneal xerophthalmia in hospitals (see Figure 9.1) and non-corneal xerophthalmia in field studies (see Figure 9.2). Of the data taken

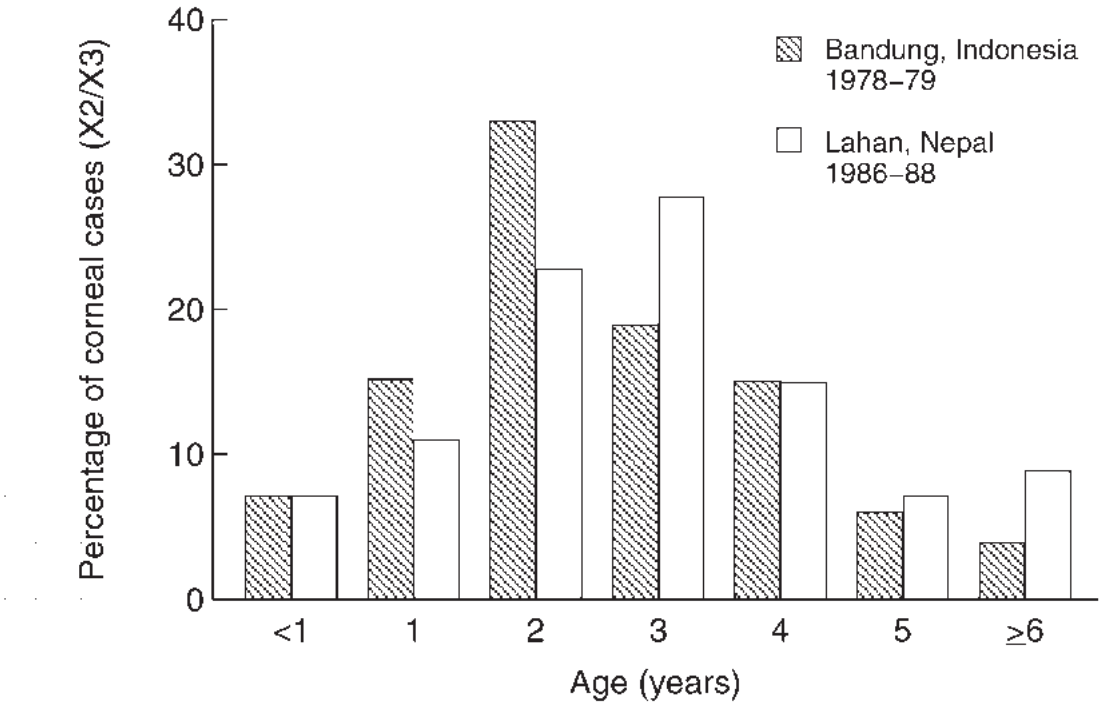


Figure 9.1. Age distribution of consecutive cases with corneal xerophthalmia (X2 or X3) presenting to the Cicendo Eye Hospital (n=162), Bandung, Indonesia, and to the Lahan Eye Hospital (n=295), Nepal (Sommer, West, 1996, p 337).

together it will be evident that the more serious lesions peak at an earlier age than the less serious.

A combination of factors probably lies behind this phenomenon. The requirements for growth of the younger child tend to be greater, while the body stores tend to be lower. The diet of the vulnerable child tends to be more restrictive and limited during infancy or just after weaning than later on. Infections have an additional adverse nutritional impact.

Keratomalacia in the infant usually proceeds rapidly without any tendency for xerosis of the conjunctiva to develop. From some parts of India there have been recent reports of increase in the occurrence of infant keratomalacia. This may be related to the increasing practice of women of low socioeconomic status to go out to work and to leave their infants at home without adequate attention (Rahmathullah, Raj, Darling et al, 1994).

School age is another period of increased susceptibility, usually involving the milder degrees of xerophthalmia (night blindness or Bitot's spots) (Khan, Haque, Khan, 1984). This may be due to increased requirements for growth, especially during the adolescent growth spurt (Ahmed, Hasan, Kabir, 1997).

Physiological status

It has long been recognized that the pregnant and lactating woman is especially vulnerable. Recently some very high rates have been reported especially from the Indian subcontinent (Katz, Khatry, West et al, 1995). In this study from Nepal 16.2% of women reported XN at some time during the pregnancy that produced the child they were breast-feeding

at the time of the interview. 8.1% reported XN at the time of the interview. The chances of XN in the current pregnancy were six times greater for those who reported XN in their previous pregnancy than for those who did not.

Only mild xerophthalmia is usually seen and the mother's health may not be permanently affected. However, low vitamin A content of breast milk will contribute to the increased susceptibility of the infant. The proposal that breast milk retinol be used as a biological indicator of vitamin A status in the community was discussed in Chapter 4.

More recent studies by the same group in Nepal have shown that XN in pregnancy may be a marker of other nutritional and health risks (Christian, West, Khatry et al, 1998). Attitudes of women towards XN have been documented (Christian, Bentley, Pradhan et al, 1998) as has the extent to which it impairs their work activities (Christian, Thorne-Lyman, West et al, 1998).

Diet

Regular ingestion of the customary dietaries around the world, both now and in the past, has provided for vitamin A requirements to be met. It has only been when consumption was limited, perhaps at certain seasons of shortage or when certain food items were omitted altogether, that problems have arisen.

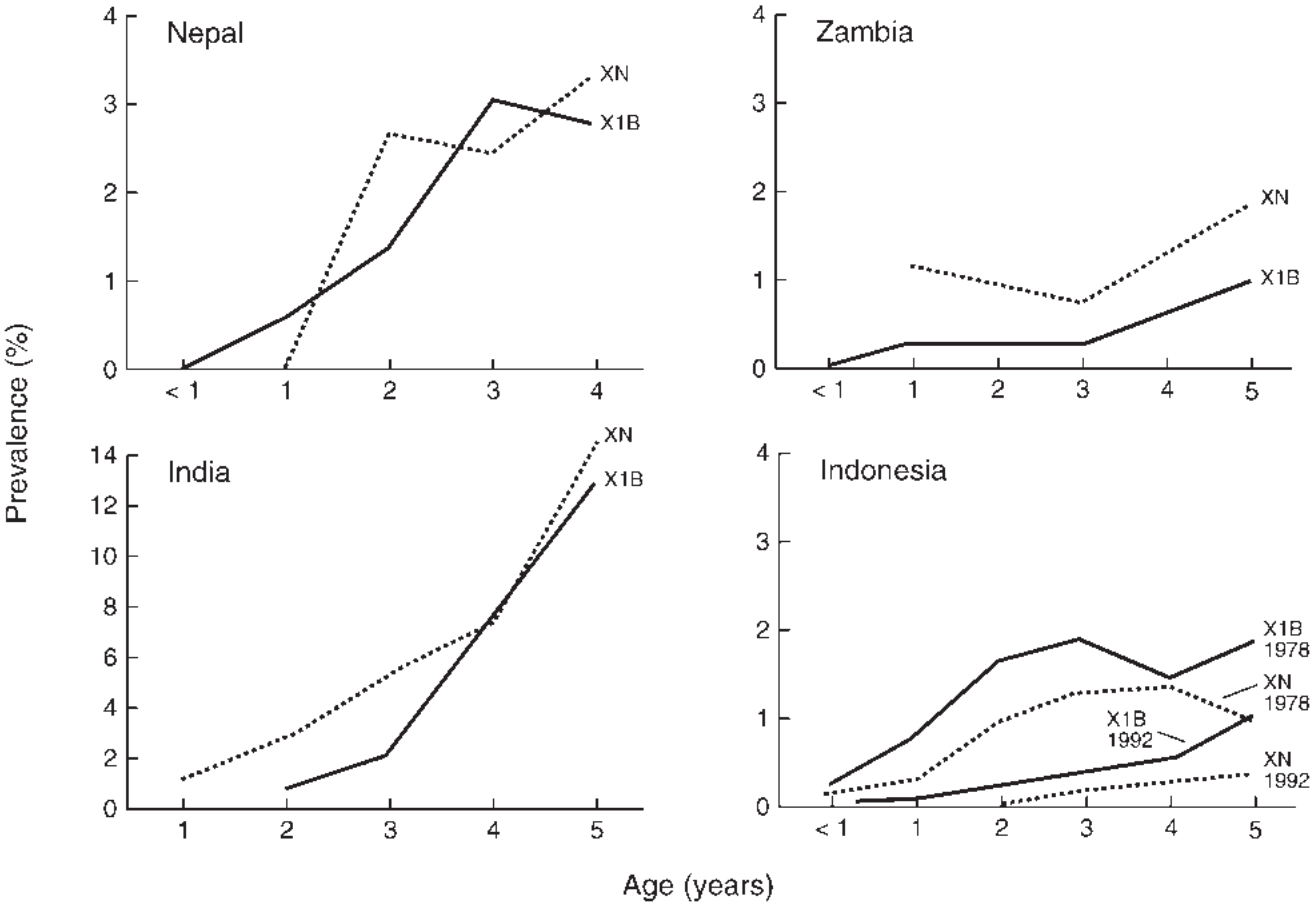


Figure 9.2. Age distribution of mild xerophthalmia in selected countries in Asia and Africa: Nepal, Zambia, India and Indonesia (showing data from both countrywide surveys in 1978–79 and 1992) (Sommer, West, 1996, p 338).

Young children have to rely on others to feed them, and pregnant and lactating women are sometimes especially targeted for food taboos.

Staple foods, mostly cereals but including some starchy roots, provide the bulk of a diet. They generally contain small concentrations of carotenoids but because they are eaten in bulk the amount supplied may be considerable. Rice is the main exception to this rule. It contains no carotenoids in the form in which it is consumed (see Chapter 11). Furthermore, it is often considered to be a complete diet for the young child by the mother. Infants find it easy to eat and satisfying. “Rice-dependent” communities, where rice and little else forms the daily diet, have proved to be especially prone to suffer from VADD. It is ironical that this situation occurs mainly in the moist tropics, where carotene is readily available on every hand. This is truly an instance of nutritional “poverty in the midst of plenty” (McLaren, 1962).

Enquiries about dietary intake should start with identification of the staple food. In this way the traditional “home” of xerophthalmia was found to be the rice-dependent areas of south and east Asia. In India the rice-eating south has been much more vulnerable than the wheat-eating north. On some of the small islands of Indonesia maize is the staple food, and xerophthalmia was virtually confined to communities that consumed white maize (carotene-free) and not seen among those that ate the yellow variety (Oomen, 1961).

Recent studies continue to provide evidence for the close relationship between VAD and the details of dietary intake and cultural practices, especially in young children (Hudelson, Dzikunu, Mensah et al, 1999). Adverse factors are large family size (Kjolhede, Stallings, Dibley et al, 1995), sharing of the food plate with an adult male rather than a female family member (Shankar, West, Gittelsohn et al, 1996) and lack of child care (Gittelsohn, Shankar, West et al, 1998). Feeding practices in infancy can influence subsequent risk for VAD (Gittelsohn, Shankar, West et al, 1997). Preformed vitamin A intake may be more important than carotenoid-containing foods for the protection of children and others (Shankar, West, Gittelsohn et al, 1996).

Breast-feeding

There is abundant evidence that breast-feeding is highly protective against xerophthalmia (Sommer, West, 1996, pp 343-345). This may be partly due to the regular supply of preformed vitamin A in the milk. Another important factor is the lower rate of infections as compared with artificially fed children. A study in south India (Ramakrishnan, Martorell, Latham et al, 1999) showed that while nonbreast-fed children met only 60% of the Indian RDA, those breast-fed met 90% during the second year of life. WHO and UNICEF recommend that all infants be exclusively breast-fed for at least 4 and if possible 6 months and that they continue to be breast-fed up to two years or beyond with the addition of adequate complementary foods from about 6 months of age.

The composition of the weaning diet is obviously of great importance. Infrequent consumption of dark green leaves or yellow fruits was associated with four- to sixfold increase in the risk (odds ratio) of xerophthalmia in one study (see Figure 9.3).

Cultural factors

The customs practised by a community are usually deeply embedded within the fabric of the society. They are not as open to understanding by outsiders as are less complicated characteristics like others considered here. Groups under study often speak a language not readily understood by the investigators,

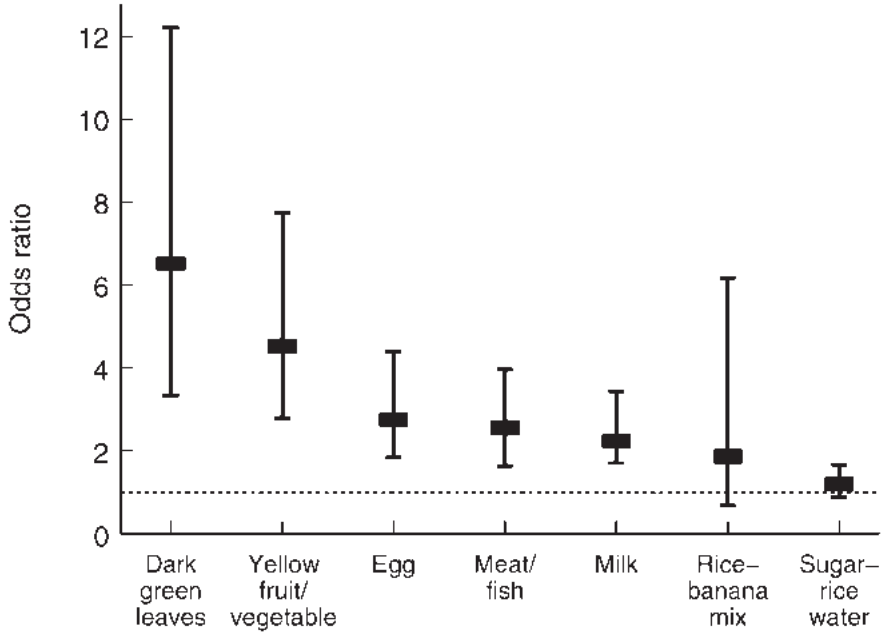


Figure 9.3. Relative risk (case-control odds ratio) (±95% CI) of mild xerophthalmia (vitamin A deficiency) by type of food reportedly consumed by children daily or every other day during their first twelve months of weaning (Mele, West, Kusdiono et al, 1991). Not eating dark green leaves e.g., increases the risk about sixfold.

even if of the same country. For outsiders, and this is what scientists investigating a community inevitably are, understanding the worldview of others is always limited.

More than forty years ago (McLaren, 1956) the apparent protection of the children of a tribal group in an area of south India where keratomalacia was highly endemic was attributable to the custom of child-spacing practised by this group, but not practised by another group among whom corneal xerophthalmia was common. In this rice-dependent state of Orissa, India, the “deposed child” was the target of keratomalacia. This phenomenon had first been described in Ghana (Williams, 1933) where the victim of kwashiorkor was the child who had been abruptly weaned when the mother realised she was pregnant again. It is of considerable interest that in both instances the investigators made their contributions as by-products of their primary work and from long and intimate contact with the people concerned.

Infectious diseases

As was noted earlier, when the topic of the impact on infections of vitamin A deficiency was considered (see Chapter 6), the relationship is complex. Infections also predispose to the development of vitamin A deficiency, as will be considered here.

Systemic infections

Sommer and West (1996, pp 191-220) have reviewed the extensive evidence that a variety of infections are associated with a decline, often dramatic, in serum retinol levels. It is only in recent years that this phenomenon has received the attention it deserves. The fall in

serum retinol is usually greatest in acute and severe infections and there is a return to pre-infection levels during recovery. However, even a normally trivial illness like chicken pox may have a dramatic and prolonged effect in reducing serum retinol, even in children who previously appeared healthy.

The fall in serum retinol appears to be part of what is known as the acute-phase protein reaction (Smith, Goodman, 1971). Serum levels of C-reactive protein and other proteins rise in response to significant infections and other insults. It is thought that these responses may be set in motion by proinflammatory cytokines produced early on by the infection. On the other hand there is evidence (Rosales, Ritter, Zolfaghari et al, 1996) that this results in suppression of the synthesis of transport proteins like albumin and RBP by hepatocytes. These proteins may also be diverted from the plasma into the extra-cellular space, resulting in decreased serum levels. This phenomenon has been studied in pregnant women in relation to their experiencing illness symptoms and night blindness (Christian, West, Khatry et al, 1998a).

An important implication of these observations is that care must be exercised in the interpretation of serum retinol levels, in relation to the assessment of vitamin A status, including their use in relative dose-response tests, in subjects who may be harbouring an infection at the time.

There is every likelihood that vulnerable children frequently become trapped in a cycle of infectious disease and increasing vitamin A deficiency which potentiate the effect of each other. This can lead to serious consequences of morbidity and death.

Diarrhoeal diseases

There is strong evidence from both clinical and field studies that diarrhoeal disease is closely associated with xerophthalmia and with impaired vitamin A status.

Considerable interest was aroused by the report from Peru (Alvarez, Salazar-Lindo, Kohatsu et al, 1995) that diarrhoeal disease, especially that due to rotavirus infection and with accompanying high fever, may lead to as much as a tenfold increase in the urinary excretion of vitamin A (see also Table 10.1). Alvarez and colleagues reported a similar urinary loss of vitamin A in adults with sepsis and pneumonia in the United States and have extended their studies to Bangladesh (Mitra, Alvarez, Stephensen, 1998). In shigellosis urinary retinol loss was proportional to the severity of the disease, and impaired tubular reabsorption of low molecular weight proteins like RBP appeared to be the cause.

Intestinal parasites

Giardia lamblia, *Ascaris lumbricoides*, (roundworm) and *Ankylostoma duodenale* (hookworm) have all been shown to reduce vitamin A absorption, and in some instances to be associated with clinical VAD (Curtale, Pokhrel, Tilden et al, 1995). The impact may be greater on absorption of carotenoids than on absorption of preformed vitamin A. Most of the population becomes infested where sanitation is lacking. Deworming programmes fail to have a long-term impact in the absence of improvements in sanitation and the breaking of the cycle of transmission.

Respiratory diseases

As was the case for diarrhoeal diseases, also for respiratory infections there is good evidence from field and hospital studies that they worsen vitamin A status at all levels. Several

Table 9.1. Corneal ulceration within one month of measles infection by cause and age as a percentage of all ulcers in Tanzanian children (Foster, Sommer, 1987)

Age (years)	Xerophthalmia			Herpes simplex			Traditional eye medicine		
	N	n	%	N	n	%	N	n	%
<2	16	10	62	24	3	12	8	1	12
2–4	12	11	92	10	3	30	5	5	100
5–10	6	3	50	13	4	31	5	2	40
Total	34	24	71	47	10	21	18	8	44

N=total numer of children seen with corneal ulceration due to a specific cause; n=number of children seen with corneal ulceration due to a specific cause which developed within one month of measles infection

possible mechanisms may be at work including loss in urine and impaired absorption of both vitamin A and carotenoids (Sommer, West, 1996, pp 191-220).

Measles and associated conditions

The effect of vitamin A deficiency on the course of measles infection was discussed earlier (see Chapter 6). Decades ago measles was recognized to carry a high mortality risk in children in Africa with severe PEM, and involvement of the eye was occasionally noted. It took many years to provide the evidence that was necessary to bring about general acceptance of the fact that vitamin A deficiency is frequently responsible for the blindness that sometimes followed an attack of measles. There is evidence from other parts of the world, as well as from several regions of Africa, that about one quarter to one half of all cases of corneal blindness in young children are associated with measles (Sommer, West, 1996, pp 191-220).

It is now recognized that often there is not only the combination of VAD and measles but that other factors may also play a part. Sometimes the initial eye involvement is treated by the application of traditional eye medicines that usually serve to increase the damage. Ulceration in the periphery of the cornea, especially in otherwise quiet eyes, is then characteristic (Lewallen, Courtright, 1995). Severe involvement of the cornea may be due to superadded infection with the herpes simplex virus (see Table 9.1). In this series of cases of corneal ulceration treated in hospital there is a higher proportion of cases following measles infection in the group with xerophthalmia as a specific cause than in those in which herpes simplex or traditional eye medicine was the cause. This is perhaps not surprising as severe measles tends to precipitate nutritional deficiency.

This combination has been reported from several parts of Africa but is not known to have the same importance elsewhere. Malaria has also been reported to be a precipitating factor in some places (Yorston, Foster, 1992; Genton,

Al-Yaman, Semba et al, 1994). This merits investigation elsewhere in view of the widespread occurrence of drug-resistant malaria.

HIV/AIDS

Only in recent years has the association between HIV infection and vitamin A status begun to be investigated (see Chapter 5). There is no evidence yet as to whether infection impairs vitamin A status. It is likely that a serious disease like AIDS would have such an effect, and the reverse is certainly so.

Protein-energy malnutrition (PEM)

Impaired growth usually accompanies xerophthalmia and tends to increase *pari passu* with the degree of vitamin A deficiency (see Chapter 7). Severe degrees of PEM, evidenced clinically as marasmus, marasmic-kwashiorkor, or kwashiorkor, are not necessarily associated with eye signs of xerophthalmia, although serum retinol is usually depressed. Corneal xerophthalmia on the other hand is usually accompanied by severe PEM in some form (McLaren, Shirajian, Tchalian et al, 1965).

Much of the association can be explained by dietary habits and disease patterns that at the same time adversely affect both protein energy and vitamin A status. In addition, there is both experimental and clinical evidence that low protein status can impair RBP synthesis and its release from the liver. Therefore the RBP response to a large dose of vitamin A is reduced (Sommer, West, 1996, p 207). Low protein status can impair response to vitamin A therapy and delay recovery of corneal xerophthalmia (Sommer, West, 1996, p 207). However, in chronic protein deficiency growth and metabolic demands are inhibited and when protein is given therapeutically vitamin A demands may be increased and xerophthalmia precipitated.

Season

Fluctuation in the amount of vitamin A and carotene available in the diet throughout the year is well documented (Moore, 1957). Where sources are limited the change is reflected in variations in plasma levels of vitamin A and the seasonal occurrence of signs of vitamin A deficiency. Cattle fed on winter fodder produce milk with a lower vitamin A content than when they receive summer pasture. In developing countries yellow fruits like mangoes and papaya are usually consumed during their quite short seasons and may not be stored. Leafy vegetables have longer, but still limited, seasons. Communities often experience an annual dry, “hungry” season when young children and their mothers tend to suffer most (Oomen, 1969). Failure of the rains or, conversely, excess flooding for several years in succession can lead to serious food shortage or even famine. Under such conditions outbreaks of night blindness and other deficiencies tend to affect a high proportion of the total population.

Among communities that subsist in semi-desertic terrain, as in the deserts of Rajasthan (Desai, Desai, Desai, 1992), a single season with a lack of monsoon rainfall can spell nutritional disaster (Fig 9.4).

Frequently superimposed upon the seasonal pattern described above there is the added effect of seasonal outbreaks of infectious diseases (Oomen, McLaren, Escapini, 1964; McLaren, Shirajian, Tchalian et al, 1965). The most important of these is the group of diarrhoeal diseases, gastro-enteritis, often known locally as “summer diarrhoea” because of its peak season. VAD is usually accompanied by PEM and emerges towards

the end of the diarrhoea season, as the effects of prolonged and repeated attacks of diarrhoea take their toll.

Respiratory infections tend to peak in the winter, which in some developing countries with a continental climate or at high altitude may be bitterly cold.

The most precise documentation of seasonal patterns and VADD was made by Sinha, who resided for two years in the West Bengal village area of Ichag (see Figure 9.5).

Socio-economic status

Economic deprivation was found in the countrywide survey in Indonesia (Sommer, 1982) to correlate closely with vitamin A status. Other indices of health have shown the same thing, as did for example growth of young children in Lebanon (Kanawati, McLaren, 1973).

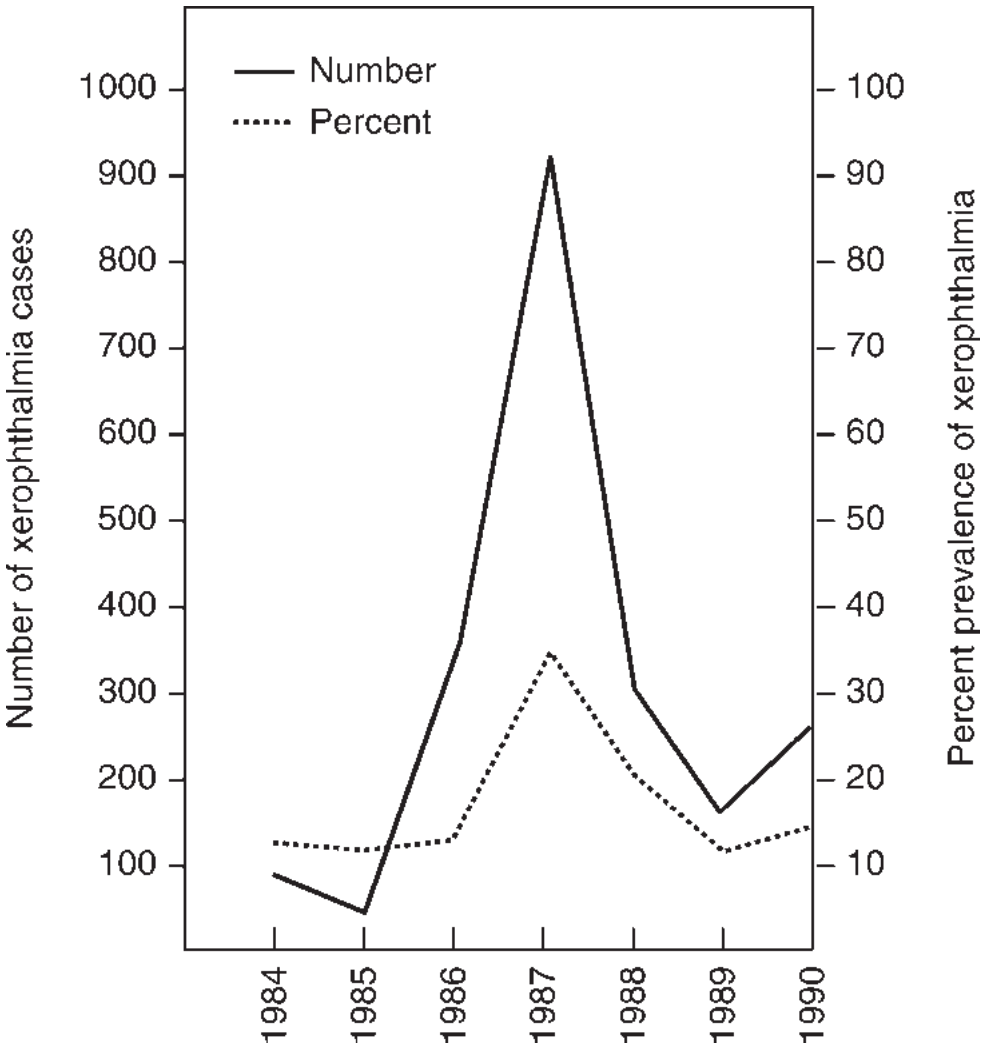


Figure 9.4. The yearly prevalence of xerophthalmia in a population of children aged less than 10 years shows a peak of 34.3% in 1987, the year of severe drought in western Rajasthan (Desai, Desai, Desai, 1992).

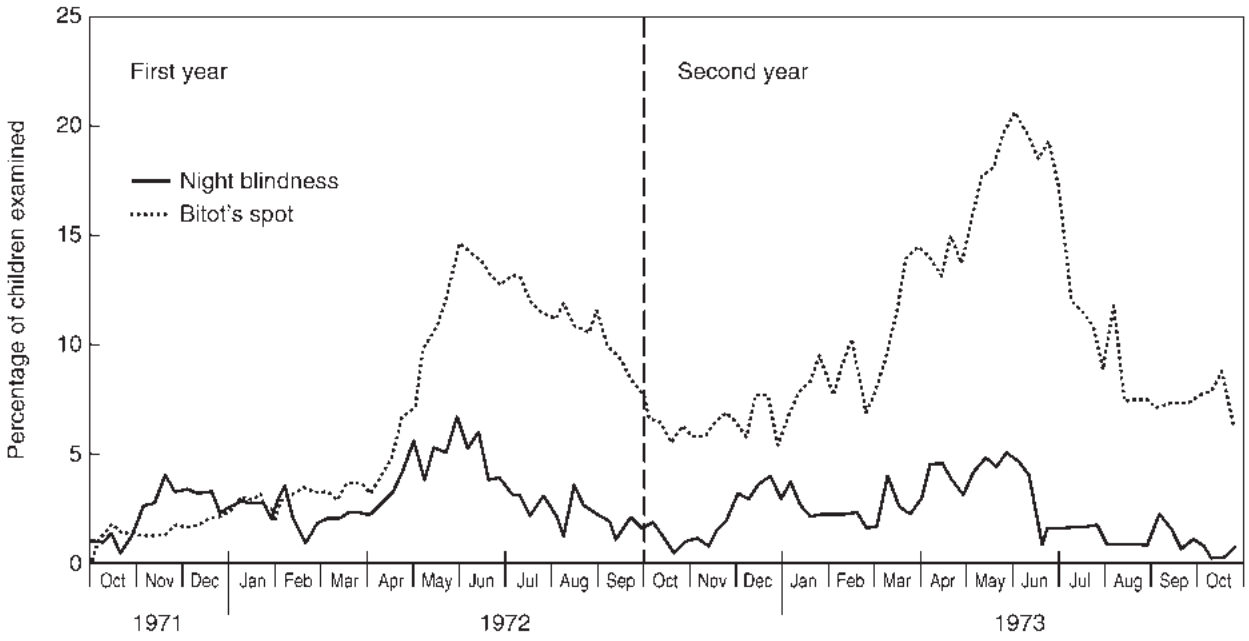


Figure 9.5. Seasonal prevalence of vitamin A deficiency signs, Ichag, 1971–73 (Sinha, Bang, 1976).

Location

Sommer and West (1996, pp 335-354) summarize a large body of evidence that shows that VADD tends to cluster at a variety of levels. This phenomenon applies to provinces, districts, subdistricts, villages and households (IVACG, 1996a). Figure 9.6 shows this clustering in regions in Bangladesh.

In some countries childhood blindness has long been known to be a special feature of certain remote regions. The Luapula Valley in Zambia (Sukwa, Mwandu, Kapui et al, 1988) and the Lower Shire Valley in Malawi (West, Chirambo, Katz et al, 1986) are two especially well studied examples. Vitamin A deficiency has proved to be the major cause of childhood blindness in both places. The underlying reasons have proved to be complex.

As for any disease, the occurrence of clusters may be seen as the probable presence at one time and place of a combination in some degree of intensity of several of the risk

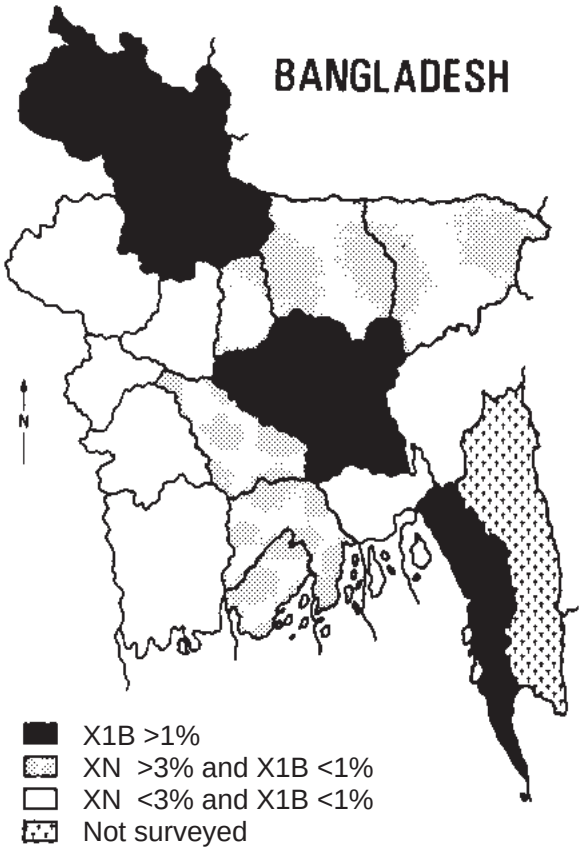


Figure 9.6. Regional clustering of VAD by severity and district in Bangladesh (Cohen, Rahman, Mitra et al, 1987).

factors considered here. There might also be other variables that are as yet unrecognized.

Katz, Zeger, West et al (1993) have studied clustering of xerophthalmia within households and villages in Malawi, Zambia, Indonesia and Nepal. The magnitude of clustering varied between countries and in all places studied it was much greater within households than within villages. Infectious diseases appeared not to explain much of the clustering. Other household factors related to diet and nutrition are suspected to be more important.

Identification of clustering can considerably influence the implementation of an intervention. In Nepal it was estimated that measures to prevent xerophthalmia were 7–34 times more efficient in high-risk, highly populated areas than in remoter communities (West, Pokhrel, Khatry et al, 1992).

WHO (1996) has proposed a number of ecological indicators of VAD assessment to be used in conjunction with biological indicators (see Chapter 4). Most of these have been referred to elsewhere in this chapter.

Sex

Most of the evidence from animals and humans suggests that males are more susceptible to VAD than females. In healthy human adults plasma retinol and RBP are both about 20% higher in males, but the significance of this is unclear (Pilch, 1987; Smith, Goodman, 1971). Night blindness and Bitot's spot are almost uniformly reported to be from 1.2 to 10 times more common in males (Paton, McLaren, 1960; ten Doesschate, 1968; Solon, Popkin, Fernandez et al, 1978). These selected samples were supported by the countrywide survey in Indonesia (Sommer,

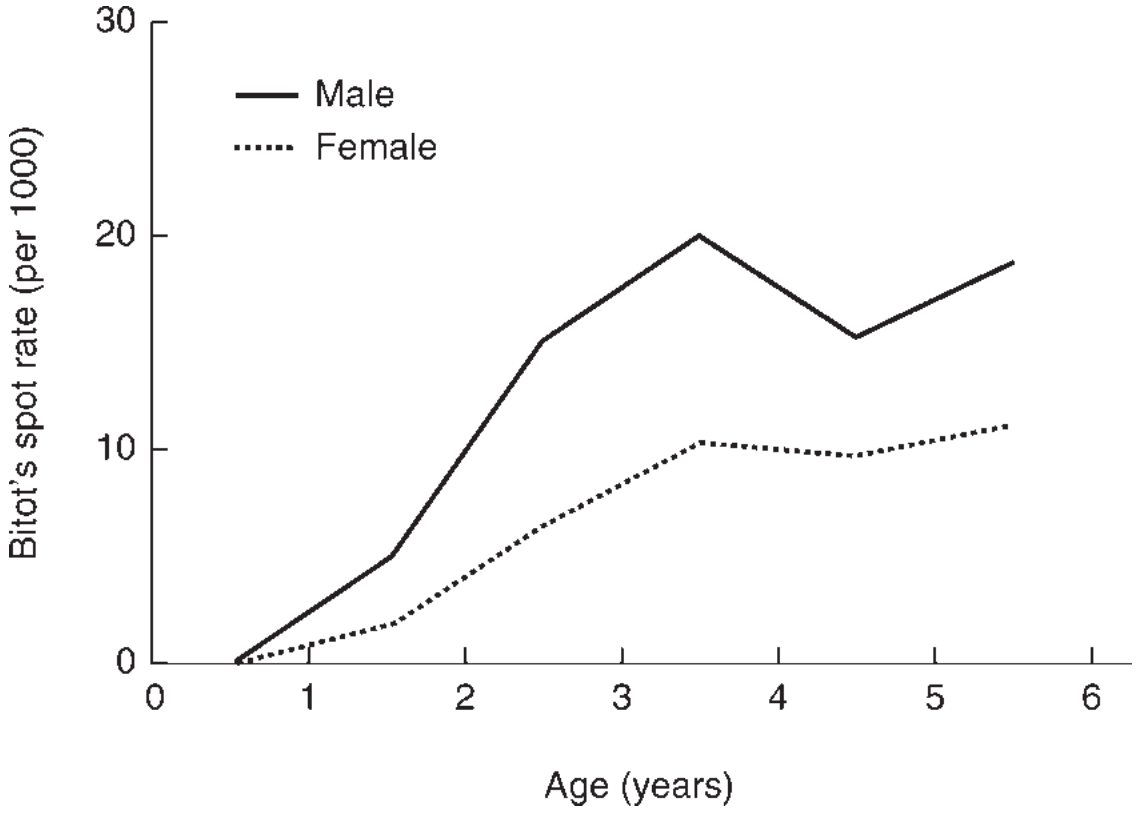


Figure 9.7. The prevalence of Bitot's spots increased significantly (p >0.01) at 1, 2, and 3 years of age. Bitot's spots were more common among boys (12.5/1000) than among girls (7.2/1000) (p <0.001) (Sommer, 1982).

1982) with about twice as many Bitot's spots in males as in females (see Figure 9.7).

Interestingly, no evident sex difference was found in the detailed longitudinal study of Sinha and Bang (1973) (see Figure 9.8). Prevalence of VAD was extremely high in this area. Not only did it reach an astonishing peak of 15 to 16% in both sexes at the beginning of the rainy season; it never fell to a rate below that of the WHO criteria (1% and 0.5%).

Male preponderance was again found in the largest hospital series ever reported (Oomen, 1961) from the eye hospital of Dr Yap Kie Tiong in Jogjakarta, Indonesia (see Figure 9.9).

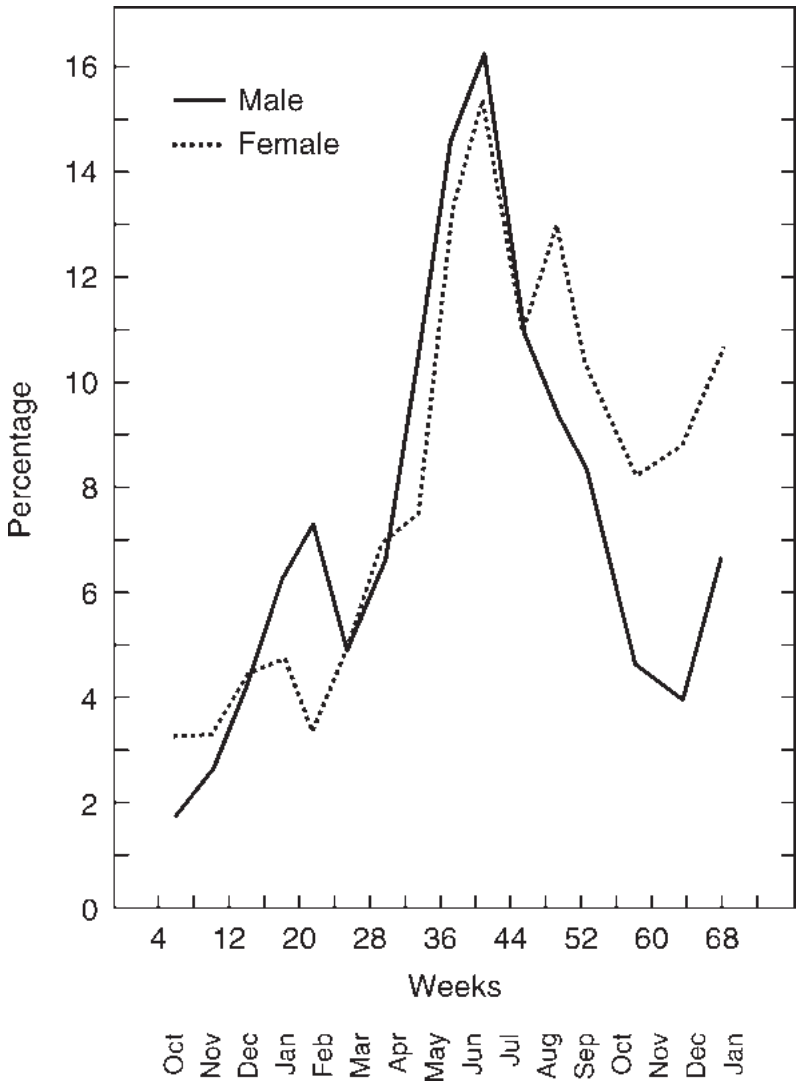


Figure 9.8. Seasonal prevalence of vitamin A deficiency (night blindness and/or Bitot's spot) according to sex, Ichag, 1971–73 (Sinha, Bang, 1973).

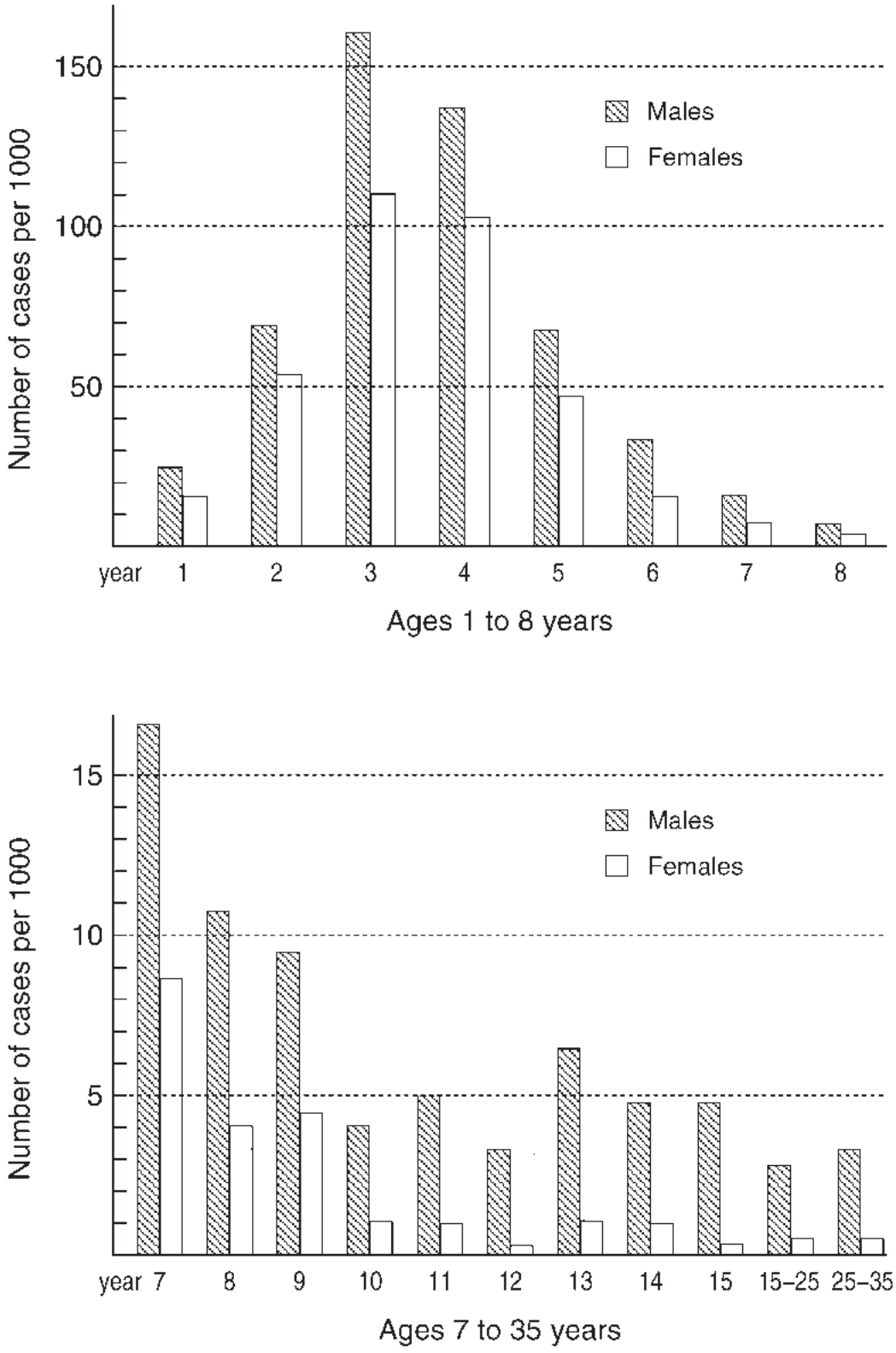


Figure 9.9. Age distribution of 6300 cases of xerophthalmia in the Yap Eye Hospital at Jogjakarta, Indonesia, from 1935 to 1954. The male preponderance throughout increases with age (Oomen, 1961).

Among the 6300 cases the male:female ratio varied with age. It was 1.4:1.0 in the pre-school age period, and 6.0:1.0 at around 10 years of age.

In some cultures in hospital studies male children will tend to appear preferentially because medical attention is more likely to be sought for them.

The Vitamin A Deficiency Disorders (VADD) cycle

This flow diagram (see Figure 9.10) has been devised to give some impression of the kind of combinations of risk factors that conspire together at different stages of the life cycle to predispose to the development and persistence of a VADD problem in a community.

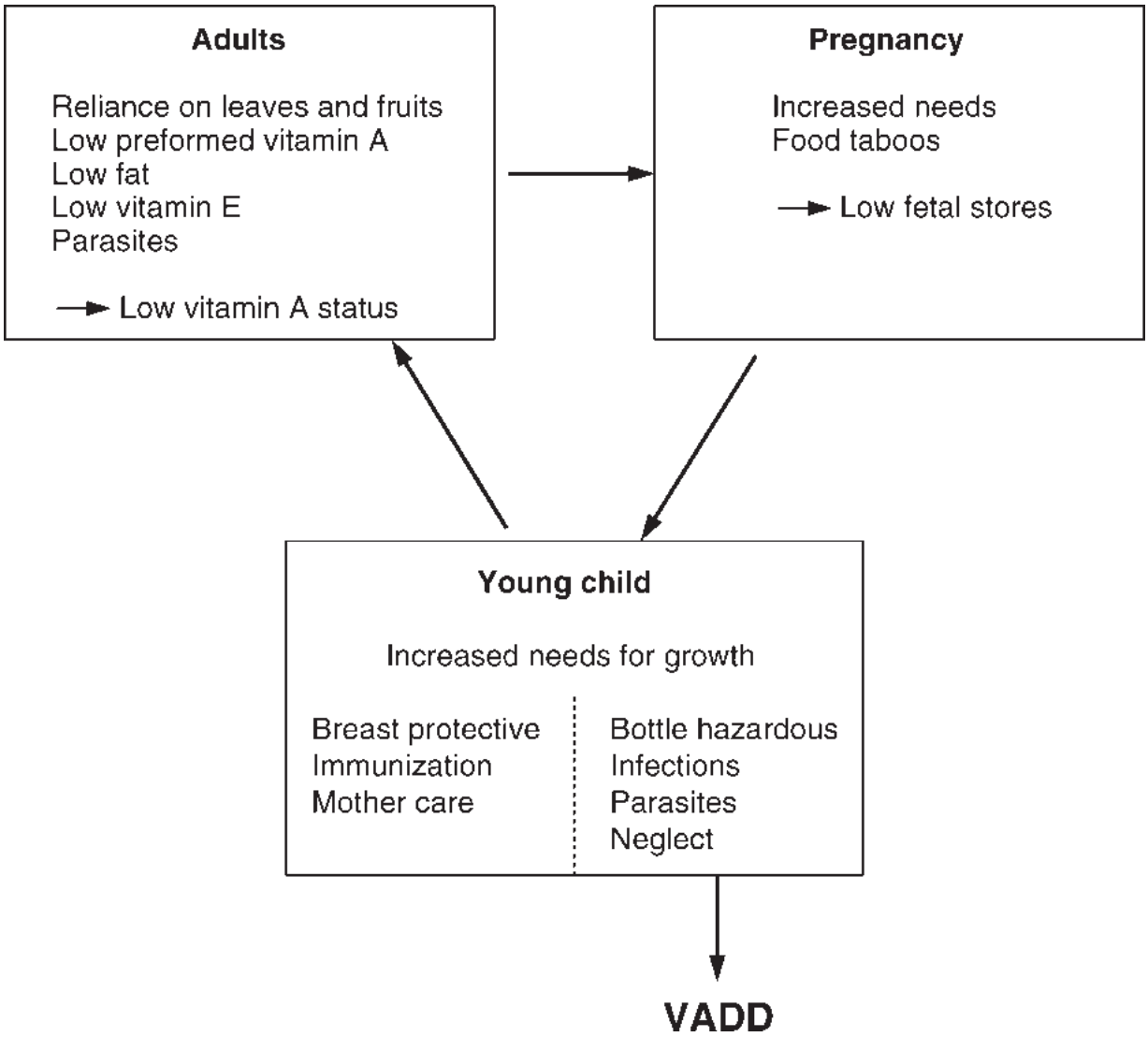


Figure 9.10. The Vitamin A Deficiency Disorders (VADD) cycle.

RETINOIDS IN GENERAL MEDICINE

Introduction

In addition to the various aspects of the global problem of VADD with which this Manual is primarily concerned, there are other closely related and rapidly expanding areas of interest that concern retinol and other retinoids in a human health context. The intent in this chapter is to touch on these and to indicate where more detailed information can be obtained if desired.

In addition to arising from some deficiency in the dietary intake of vitamin A, VADD may also occur as a result of some defect in the the body's physiology or metabolism. This may be called secondary or endogenous, as opposed to exogenous VAD, and attention will be given here to a variety of forms that it can take.

There is an increasing number of diseases in which dosing with vitamin A appears to have a beneficial effect, even in the absence of any deficiency. This area is clearly a part of the vitamin A story and is rightly receiving considerable interest at present.

Vitamin A is one of the vitamins that can lead to disease as the result of not only deficient, but also excessive, intake. This is usually known as hypervitaminosis A. This may arise as the result of prophylactic or therapeutic use of vitamin A as will be discussed later in Chapter 11. Some aspects of the excessive intake of retinol or other retinoids are more appropriately dealt with here.

As mentioned (see p 2) when the chemistry of retinoids was introduced, large numbers of them have been synthesized for use in various diseases, especially those in which epithelial cell differentiation has been disturbed. Some attention will be paid to the topic here.

Finally, many of the carotenoids, irrespective of whether they are provitamins or nonprovitamins, have been shown in recent years to have the potential to play a protective part, illunderstood at present, in a number of common diseases. There is growing evidence that habitual consumption of diets that are rich sources of these carotenoids are associated with low rates of some of these diseases.

Secondary VAD

Table 10.1 summarizes the various different kinds of secondary VAD that have been reported to date, together with the mechanisms. In most instances the degree of vitamin A deficiency is not severe. As little attention is given to VAD or any other nutritional deficiency in general medicine it is usually not until some clinical manifestation, like night blindness or conjunctival xerosis, occurs that deficiency is suspected. Consequently it is

Table 10.1. Secondary or endogenous causes of vitamin A deficiency

Diseases	Mechanisms
Coeliac disease, sprue, obstructive jaundice, ascariasis, giardiasis, partial or total gastrectomy	Impaired absorption of lipids, including vitamin A
Chronic pancreatitis	In some cases secondary to zinc deficiency
Chronic liver disease, especially cirrhosis	Storage impaired by damage to liver cells; zinc deficiency enhances the effect
(1) Severe infection	Loss of RBP in urine
(2) Cystic fibrosis	Excessive faecal loss, unrelated to degree of fat in stools
(3) Enzyme defect	Failure to cleave β-carotene in small intestine
(4) Heterozygotic reduction of plasma RBP	One case reported of keratomalacia due to reduced transport
(5) Mutations in the gene for RBP	Biochemical but not clinical deficiency

(1) Alvarez, De Andres, Yubero et al (1995)
(2) Ahmed, Ellis, Murphy et al (1990)
(3) McLaren, Zekian (1971)
(4) Matsuo, Matsuo, Shirago et al (1988)
(5) Biesalski, Frank, Beck et al (1999)

really not known how common deficiency is in these conditions, especially at the subclinical level. The various causes of malabsorption are by no means uncommon and most cases of secondary VAD have this basis. On the other hand only single cases have so far been reported of the enzyme defect, the transport abnormality and the genetic problem in twins. However, there are indications that genetic predisposition to VAD may be of more importance than has hitherto been generally recognized (Ward, MacGowan, Hornby et al, 2000). Larger doses than normal of vitamin A are required to overcome the metabolic problems present, and where there is impaired fat absorption a water-miscible preparation should be given by mouth and perhaps initially intramuscularly. It is often not appreciated that the usual form of oily vitamin A given intramuscularly stays in the muscle and is not released to any extent into the circulation (McLaren, 1969).

Non-nutritional diseases responsive to vitamin A

Bronchopulmonary dysplasia (BPD) is the leading cause of chronic lung disease in infancy and affects particularly very low birth weight infants. In the United States among the 3.9 million or so births annually approximately 55,000 newborns weigh <1500 g at birth. These are classified as very low birth weight (VLBW). About 49,000 survive hospitalization and about 24% of these suffer from BPD. The histological changes in the lungs that accompany this condition are similar to those described in children dying with xerophthalmia. Most of these infants have serum retinol and RBP in the deficient range. They are also born with low liver reserves. Several trials have shown benefit from dosing with vitamin A. The usual regime is 5000 IU of water-miscible vitamin A intramuscularly three times a week for four weeks (Shenai, 1999). The precise requirement of vitamin A, the

optimal mode and duration of its administration need further investigation.

Experimental model of emphysema

Emphysema is another common lung disease, affecting about 2 million Americans and causing the deaths of 17,000 each year. Most cases are associated with cigarette smoking but about 5% are caused by a deficiency of the enzyme alpha-1-antitrypsin. Emphysema has been induced in rats by intratracheal instillation of a saline solution of elastase which destroys elastin fibres leading to collapse of alveolar walls in the lu“ngs and resulting in emphysema. It has been shown (Massaro, Massaro, 1997) that retinoic acid can reverse the action of elastase and permits the growth of new alveoli. This work holds out hope for the development in future of a treatment in human disease. Chytil, (1992) has reviewed the role of retinoids in lung physiology.

Retinitis pigmentosa and some other retinopathies

As was pointed out in Chapter 4 in addition to VAD several other diseases affecting the retina may also have night blindness as part of their symptomatology. Almost all of these are rare genetic disorders. Retinitis pigmentosa (RP) affects about 1 in 4000 worldwide and eventually leads to total blindness. There are many different genetic forms of the disease. Over many years claims have been made for improved vision with vitamin A and other treatments, but the only large, fully controlled trial with vitamin A over a prolonged period was carried out in the United States (Berson, Rosner, Sandberg et al, 1993). The claim was made that patients receiving vitamin A tended to experience a slowing in the rate of deterioration of their electroretinogram, compared with those who did not. There was no improvement in vision. There was considerable controversy about these findings at the time. The publicity the report received has unfortunately meant that many patients have started self-medication with vitamin A in the absence of any proven treatment. The equivocal nature of the results may have been at least in part due to the mixed nature of RP cases included in the trial. Recent evidence suggests that some forms of RP may be more likely to respond than others (Fariss, Zong-Yi, Millam, 2000). Other rare diseases in which vitamin A has brought about some improvement in rod function are Bassen-Kornzweig Syndrome and Sorsby Fundus Dystrophy (Berson, 1999).This whole complex subject was discussed at greater length recently (McLaren, 2000).

Hypervitaminosis A

Vitamin A toxicity may be either acute or chronic. Acute toxicity usually follows a large single dose of vitamin A and is recognised by symptoms that suggest an acute rise in intracranial pressure – nausea, vomiting and headache. Providing no further vitamin A is given symptoms rapidly subside and there are no permanent ill effects (see Chapter 11). The subjects have usually been young children in a supplementation programme or arctic explorers short of food who have consumed the liver of polar bear, seal or their own sled dogs.

Chronic vitamin A toxicity is uncommon but may be very difficult to diagnose. This is partly because the physician may neglect to take a careful dietary history and routine clinical laboratories are not usually capable of measuring serum retinol. Perhaps even more important is the fact that the symptomatology of hypervitaminosis A may be very varied, affecting a number of different systems and sometimes mimicking other diseases. Common symptoms are headache, vomiting, diplopia, alopecia, dryness of mucus membranes, desquamation, bone and joint

pain, liver damage including cirrhosis, haemorrhages into the skin and coma. If not correctly diagnosed and further excessive vitamin A intake is prevented, death can result.

Congenital malformations

In experimental animals both vitamin A deficiency and toxicity have been known for a long time to induce congenital malformations. In several animal models similar malformations have been regularly produced by synthetic retinoids.

In humans the evidence for the involvement of deficiency of vitamin A is slender, amounting mainly to isolated case reports. A definitive report of a study of the effect of maternal vitamin A or β-carotene supplementation on the incidence of birth defects in Nepalese infants (Khatry, LeClerq, Adhikari et al, 1997) is awaited with interest. In striking contrast, there are numerous reports of high incidences (>20%) of spontaneous abortions and birth defects in the fetuses of women ingesting therapeutic doses of 13-*cis* RA and other retinoids during the first trimester of pregnancy (Ross, 1999).

The most controversial area has been the possible teratogenic effect of excess vitamin A intake during early pregnancy. The present consensus is that in communities where VAD is known to be present vitamin A supplementation throughout pregnancy should not exceed 10,000 IU per day (see also Chapter 11). Where the diet supplies the RDA and more, even this level may be excessive. Even so, a recent Europe-wide study found no association between high vitamin A intake in early pregnancy and major malformations (Mastroiacovo, Mazzone, Addis et al, 1999). 120 infants exposed to more than 50,000 IU daily showed no malformations. Another very large study from Europe (Czeizel, Rockenbauer, 1998) reported on the population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities for 1980–94. This included 35,727 normal infants and 20,830 infants with congenital abnormalities of all kinds. The mothers of 3399 (9.5%) of the normal infants received vitamin A supplements, as did 1642 (7.9%) of those who had infants with malformations. The difference was highly significant ($p<0.001$). It was suggested that this analysis was in favour of low or moderate doses of vitamin A being protective, and not teratogenic. An additional analysis, not pursued by the authors, is to group the infants according to whether or not their mothers received vitamin A and to compare the numbers of those with malformations in each group. Of 5041 receiving vitamin A 1642 (32.5%) had malformations ; of 51,516 not receiving vitamin A 19,188 (37.2%) had malformations. This difference is also highly significant at the $p<0.001$ level and also suggests that vitamin A might be protective. All of the evidence available to date is reassuring with regard to the safety of the use of vitamin A supplements according to generally accepted practice.

Pharmacological use of synthetic retinoids

Dermatology

Natural and synthetic retinoids influence epithelial cell proliferation and differentiation and are increasingly being used in dermatological practice to treat hyperkeratotic disorders. Etretinate and acitretin are very effective in psoriasis. 13-*cis* retinoic acid and retinoyl-β-glucuronide suppress sebum production and are used in acne and acne-related disorders (Figure 10.1). Tretinoin has a beneficial effect in some cases of actinic keratosis, caused by chronic exposure to sunlight. At present only three retinoids are approved for oral administration in many countries; isotretinoin, etretinate, and acitretin. Their teratogenic effects were discussed above.

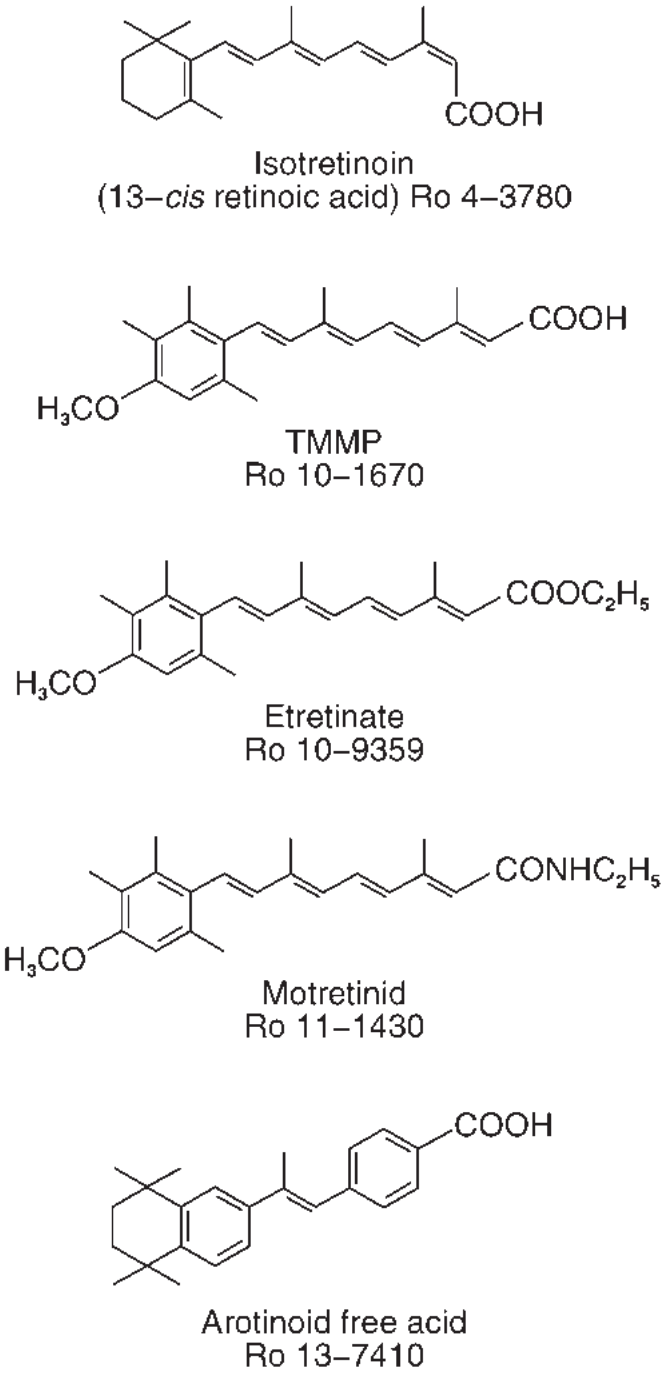


Figure 10.1 Formulae of some highly active synthetic retinoids.

Cancer chemoprevention and treatment

Many studies in experimental animals have shown the ability of both natural and synthetic retinoids to reduce carcinogenesis in organs such as breast, skin, liver, colon, prostate, lung and other sites. Natural retinoids are too toxic for prolonged use in man, but some synthetic retinoids are well tolerated and have the potential for prophylactic use. In recent years attention in this field has turned more to the potential of some carotenoids to act as agents in chemoprevention of cancer and some other conditions (see below).

At present retinoid treatment of cancer is confined to one retinoid and one form of cancer. Acute promyelocytic leukaemia (APL) is a form of leukaemia which is a group of malignant neoplasms affecting the cells of the blood-forming tissues. In APL chromosome 15 is abnormal with a translocation that affects the gene for RAR-alpha (Norum, 1994). All-*trans* RA is highly effective in bringing about remission of the disease, but the exact reasons for the success of this “differentiation therapy” are not fully understood. Unfortunately some patients react badly to high-dose treatment with all-*trans* RA and some become resistant.

Carotenoids and chronic diseases

There is a great deal of evidence to show that serum levels of β-carotene are lower than usual in many disease states. It has usually been assumed that this indication of what might be called low “β-carotene status” is an adverse effect and if corrected should lead to some health benefit. Surprise has been expressed that β-carotene supplementation under some circumstances, such as in cigarette smokers with lung cancer, has been related to deterioration rather than benefit (The α-Tocopherol, β-Carotene Cancer Prevention

Study Group, 1994; Omenn, Goodman, Thornquist et al, 1996). The hypothesis has recently been put forward (Jandacek, 2000) that low β-carotene reflects the result of disease, serves as an indicator of the presence of disease, and is in no way causally related. This might be an explanation of some of the anomalous results that have been obtained, but much more work needs to be done.

On the other hand it has been suggested, on the basis of work in experimental animals, that abnormal oxidation products of β-carotene might be responsible for the adverse effects of large doses mentioned above. Until recently it has always been assumed that hypercarotenosis had no harmful effects but only caused high blood levels and pigmentation of the skin. It seems that there is still a lot to learn about both the safety as well as the benefits of carotenoid ingestion.

The skin

Erythropoietic porphyria is an uncommon disorder of porphyrin metabolism in which a severe dermatosis may affect areas of the skin exposed to sunlight. The regular ingestion of very high levels of β-carotene leads to hypercarotenosis in which the blood level is high and deposition occurs in the skin. This was shown to be protective in this disease and the recommended dose is about 180 mg per day.

The eye

In Chapter 1 it was mentioned that carotenoids commonly accumulate in various tissues and certain carotenoids appear to have a predilection for certain tissues. β-carotene preferentially accumulates in the human ovary but the significance of this is unclear. In primates there is a specific uptake of two nonprovitamin A carotenoids, lutein and zeaxanthin, from the diet by the macula region of the retina. This accounts for the yellow colour of the macula which is the site of maximal visual acuity (Harnois, Samson, Malenfaut et al, 1989; Demmig-Adams, Gilmore, Adams III, 1996).

Age-related macular degeneration (AMD) is a disease, or group of diseases, that causes a devastating loss of vision in the elderly. It is the commonest cause of blindness in the over 75 years age group. The cause is unknown but there is now intensive research going on, both epidemiological and clinical, in which it is hypothesized that diet plays a part. Dietary intake of lutein and zeaxanthin, the two macula pigments, does not appear to be related and there is some evidence that another carotenoid, lycopene, in high concentration in tomatoes, may be protective. Other nutrients such as zinc and some antioxidants may be involved (Taylor, 1999; Schalch, 2000).

Cataract, which consists of a gradual opacification of the lens of the eye leading eventually to blindness, has hitherto only been capable of treatment by removal of the damaged lens. On the basis that oxidative damage may be responsible the possible association of dietary intake of antioxidant nutrients, like the carotenoids, and vitamins C and E is being studied. As in the case of AMD there is no conclusive evidence either way at present (Taylor, 1999). A recent study of monozygotic and dizygotic twins in the UK showed that almost 50% of the variation in severity of nuclear cataract could be explained by heredity, about 38% by age and only 14% by environment (Hammond, Snieder, Spector et al, 2000). In other parts of the world where nutritional deficiencies and other environmental factors are very different, results might also be very different.

Cancer

As was the case with vitamin A (see above) so with the carotenoids the types of cancer that appear to be most closely related to

carotenoid intake are those of epithelial cell origin – lung, head and neck, oesophagus and stomach, colorectal, breast, cervix, and prostate cancers. β-carotene has been most studied, but in the case of prostate cancer lycopene appears to be most promising. Trials of β-carotene supplementation in high-risk groups like asbestos workers and cigarette smokers have given unexpected results that showed either negative or even adverse effects of supplementation. It is generally concluded that supplementation with specific nutrients, especially in large doses, is not advisable. Increased intake of fruit and vegetables provides a variety of antioxidant and other beneficial substances (Orfanos, Braun-Falco, Farber et al, 1981).

Cardiovascular disease

The results of numerous epidemiological studies involving β-carotene and other micronutrients in relation to the occurrence of ischaemic heart disease are conflicting. A possible mechanism – if there is an effect – is reduced oxidation of low density lipoproteins, which seem to play a part in atherogenesis. Other carotenoids rather than β-carotene itself may be effective but a great deal more research is required in this and other related areas touched on here.

CONTROL

Introduction

At the World Summit for Children in 1990 and at the International Conference on Nutrition in 1992 the goal agreed upon was to "virtually eliminate vitamin A deficiency and all its consequences including blindness by the year 2000". 1995 passed without the mid-decade goal of "ensuring that at least 80% of children under 24 months of age receive adequate vitamin A through a combination of strategies" being met. In the first edition it was noted that " With very little time remaining the goal for the year 2000 is most unlikely to be reached in its entirety". At that time the latest figures (Underwood, 1996) suggested that clinical xerophthalmia had diminished to a figure of about 3 million annually. The prevalence of subclinical deficiency was estimated to have risen to about 230 million. Data are still unavailable from a number of countries and these figures are likely to be a considerable underestimation (see Chapter 8).

Among the criticisms that were levelled at the first edition of the Manual one recurred much more frequently than any other. This was that the chapter on Control should be expanded considerably in any later version. This point has been well taken and what follows is meant to satisfy this need.

There are several distinct types of intervention designed to lead to the control of VADD and these will be briefly introduced before they are considered at greater length later.

Treatment

For the forseeable future control of clinical VAD will include treatment of established cases in hospitals and clinics. Central to this process is the administration of high-dose vitamin A preparations as capsules or in other forms. Provision of a regular supply of vitamin A in these forms where they are needed has yet to be achieved in vast areas of the world where VADD are a serious public health problem. In addition to provision on a routine basis of vitamin A preparations, there is widespread need for training in the recognition of the various stages of VADD and in simple measures for preventing recurrence of the problem. Achievement of even these modest goals is an important part of control. In recent years it has been recognized that children with subclinical deficiency have an increased risk of dying. Many of these have severe measles, diarrhoea, and/or PEM and have been recommended in the past for what has been termed "targeted prevention" with vitamin A supplementation. In these circumstances they should be considered to be subjects of treatment which includes vitamin A.

Prophylaxis

Periodic high-dose vitamin A distribution or supplementation in the community may be viewed as a prophylactic extension of treatment in hospital. The form of vitamin A is usually the same – capsules. The aim here is short-term prevention. The measure, like

treatment of the established case, is an emergency one, to be superseded or supplemented by something of longer-term effect as soon as possible.

Control of infections

In recent years the close two-way association between VADD and infectious diseases has become well recognized (see Chapters 6 and 9). This has led to the view that the combination of vitamin A supplementation with immunization has distinct theoretical and practical advantages. In recent years this combined approach has been tested and found not to have important adverse effects. The promotion of National Immunization Days (NIDs) by many countries has provided a novel opportunity for vitamin A supplementation of young children on a large scale. This and other aspects of the subject are discussed further below.

Food fortification

Food fortification has a long history, starting in industrialized countries and spreading more recently to developing countries. It is arguably justified to add nutrients to widely consumed foodstuffs if vulnerable groups are unlikely to obtain their nutrient requirements in any other way. Certain conditions need to be met if a programme is to be successfully sustained. In the past few years this form of VAD control has grown considerably and recently multimicronutrient fortification has been introduced.

Dietary interventions

Dietary interventions of various kinds would seem to be the logical approach to the problem in most circumstances. For those who approach the problem for the first time it is usually a surprise and a shock to learn that the vast majority of young children going blind and dying with vitamin A deficiency do so surrounded by readily available sources of the vitamin. There are indeed “many slips between cup and lip”: problems in getting the vitamin out of the food and into the child. The evidence produced in recent years that the bioavailability of provitamin A carotenoids from fruit and vegetables is usually less than previously thought (Chapter 2) has tended to increase the difficulties of VAD control by means of dietary improvement. As a consequence, greater emphasis is being placed on the value of promoting the much more readily bioavailable preformed vitamin A sources wherever this is feasible.

New plants

Plant breeding and genetic modification: Very recently plant breeding has been applied to the development of a number of high-carotene foods. The genetic modification of rice to provide β-carotene has attracted worldwide attention and requires careful consideration in relation to the control of VADD.

Disaster relief

Disaster relief victims of natural and man-made disasters are especially susceptible to hunger and malnutrition. In these unnatural and emergency situations it is important that those who take on their care are able to provide an adequate and balanced diet.

General considerations

In a recent report by the Administrative Committee on Coordination, Subcommittee on Nutrition (ACC/SCN Consultative Group, 1994) on Controlling Vitamin A Deficiency results were reported of a comparative evaluation of different interventions, drawing on 46 individual evaluations. 25 were of supplementation interventions (vitamin A dosing), 13 of dietary modification, 4 of fortification, 2 of public health, and 2 of breast-feeding. Supplementation is easiest to implement and to

evaluate. It is difficult to make valid comparisons between the different types of intervention. In addition to this it should be realized that some studies have been carried out under carefully controlled research conditions while others have been of a more routine nature. What goes on in strictly routine programmes is very unlikely to be reported in the scientific literature.

In practice choices will need to be made at the local level between the various control measures on offer that have been outlined above. Wherever possible this choice should be made after the situation has been assessed in some way (see Table 8.2). A baseline may then be established and the efficacy of measures introduced evaluated to some extent. Treatment of cases and prophylaxis for vulnerable groups can be instituted almost right away. Other measures take much longer to be implemented fully. However, it should always be possible to start some nutrition and health education *pari passu* with capsule use.

It is important to bear in mind that interventions of this nature are taking place in a situation that can never be fully characterized and that is constantly undergoing change. It should therefore be evident that it will rarely be possible to attribute any benefit observed directly to the intervention. Good examples of this are provided by the improvements that have been documented in Bangladesh, India and Indonesia (see Figure 8.8).

The wider implications of health interventions should be constantly at the back of the minds of those engaged in the control of VADD and others like them. If health is to be a sustainable state (King, 1990) then in circumstances where communities have outgrown, or are in the process of outgrowing, the carrying capacity of their ecosystem the ethical implications of applying effective health interventions like vitamin A supplementation should not be shirked (King, Elliott, 1993).

Finally, it may be helpful to point out that the *SIGHT AND LIFE Newsletter* regularly provides examples of what is going on all around the world with regard to the routine combat of VADD. We hope that some readers may be encouraged by reading about some of these accounts to “have a go” themselves.

Treatment

On several occasions WHO has made recommendations for the treatment of xerophthalmia. The latest schedule is shown in Table 11.1.

Retinyl palmitate in oily solution (as contained in capsules) by mouth is the preferred form and route of administration. The full dosing schedule should be given for all stages of clinical xerophthalmia, not just for the more severe. This should also apply to those eligible for what was termed “targeted prevention” referred to above.

In rare cases where there is persistent vomiting or severe diarrhoea that might prevent ingestion and absorption of the vitamin, intramuscular water-miscible vitamin A may be given. Recently, an aerosol of retinyl palmitate for inhalation has been successfully field tested in preschool children in Ethiopia (Biesalski, Reifen, Fürst et al, 1999). Vitamin A was well absorbed through the respiratory tract and might be preferable to injection.

Because of the known teratogenic effects of large doses of vitamin A, care has to be exercised in the treatment of xerophthalmia in pregnant women. Active corneal lesions should receive the full treatment, but XN and X1B are treated with 10,000 IU (3.0 mg retinol, see Table 11.1) daily for two weeks (WHO, UNICEF, IVACG Task Force, 1988).

Associated medical conditions, such as PEM, measles, diarrhoea, should receive

Table 11.1. Treatment schedule for xerophthalmia for all age groups except women of reproductive age[a] (WHO, UNICEF, IVACG Task Force, 1997)

Timing	Vitamin A dosage[b]
Immediately on diagnosis:	
<6 months of age	50,000 IU
6–12 months of age	100,000 IU
>12 months of age[a]	200,000 IU
Next day	Same age-specific dose[c]
At least 2 weeks later	Same age-specific dose[d]

[a] Caution: Women of reproductive age with night blindness or Bitot's spots should receive daily doses ≤10,000 IU, or weekly doses of ≤25,000 IU. However, all women of reproductive age, whether or not pregnant, who exhibit severe signs of active xerophthalmia (i.e. acute corneal lesions) should be treated as above.
[b] For oral administration, preferably in an oil-based preparation.
[c] The mother or another responsible person can administer the next-day dose at home.
[d] To be administered at a subsequent health-service contact with the indivldual.

appropriate treatment. Frequently, secondary infections of the eye are also present and should receive local and/or systemic treatment.

Prophylaxis

Children under the age of 6 years and pregnant and lactating women constitute the main vulnerable groups in communities where VAD has been identified as a public health problem. They should participate in a supplementation programme wherever this is deemed to be appropriate (see Table 11.2).

A number of studies carried out recently in several developing countries have shed additional light on some aspects of vitamin A supplementation programmes. In Nepal (Christian, Schulze, Stoltzfus et al, 1998) supplementation with weekly dosage of vitamin A or β-carotene at about the RDA level failed to eliminate maternal night blindness in about 60%. It is possible that some other factor besides vitamin A may also play a part, such as deficiency of zinc (see Chapter 7). In a similar study in Bangladesh (Rice, Stoltzfus, de Francisco et al, 1999) vitamin A or β-carotene failed to prevent subclinical vitamin A deficiency in lactating mothers and their infants. Universal distribution of vitamin A capsules in Bangladesh was reviewed (Bloem, Hye, Wijnroks et al, 1995) and was shown to be an effective strategy for reducing night blindness incidence, but weaknesses were revealed in the rural areas of the programme.

There are two main areas of concern with regard to the safety of use of vitamin A prophylaxis (Sommer, West, 1996, pp 394-399). The first of these relates to acute adverse effects in children. Nausea, vomiting and headache have been reported in several per cent of children taking part in large-dose programmes (30, 60 mg vitamin A). Severe vomiting (1.2%) was limited to the children given 60 mg vitamin A; symptoms lasted for almost all no longer than 12–24 hours. In young infants there may also be observed bulging of the anterior fontanelle of the skull, which is still

Table 11.2. High-dose universal-distribution schedule for prevention of vitamin A deficiency (WHO, UNICEF, IVACG Task Force 1997)

Infants <6 months of age[a]	
Non-breast-fed infants	50,000 IU orally
Breast-fed infants whose mothers have not received supplemental vitamin A	50,000 lU orally
Infants 6–12 months of age	100,000 lU orally, every 4–6 months[b]
Children >12 months of age	200,000 IU orally, every 4–6 months[b]
Mothers	200,000 IU orally, within 8 weeks of delivery

[a] Programmes should ensure that infants <6 months of age do not receive the larger dose intended for mothers. It may therefore be preferable to dose infants with a liquid dispenser to avoid possible confusion between capsules of different dosages.

[b] Evidence suggests that vitamin A reserves in deficient individuals can fall below optimal levels 3–6 months following a high dose; however, dosing at 4–6 months intervals should be sufficient to prevent serious consequences of vitamin A deficiency.

open at that age. Like the other symptoms the effect is transient and there is no evidence of after-effects (de Francisco, Chakraboty, Chowdhury et al, 1993). Several controlled trials have been carried out and the consensus is that the extremely slight risk is fully justified in view of the potential benefit to life and health (Florentino, Tanchoco, Ramos et al, 1990). The effects of neonatal vitamin A supplementation was followed over a period of many months and no evidence was found for an adverse effect on development or growth (van Dillen, de Francisco, Wouterina et al, 1996; Humphrey, Agoestina, Juliana et al, 1998).

A very recent review of the vitamin A supplementation of young infants (Humphrey, Rice, 2000) concludes that “a regimen in which mothers are given 200,000 IU vitamin A at delivery and their infants receive four doses of 50,000 IU (i.e. at birth and with their immunization contacts) would allow nearly all infants to enter the second half of infancy in adequate vitamin A status”.

The second issue concerns vitamin A prophylaxis during and shortly after pregnancy. Vitamin A and related compounds in large doses are known to be teratogenic in early pregnancy (Nau, Chahoud, Dencker et al, 1994). High-dose vitamin A given at or shortly after delivery has been shown to raise breast milk levels considerably for a number of months. Frequently this is a rare opportunity to apply prophylaxis. Present recommendations are that the high-dose prophylaxis (200,000 IU) would be given only to breast-feeding women at delivery or during the infertile postpartum period, currently thought to last 4–6 weeks. IVACG (1998a) has issued a statement on “Safe doses of vitamin A during pregnancy and lactation”.

WHO currently recommends that the relatively small increased need for vitamin A during pregnancy be met by diet, or a supplement not exceeding 10,000 IU daily throughout gestation (WHO, UNICEF, IVACG Task Force 1997). This is a second edition of its guide to the use of vitamin A supplements and

prevention of vitamin A deficiency and xerophthalmia. This was followed in by recommendations and a report of a consultation on "Safe vitamin A dosage during pregnancy and lactation" (WHO, 1998).

As mentioned above, supplementation does not address the underlying cause(s) and is an emergency measure. It should be accompanied by dietary counselling. Research studies (Sommer, West, 1996, pp 388-409) have shown that full implementation of the schedule is very efficacious. However, experience has shown that once a supplementation programme has been integrated into the routine primary healthcare system efficiency tends to fall to unacceptably low levels (West, Sommer, 1984). As was noted previously (see Chapter 6) those who are not covered in the first place or who drop out subsequently are usually those in greatest need of the service.

Targeted distribution offers the greatest flexibility and cost-effectiveness and best utilizes existing contacts between health providers and the community. This requires planning, coordination and needs to be sustained if the results are to be better than those that come from passive targeting of only those children who attend health clinics.

Universal distribution requires dosing of all children of the vulnerable age group in a high-risk area, usually on a semi-annual basis. It is particularly this type of distribution that often suffers from low coverage, as mentioned above, due to all kinds of logistic problems.

Prevention and management of infectious diseases

Attention was drawn earlier to the inter-relationships of infections and vitamin A status (see Chapters 6 and 9) that need to be taken into account in the control of VADD. Immunizations that have been developed for certain infectious diseases, especially measles, may be seen as providing an opportunity for a joint approach. The Expanded Programme on Immunization (EPI) of WHO advises that "any EPI contact after the age of six months is appropriate for supplementing the infant or young child (with vitamin A). The visit for measles vaccine at around 9–11 months is especially suitable" (WHO, 1994).

It has been demonstrated on a number of occasions that integration of vitamin A supplementation into a successful immunization programme, like EPI, can result in greatly increased coverage (Karim, Shahjahan, Begum et al, 1996). There is evidence that immunization against measles is already having a favourable impact on reducing corneal blindness in young children. High-dose vitamin A was shown to interfere to some extent with seroconversion to measles (Semba, Munasir, Beeler et al, 1995). Subsequent studies have failed to confirm these initial concerns for measles (Benn, Aaby, Balé et al, 1997), DPT (van Dillen, de Francisco, Overweg-Plandsoen, 1996) or polio (Semba, Muhilal, Mohgaddam et al, 1999). A large study in Ghana, India and Peru with DPT, polio and measles immunization (WHO/CHD Immunization-Linked Vitamin A Supplementation Study Group, 1998) confirmed the safety of the intervention. Unfortunately it failed to find any sustained benefits in terms of vitamin A status beyond the age of 6 months or of infant morbidity.

It was reported at the XIX Meeting of IVACG in Durban (1999) that of the 64 countries that carried out National Immunization Days (NIDs) in 1998 43 or 67% added vitamin A distribution to one of the immunization days. Thirteen more countries were planning to add vitamin A supplementation to their NIDs in 1999. Reports of a number of successful such programmes were presented at the meeting. As NIDs are being phased out since polio-

myelitis is being eradicated there is urgent need for other effective ways to increase vitamin A supplementation coverage, such as intensified measles and other infectious disease immunization campaigns.

Food fortification

In industrialized countries food fortification has long been an accepted strategy for improving micronutrient nutriture, including that of vitamin A. In Denmark, during World War I, an epidemic of xerophthalmia paralleled the substitution of butter by margarine, which lacked vitamin A. Today, margarine, is among those food items most frequently fortified with vitamin A in the world.

Potentially, food fortification offers a direct, effective and sustainable way to correct VAD. However, in practice it has sometimes proved difficult to meet all the necessary criteria. In the early efforts to fortify staple foods technological obstacles had to be overcome. Today such problems are not considered to be limiting factors. Although technologically possible, implementation of food fortification has proved to be a complicated and long lasting process.

A food item to be fortified should be consumed regularly by most of the target population in certain quantities. There should be no risk of overdosing for those consuming the highest quantities. Further criteria are that the vitamin A should not affect the appearance, colour, texture, or organoleptic properties of the food in order to be acceptable to the consumer. The stability of the vitamin A should remain at an acceptable level during processing, transport, storage and cooking.

It is evident that fortification is only possible if processing of the food in question is to some extent centralized. This is also necessary in order for adequate quality control to be provided. Difficulties have to be overcome in relation to such matters as the passing and implementation of food laws and regulations and their continuing enforcement. The long-term financing of all the costs involved in fortification has proved to be a stumbling block in some programmes.

Like supplementation, food fortification may be universal or targeted. In the former, fortification is applied throughout the population. In the latter it applies to specific groups, e.g. to supplementary feeding programmes for pregnant women, welfare recipients, school children, or those receiving complementary foods. Fortification of food aid is an important issue (see p 134). Several research studies (Sommer, West, 1996, pp 410-430) have demonstrated conclusively that fortification can significantly improve vitamin A status of a whole population.

In developing countries many foods have been fortified with vitamin A or imported as fortified products. These include wheat, rice and other grain products, tea, dairy foods (especially dried skim milk), margarine, edible oils, formula foods and speciality items.

Sugar in Latin America and monosodium glutamate (MSG, a popular flavour enhancer) in South-East Asia were the first vehicles for vitamin A fortification to be extensively tested, widely distributed and evaluated for their public health impact (Sommer, West, 1996, pp 411-425). Neither of these food substances is an ideal vehicle from the nutritional point of view, but even so sugar fortification is in operation at the present time in several countries in Central America and is in the process of being introduced in some countries in Latin America and in Africa.

From the early 1970s fortification of white, refined sugar with vitamin A was proceeded within a number of Central and South American countries, with much of the development taking place in Guatemala. Surveillance over a number of years demonstrated a positive

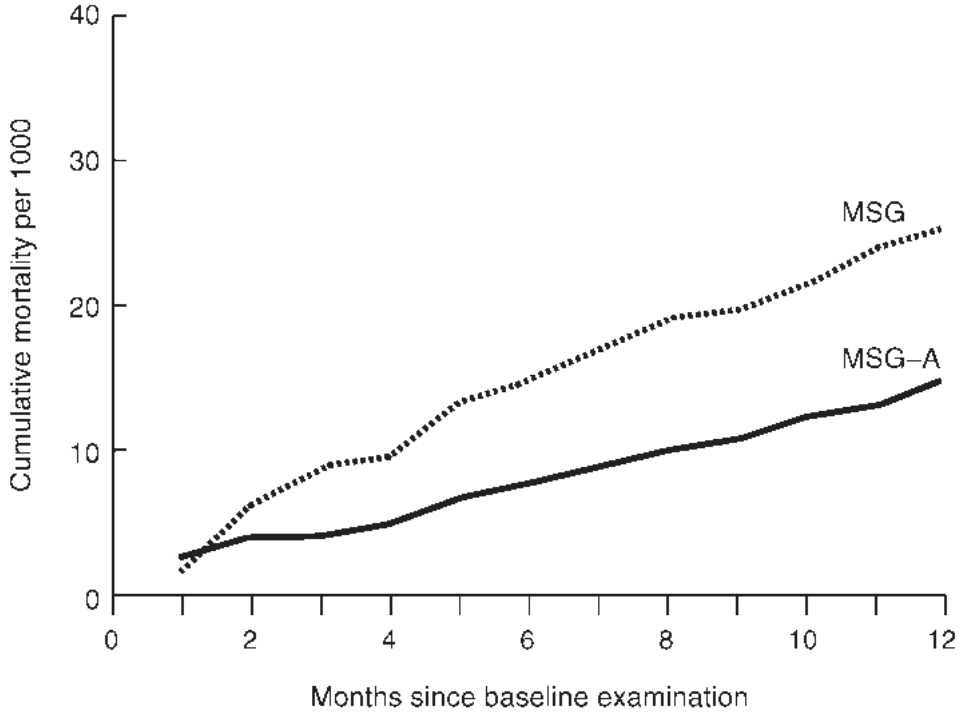


Figure 11.1. Effect of MSG (monosodium glutamate) fortification (Muhilal, Permaesih, Idjradinata et al, 1988).

impact of the programme (Arroyave, 1986). Adverse internal and external circumstances halted the programme for nearly eight years. In 1988, six months after a programme restart there was significant reduction in the number of children with low serum retinol (26% to 10%) and of those with an abnormal RDR (33% to 14%) (Pineda, 1993).

In the 1970s, fortification of MSG was pursued in the Philippines and in Indonesia. The programmes proved to be efficacious in children to raise serum retinol levels, reduce the prevalence of xerophthalmia, improve linear growth, and reduce mortality (Solon, Latham, Guirriec et al, 1985; Muhilal, Permaesih, Idjradinata et al, 1988) (Figure 11.1). After nearly twenty years of research and development in both countries, MSG fortification with vitamin A has not been implemented.

As consequences of the greatly increased interest in micronutrient fortification of foods in developing countries in recent years several trends have become evident that are likely to pose difficulties in the near future. Multimicronutrient fortification is rapidly on the increase. For example, biscuits for primary school children in South Africa are being fortified with iron, iodine, and β-carotene (van Stuijvenberg, Kvalsvig, Faber et al, 1999). The evaluation of the effects of such studies and comparison of results from different studies is a complex matter. In some countries there are now more than 20 different food items fortified with vitamin A at a high level, posing possible problems of excess intake (IVACG, 1999, p 54). One study from Guatemala (Krause, Delisle, Solomons, 1998) showed that poor urban toddlers were obtaining about half their recommended vitamin A intake from fortified foods. Some evidence from Central America suggests that even prolonged fortification of a single food item may not have been sufficient to make a lasting impact on a problem of VAD there (IVACG, 1999, p 54). It is being suggested that fortification and supplementation may both be required.

Despite the limitations and the drawbacks the examples given here have demonstrated that food fortification can be a very powerful strategy. In order for food fortification to be successful, effective collaboration of all parties involved, including scientists, industry, public advocacy groups, legislators and politicians, is essential. Sommer and West (1996, pp 410-430) end their review of the subject "...implementing a national fortification program is a major undertaking that requires sound scientific rationale, industrial capacity, training, advocacy, adequate legislative support, economic viability, community acceptance and long-term sustainability, monitoring and quality control".

Dietary modification

The ACC/SCN Consultative Group (1994) pointed out that there are four types of strategies aimed at achieving the goal of dietary modification:

1) Nutrition education or communication, often using a social marketing approach, to improve practices related to the consumption of available vitamin A-rich food sources.

2) Horticultural interventions (or home food provisioning), e.g. home gardening, that aim to increase availability of vitamin A-rich foods.

3) Economic/food policies affecting availability, price and effective demand of vitamin A-rich foods.

4) Technological advances concerning food preservation, plant breeding etc.

Strategies 2–4 aim to improve the availability of vitamin A-rich foods. Strategy 1 aims to improve their consumption.

The report goes on to review 13 dietary modification evaluations. Nine of these incorporated some education/communication project activities, 7 included home gardening (4 of which were combined with social marketing activities), and one study examined changes in consumption in response to naturally occurring changes in prices and availability of vitamin A-rich foods. Most of the evaluations were of pilot projects or field trials.

It was possible to demonstrate positive change, to varying degrees in several projects. These included improvements in knowledge, attitude and practices (KAP) in North-East Thailand (Smitasiri, Attig, Dhanamitta, 1992) and West Sumatra (Pollard, van der Pasch, 1990). In Bangladesh there was a 40–60% increase in production and consumption by young children of green leafy vegetables and yellow fruits. There was increased awareness of night blindness and prevalence fell (Institute of Nutrition and Food Science, Bangladesh, 1990). However, documentation of the extent of improvement comparable to the documentation of the efficacy of fortification or capsule programmes is lacking.

In general it appears that consumption of more than 40% of total vitamin A in the form of preformed vitamin A is highly protective (FAO/WHO Expert Consultation, 1988).

Dietary interventions are usually targeted towards vulnerable groups – infants, preschool age children and their pregnant and lactating mothers. School age children might also be included if shown to be particularly at risk. The food categories to be promoted will vary with the vulnerable group (Figure 11.2).

Surveys have shown that young children frequently eat less than 15 g of green leafy vegetables in a day. It was shown in Bangladesh (Rahman, Mahalanabis, Islam et al, 1993) that 40 g of dark green leaves would be readily consumed daily if prepared attrac-

tively. This is sufficient to provide the daily requirement of vitamin A in one meal.

Most yellow fruits and some green leaves are usually available only at certain seasons. Village-based food processing and preservation can extend the availability and acceptability (Sommer, West, 1996, pp 355-387).

In dietary interventions, more so than in other kinds, it is of special importance to take into account the efficacy or effectiveness of the intervention. An intervention is effective if it can be shown to have produced the desired effect; in this case to have improved vitamin A nutriture. Consequently, to have increased the vitamin A (usually provitamin A) intake does not satisfy this criterion. Improving vitamin A status (measured by serum retinol or some other method) may be considered to have done so.

Recent work suggests that provitamin A carotenoids, in their natural form in foods, are not as effective as has often been assumed in the past (de Pee, West, Muhilal et al, 1995; Bulux, Quan de Serrano, Giuliano, 1994). The nature of the matrix may be important and some diets of vulnerable communities are low in fat. Dietary fat appears to be more important for the absorption of carotene than for that of preformed vitamin A. More attention is now being given to encouraging even a small increase in preformed vitamin A intake from such readily available sources as eggs and perhaps milk or fortified foods.

Dietary modification can be brought about by a variety of means, and if several can be employed so much the better. In Vietnam (English, Badcock, Giay et al 1997) home garden production and nutrition education were combined and highly significant improve-

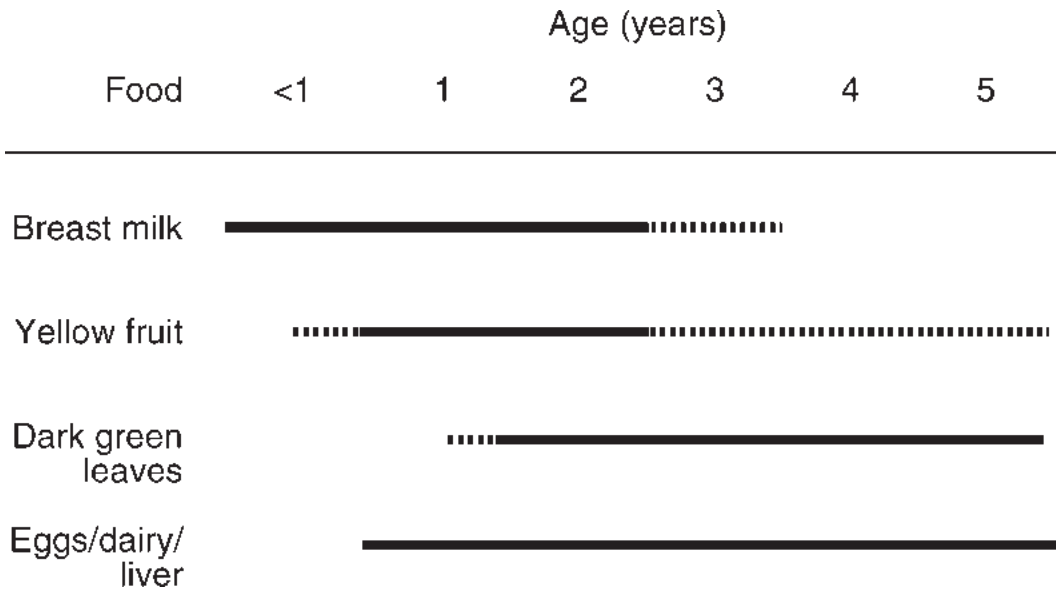


Figure 11.2. Composite profile of age-specific protection against xerophthalmia and low serum retinol levels conferred by dietary intakes of selected types of foods. Solid bar denotes ages for which epidemiologic evidence is strong. Dashed bar denotes ages at which some evidence of protection exists for a food (Sommer, West, 1996, pp 130-137).

ment in the incidence of diarrhoeal disease and respiratory infections (but see p 77) resulted. Social marketing of vitamin A -rich foods in Thailand to bring about behavioural change, with especial emphasis of use of the readily available ivy gourd, could be readily applied in the region (Smitasiri, Attig, Valyasevi et al 1993). The XIX Meeting of IVACG (see p 55) reviewed a series of papers presented at the meeting on experiences with "communicating micronutrients to the public".

With recent concerns being expressed about the bioavailability of provitamin A carotenoids (see Chapter 2) renewed interest has been aroused in red palm oil as a potent source of vitamin A (Mahapatra, Manorama, 1997; Solomons, 1998). There is urgent need for more thorough assessment of the value of this and other palm products as underexploited sources of the vitamin.

General issues

Multiple strategies are now being increasingly employed and "sorting out the wood from the trees" is becoming increasingly difficult. The Proceedings of the XIX Meeting of IVACG once again has come to the rescue (IVACG 1999, pp 57-61) with a three-page table and accompanying text.

Cost-effectiveness has always been an important issue but is only recently being addressed by well-designed and executed studies. Targeting vitamin A supplements to high-risk children was found not to be an efficient use of resources. It was concluded that, like immunization, vitamin A should be provided to all preschool age children in developing countries (Loevinsohn, Sutter, Costales, 1997). In a comparison of nutrition education and vitamin A supplementation it was found that the latter was quicker and cheaper, but education had longer lasting effects. It was concluded that both were needed for a comprehensive national programme (Pant, Pokharel, Curtale et al, 1996). Under ideal circumstances vitamin A fortification can be cheaper and more efficient than either capsule distribution or home food production with nutrition education (Phillips, Sanghvi, Suaréz, 1996).

Plant breeding and genetic modification

Traditional methods of plant breeding have been applied quite extensively to the enhancement of the β-carotene content of foods since this topic was raised in the first edition of the Manual (Bouis, 1996). These include higher-yielding varieties of amaranth in India, tomatoes with a high β-carotene content in Taiwan, high-carotene sweet potato in Africa, and red canola oil with a unique blend of several carotenoids (Reddy, 2000).

Genetically modified (GM) rice, aimed at improving the supply of iron and vitamin A in the human diet was first announced at the XVI International Botanical Congress on 3 August 1999. The definitive publication of this ground-breaking research was made a few months later (Ye, Al-Babali, Klöti et al, 2000). The research involved collaboration between the Institute for Plant Sciences of the Swiss Federal Institute of Technology, Zurich, Switzerland and the Center for Applied Biosciences of the University of Freiburg, Freiburg, Germany.

Rice *(Oryza sativa)* is usually milled to remove the oil-rich aleurone layer that turns rancid upon storage especially in tropical areas. This layer contains some β-carotene and some other nutrients which do not occur in the remaining edible portion of the grain, the endosperm. In this research recombinant DNA technology, with a combination of transgenes, was used to enable biosynthesis of provitamin A in the endosperm to occur. Immature rice endosperm can synthesize

the early intermediate geranylgeranyldiphosphate (GGPP – see also Chapter 1). This can be used to produce the uncoloured carotene phytoene by expressing the enzyme phytoene synthase in rice endosperm. For the synthetic pathway to be completed towards β-carotene three additional plant enzymes are required – phytoene desaturase and ζ-carotene desaturase, each of which catalyzes the introduction of two double bonds, and lycopene β-cyclase, encoded by the *lcy* gene. Four transgenes were required; all were obtained from daffodil *(Narcissus pseudonarcissus)*.

In most cases the transformed endosperms of the rice were yellow in colour, indicating the formation of carotenoid. There was some variation in different seed lines concerning the carotenoids produced. Often this was only β-carotene, but occasionally lutein and zeaxanthin were also detected. From the results so far it seems that the yield of provitamin A will be at least 2 μg/g of rice as consumed. The authors suggest that a daily intake of 300 g of rice will provide 100 μg RE. This would be equivalent to about 25% of the adult's RDA. This assumes that the bioavailability of β-carotene would be $^1/_6$ that of retinol, but this remains to be proved.

There are still many technological, sociological and other considerations to be addressed before there is any possibility of GM rice becoming the staple food of hundreds of millions of the world's poor. A diet of ordinary rice adequately supplemented with vegetables and fruits is capable of providing a balanced supply of known nutrients and other health-promoting substances. The overriding goal of attaining full nutrition for all by practical and realistic means should not be allowed to be obscured by any "quick fix" approaches.

Disaster relief

Over the past two decades the number of refugees registered by the UN High Commission has risen steadily and now approximates 20 million. In addition, the estimated number of people forced from their homes but not from their countries is now nearly 30 million. About half of these groups are children under the age of 15 years and are especially vulnerable. Experience has shown that they are not only particularly susceptible to infections and protein-energy malnutrition but also to vitamin A and other vitamin deficiencies (McLaren, 1987).

If agencies involved in disaster relief operations are made aware of the problem, there is no reason why it should not be averted. It should be ensured that the vitamin A content of food rations supplied is adequate. In particular skim milk must be fortified, as it usually is, with vitamin A. The most readily implemented single measure is the distribution of vitamin A capsules to all young children. Mothers should be encouraged to breast-feed their infants. In more settled circumstances the growing of green leaves and yellow fruits should be encouraged.

Some country experiences

These are a few examples of the reports that appear regularly in *SIGHT AND LIFE Newsletter*. They are not meant to be representative in any way but were chosen because they were seen to illustrate the variety of approaches to the problem of the control of VADD, some of the difficulties involved and the attempts to overcome these. Earlier on most of the accounts related to the problem of xerophthalmia and nutritional blindness. Although severe VAD has not yet been eliminated along with most other severe forms of nutritional deficiency it has diminished considerably worldwide. The emphasis now needs

to be placed on the much more widespread subclinical VAD that threatens survival and general health. If this is successfully tackled xerophthalmia will automatically decrease. The reports have been condensed and it is hoped that readers will turn to the full accounts for further information and also be encouraged to follow other reports as they appear in future Newsletters.

Figure 11.4. Drumstick *(Moringa oleifera)*: its lleaves, flowers and fruits are edible. It was identified as a promising crop as it requires little care, is easy to grow and yields for many years.

Involving programme participants in the evaluation and replanning of how to reach the "hard-to-reach group (HRG)" (Shamim, Ahmed, Costa et al, 1999)

This group found that the usual models advocated for homestead production of vegetables and fruits, with 3–5 varieties of vegetables grown in raised beds in small plots were inappropriate for local conditions in Debidwar, Bangladesh. Moreover, local people's opinion in planning and implementing is usually not sought. Consequently a participatory rural appraisal (PRA) method was used. Focus group discussions, interviews, conversations, observations and discussions with key informants were held. Indigenous technical knowledge about such matters as traditional food plants, the growing of plants in the homestead including use of the roof and utilization of by-products was collected. Such contacts, experience and knowledge formed the basis for future programmes. This kind of confidence-building, partnership-forming approach needs to be widely encouraged.

Figure 11.3. Production of beans on the roof of a hut. Year-round production of such crops could contribute to the nutrition.

Figure 11.5. Snacks prepared with inclusion of leaf protein.

Leaf concentrate: a good food source to control vitamin A deficiency (Joshi 1998)

Leaf protein, as it was originally called by its founder N.W. Pirie in the 1950s, was developed as an attempt to combat what was then termed the "protein crisis". For various reasons it became evident eventually that protein deficiency was not the main cause of malnutrition in young children but a global lack of nutrients and energy sources combined with infections. As "leaf concentrate" the green curd prepared from species of leaf not commonly eaten as human food is making a comeback as a rich source of provitamin A carotenoids. This account from India gives details of the equipment needed, the way the material may be prepared at home, either to feed domestic animals or to incorporate into attractive meals that people are already used to. A table is given showing typical amounts in leaf concentrate of a variety of nutrients (β-carotene is 50–180 mg/100 g of dry matter). Several studies in India and elsewhere have shown that β-carotene in leaf concentrate is well absorbed. Initial cost of the equipment and difficulty in obtaining spare parts are often quoted as barriers to long-term operation of the programme.

Figure 11.6. Camel market in Mao, Kanem Province.

Figure 11.7. A nomadic family south of N'Djaména.

Towards improved health services for nomadic people in Chad: the challenge of combining human and animal health (Zinsstag, 2000)

About 10% of the 7 million population of this desert African country are nomads. They have virtually no access to the public health system and their health status is unknown. Close contact with animals means they are vulnerable to zoonotic diseases. Their diet consists of more than 60% of milk and milk products, which should include ample vitamin A. Because man here is so dependent upon his domestic animals a joint veterinary and human medicine approach is being adopted with regard to control of diseases like tuberculosis and vaccination programmes which until now have been especially lacking on the human side.

In time of drought, always imminent in such desertic environments, livestock and humans are equally badly hit and it is difficult to see how their vitamin A and other nutritional needs can be met under these circumstances. The next experience may have an answer.

Vitamin A in milk from cows, goats and sheep related to β-carotene in fodder – Timbuktu, Mali (Jacks, 1997)

In northern Mali repeated drought over the last two decades has decimated the herds by up to 80%. Vitamin A deficiency is common in mothers and children during the dry summer season. It was found that the β-carotene content in animal milk was closely related to that of the fodder. Goat's milk has the highest β-carotene content and is customar-

Figure 11.8. Milking a goat.

ily used as a weaning food by the people. Woody fodder species suitable for the dry climate, such as *Maerua crassifolia*, *Salvadora persica*, and *Balanites aegyptica* are being promoted. The leaves of *Maerua crassifolia* have good nutritional value even as human food. It is important that innovative experiences like this should be disseminated to those in similar circumstances and that research not be unnecessarily duplicated.

Figure 11.9. Camel browsing on an Acacia tortilis and the Tuareg owner.

A food-based strategy to prevent vitamin A deficiency – the Vietnam experience. (Vuong, 2000)

It was decided to promote the use of the bright red Gac fruit *(Momordica Cochinchinnensis Spreng)*, rich in β-carotene, as a practical contribution towards the control of VADD in northern Vietnam. The seed membrane and pulp of the Gac fruit contains significant concentrations of oil, which facilitates absorption. The Gac vine commonly grows at the entrance to rural homes. Traditionally, Gac seed and pulp are mixed with cooked rice to give colour and flavour. Studies on the local children showed significant increases in serum retinol levels in groups receiving the fruit. The local preparation method of the fruit in meals caused minimal loss of β-carotene. At present the fruit is underutilized because of seasonality and lack of awareness of its value, but efforts are being made to overcome this. Such reports, like this and the one that follows, should encourage others to look for the possibilities of local answers to their VAD problems.

Figure 11.10. Schoolchildren with SIGHT AND LIFE posters.

Promotion of vitamin A-rich foods in Pohnpei, Federated States of Micronesia (Englberger, 1999)

In this case a once highly treasured traditional weaning food has been revived. It came as rather a shock when these island people learned in 1994 through a child health survey that 51% of their children had low serum levels of retinol. In 1998 another surprise, but this time a pleasant one, was the finding that the local karat banana *(Musa Troglodytarum)* is rich in vitamin A – 160 RE/100 g. A second local banana, the mangat, was found to contain 96 RE/100 g. The writer goes on to describe the programme undertaken to promote banana consumption by competitions for growing, making posters, cooking demonstrations, the use of the media and the culmination in a vitamin A campaign week.

Figure 11.11. Karat bananas fruiting straight into the air.

Figure 11.12. Schoolchildren bringing karat plants.

Surveillance and intervention of vitamin A status of pre-school children in Fu Ping county, Baoding region, China (Wang, Zhan, Hua, et al, 2000)

Fu Ping county is a remote and mountainous area where most of the over 200,000 inhabitants are farmers. Large numbers of men have left to find work in the cities, leaving women and children behind. Water is scarce and the main crops are maize, oats, potatoes and Chinese dates. VADD and iodine deficiency disorders (IDD) are common. Serum retinol levels were measured in young children in both Fu Ping and Lai Yuan counties. In both areas a severe subclinical vitamin A deficiency problem was revealed for which preventive measures are being planned.

It is very gratifying to learn that now in the most populous country on earth a widespread VAD problem is being suspected, assessment of vitamin A status is being carried out and control measures are being planned.

Figure 11.13. Children in the village Long Quan Guan waiting to receive a vitamin A supplement.

Figure 11.14. Carpet weaving.

Figure 11.15. Nursing mother weaving carpet.

Elimination of vitamin A deficiency through active surveillance of carpet children north-east of Kathmandu (Chalise, 1998)

This report describes the work of Helpless Rehabilitation Society (HRS) among the carpet factory workers, children <10 years of age and pregnant and lactating women in the north-east area of the Kathmandu Valley. Much of the country is now covered by the national vitamin A programme but these economic migrants from the ecological destruction in the hills miss out. After capsule distribution and promotion of improved diet xerophthalmia of all degrees in children fell from 13% to 1.5%. Factory owners proved very cooperative and others are asking for similar help for the elimination of a problem that was clearly impeding the efficiency of their workers. One is left wondering about the dilemma of the immorality of child labour and with their original home environments destroyed by corporate greed what the alternative might be.

Further Reading

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Glossary

Acute-phase reaction (or response) (APR) A generalized reaction of the body to acute infection. Certain proteins, positive acute-phase proteins, increase in concentration in plasma. Other proteins, which include retinol-binding protein (RBP) and transferrin, decrease in concentration in plasma and are known as negative acute-phase proteins.

Bioavailability In general the term refers to the degree to which any substance in the diet is available after ingestion for utilization by the body. In the present context, bioavailability relates to the degree to which dietary provitamin A carotenoids are utilized after ingestion.

Bitot's spot Heaping up of keratinized cells on bulbar conjunctiva. An advanced stage of conjunctival xerosis. The first description was attributed to a French physician of that name in the middle of the 19th century. Not all Bitot's spots are attributable to deficiency of vitamin A.

Carotenoids Yellow, orange, or red pigments occurring in nature. About 600 have been identified, of which less than 10 have provitamin A activity. They all have a basic C40 skeleton which is made up from successive additions of C5 isoprene units.

Chlorophyll A green pigment that imparts its colour to the leaves of plants and many vegetables. It is mainly responsible for the process of photosynthesis, whereby in the presence of sunlight carbon dioxide from the atmosphere and water are converted to carbohydrate, and oxygen is given off.

Chloroplast This structure in leaves contains the chlorophyll and carotenoids, which act as catalysts in the process of photosynthesis.

Cis/*trans* In olefin chemistry *cis*/*trans* is replaced by E/Z for the unequivocal description of a double-bond stereoisomer. For practical reasons this was not implemented in this Manual; see isomerization.

Conjunctival impression cytology (CIC) A technique whereby a cellulose acetate strip is applied gently to the surface of the bulbar conjunctiva of the eye. When the strip is removed the superficial layer of epithelial cells adheres to it. The strip with cells is processed and stained. The histological appearances are studied for evidence of early keratinization, suggestive of subclinical VAD.

Dark adaptation The ability of the rod cells of the retina of the eye to take over the function of vision under conditions of low illumination. This function is heavily dependent on an adequate vitamin A status.

Hypervitaminosis A Excessive vitamin A status in which there are excessive concentration of retinol in plasma and symptoms and signs of toxicity.

Hypovitaminosis A This is synonymous with VAD.

Isomerization A change in the spatial orientation of a chemical molecule without any change in the basic chemical structure.

Keratinization A process characteristic of epithelial tissues. The tissues undergo a complex series of hardening and drying changes. Keratinization is normal in such tissues as skin, but is abnormal in many other epithelial tissues, including conjunctiva, cornea, and epithelial linings of lungs, gut, urinary tract etc. It is synonymous with the term xerosis.

Keratomalacia This term is applied to changes in the cornea in severe VAD. In addition to keratinization (see above) of the corneal epithelium there is softening of the stroma or underlying tissue.

Meta-analysis A statistical analysis applied to the data of a group of studies which all conform to a set of criteria to ensure similarity as far as possible. The larger numbers obtained in this way provide greater statistical power.

Night blindness The subjective sensation of difficulty to identify objects under conditions of low illumination.

Provitamin A Carotenoids, like β-carotene, capable of being converted to vitamin A in the animal body.

Recommended Dietary Allowance or Intake (RDA, RDI) This relates to the level of an essential nutrient considered to be adequate to meet the known nutritional needs of practically all healthy persons in a population.

Relative dose response (RDR) A biochemical test designed to assess vitamin A status by the indirect estimate of liver vitamin A stores.

Retinoids A class of compounds consisting of four isoprenoid units joined together in a head-to-tail manner and customarily containing five conjugated double bonds. The term vitamin A is used as a generic descriptor for retinoids exhibiting qualitatively the biological activity of retinol.

Retinol equivalent (RE) This term was created to express both preformed vitamin A and provitamin A carotenoid equivalents as a single nutritive value. One mg RE is equal to 1 mg of all-*trans* retinol, or to 6 mg of all-*trans* β-carotene, or to 12 mg of other provitamin A carotenoids.

Xerophthalmia A term that applies to all clinical stages of eye disease attributable to VAD.

Xerosis See keratinization.

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