VITAMIN A DEFICIENCY

Health, Survival, and Vision

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To our wives, Jill and Marie,
but especially to our children,
Charles and Marni Sommer and Natalie West,
who have put up with peripatetic lives and fathers,

—and to all those children of the developing world
who suffered needlessly until we recognized
the source of their tragedy, and
who suffer still because governments and
the international community have yet
to respond in full measure

Preface

My first book on this subject, *Nutritional Blindness*,[1] was published in 1982 As the title suggests, it concentrated on the ocular complications of vitamin A deficiency, the primary clinical concern of half a century The next year colleagues and I published the first of a series of studies[2,3] demonstrating that vitamin A deficiency is a public menace of multiple dimensions, including reduced resistance to severe infection and markedly increased childhood mortality The ocular changes, xerophthalmia and keratomalacia, are relatively late complications of more severe deficiency among children who manage to survive These data, it turns out, merely confirm conclusions drawn by investigators over 60 years ago[4-6] but since ignored, forgotten or considered irrelevant to the problems of today's impoverished populations

It now seems clear that improving the vitamin A status of deficient children would not only prevent 5 million to 10 million cases of xerophthalmia and half a million children from going blind each year,[1,7,8] but save a million or more lives annually as well [9] In the past two years, specialized agencies of the United Nations (WHO, UNICEF, FAO) have raised their level of concern and commitment The UNICEF Governing Board, the World Health Assembly,[10] the World Summit for Children and the International Conference on Nutrition have all called for the control or elimination of vitamin A deficiency by the year 2000 [11]

This book therefore reframes the issue of vitamin A deficiency in its broader context of child health and survival, taking up the story where the original volume left off A great deal more attention is paid to the systemic complications of vitamin A deficiency and the data from which they are derived

Ocular manifestations are still dealt with in considerable depth and the information and references updated where warranted Pertinent material from the original work is retained in the present volume, either intact or rewritten depending upon the amount and substance of intervening reports But the reader is referred to *Nutritional Blindness* and the original references for detailed presentations of that data

I wrote *Nutritional Blindness* by myself The present volume is a joint effort Dr Keith P West, Jr , a close colleague and collaborator for the past dozen years has joined me as co-author The recent explosion in knowledge about the basic biochemistry of vitamin A and its impact on the immune system, which has begun to explain the clinical and public health observations at the heart of this book, requires more thorough and knowledgeable discussion than I can provide Drs James Olson and Catharine Ross have graciously assumed responsibility for these two chapters

The goal and spirit of the original volume have, I hope, been retained in this sequel

That this book will prove useful to pediatricians, nutritionists, ophthalmologists, scientists and public health practitioners and officials engaged in designing and implementing programs aimed at curbing vitamin A deficiency and its consequences and reducing the terrible and needless loss of sight and life

Baltimore A S
May 1995

Acknowledgments

It has been two decades since one of us (A S) first turned his attention to the problems related to vitamin A deficiency and its control The past twelve years have witnessed an ever-widening circle of friends and colleagues concerned and involved with these issues In particular, thanks are due to the staff and faculty of the Dana Center for Preventive Ophthalmology, fellow travelers in the field In particular, Drs James Tielsch, Joanne Katz, Jean Humphrey, Hugh Taylor, and Richard Semba, Ms Lisa Mele, Kate Burns, Liliana Clement, Agatha Rider, Lee Shu-Fune Wu, Elizabeth Kimbrough Pradhan, and Messrs Paul Conner and Steve LeClerq

Our work could not have been conducted without the active collaboration of colleagues in over a dozen countries around the world While it is impossible to mention them all, our seminal studies would never have taken place without our family of Indonesian friends and co-investigators (Drs I Tarwotjo, D Karyadi, Muhilal, S K M Soekirman, G Natadisastra, A Pandji, E Djunaedi, and Kusdiono), Drs Florentino Solon, Rudi Florentino, and Luluth Lucero in the Philippines, Drs R P Pokhrel, M R Pandey, S K Khatry, B D Chataut, R K Adhikari, Nils Daulaire, and Mr S R Shrestha in Nepal, Dr G Venkataswamy and Mr Thulasiraj of India, Drs Festo Kavishe, Allen Foster, Andrew Barclay, and Joe Taylor of Tanzania, Dr Moses Chirambo of Malawi, and Drs T Sukwa, D Kwendakwena, and M Mukunyandela of Zambia

Many of our major undertakings were facilitated by colleagues and friends at Helen Keller International, particularly Messrs Jeff Watson and Rolf Klemm (Philippines), Mr Steve Wilbur and Dr Robert Tilden (Indonesia), Dr Susan Pettiss, Dr Martin Bloem, Ms Anne Ralte, and Executive Director Mr John M Palmer III

The thoughts, analyses, and conclusions presented in this book have been influenced over the years by colleagues at the International Vitamin A Consultative Group (IVACG) Professor Abraham Horwitz—its Chairman, Director-General Emeritus of the Pan American Health Organization, and Chairman of

the United Nations Administrative Committee on Coordination/Subcommittee on Nutrition—has been a consistent source of inspiration and encouragement

Further, our work might not have been possible at all without the unflagging support of the Office of Nutrition, United States Agency for International Development (USAID) Its former Director, the late Dr Martin Foreman, was personally responsible for identifying vitamin A deficiency and its control as an important international priority His staff, especially Drs Frances Davidson and John McKigney, developed the mechanisms that facilitated programming of our activities and offered useful and substantive advice throughout USAID Mission Health Officers, particularly Dr David Calde, Mr David Piet, and Ms Molly Gingrich, have been pillars of support

The House of Representatives Select Committee on Hunger, under the chairmanship of the late Congressman Mickey Leland, is responsible for recognizing the importance and promise of our work, and for the priority status now accorded control of vitamin A deficiency by the U S Congress The current Chairman of the Committee, Congressman Tony Hall, continues the passionately supportive legacy of his predecessor

Among the numerous foundations and other agencies to which we are indebted, special recognition is due Task Force Sight and Life of Hoffman LaRoche, Basle, its past Director Dr John Gmunder, and its past President Dr Guido Richterich, and the Rockefeller Foundation for giving one of us (A S) the opportunity to think and write at its Study Center on the inspiring shores of Lake Como

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1

Introduction

While they were saying among themselves it could not be done, behold it was done

—Helen Keller

1

Background

> We feel confident that these cases of xerophthalmia reported by Mori and Bloch should be looked upon as a deficiency disease not hitherto recognized in its true relationship to diet
>
> —E V McCollum, 1917[12]

> The disease [vitamin A deficiency] is ushered in by failure to gain weight These children are liable to be attacked by some infectious disease These infections are extraordinarily persistent and often have a fatal issue An increased susceptibility and a diminished power of resistance to infections are therefore present The eye lesion does not appear as a rule until late in the disease
>
> —C E Bloch, 1924[6]

> Vitamin A Suspicion Confirmed There is no longer any reason to wait Vitamin A supplements have taken their place alongside the handful of other low-cost strategies that could now significantly reduce illness and death among the children of the developing world
>
> —The State of the World's Children, 1993[13]

Historical Development

Vitamin A deficiency is one of the oldest recorded medical conditions, long recognized for its striking and unique ocular manifestations Nightblindness and its successful treatment with animal liver was known to the ancient Egyptians at least 3500 years ago[14], and is mentioned by Hippocrates Corneal destruction resulting in permanent blindness was certainly recognized by eighteenth and nineteenth century physicians,[15–17] usually in association with severe systemic

illnesses like meningitis, tuberculosis, typhoid, and severe protein energy malnutrition

In 1816, Magendie[18] demonstrated that dogs "starved" on sugar and distilled water suffered corneal ulceration, suggesting a specific nutritional basis for the ocular lesions Hubbenet[19] observed children in a French orphanage and described the progression of xerophthalmia from nightblindness through conjunctival and corneal involvement, attributing it to a faulty diet

By the mid-1800s, xerophthalmia was recognized in many areas of Europe,[20–24] particularly in Russia during the long Lenten fasts,[25] the United States,[26] and elsewhere around the world [27–31] In a particularly thoughtful and well-documented study published in 1881, Snell[32] demonstrated that cod liver oil would cure both nightblindness and Bitot's spots Within a decade, meat, milk, and cod liver oil were routinely administered for corneal ulceration and dissolution (keratomalacia) [33]

In 1904, Mori published an exhaustive description of "Hikan," as the disease was known in Japan [31] Not only did he recognize the entire sequence of ocular changes constituting xerophthalmia, but also the central role of dietary deficiency of fats (particularly fish liver oils) resulting from either a faulty diet or faulty absorption, the role of diarrhea, kwashiorkor, and other contributory and precipitating events, and the curative value of cod liver oil administered orally or intramuscularly

The first two decades of the 1900s witnessed the flowering of "basic" nutritional research the use of animal models in the search for specific dietary factors and their role in human health and disease Hopkins, McCollum, and Osborne and Mendel led the way Animals fed "pure" diets of fats, starch, protein and inorganic salts ceased to grow, lost weight, became susceptible to infections, and generally died of overwhelming sepsis, only those that survived developed inflammatory corneal ulceration [34–37] Administration of "accessory factors" present in the lipid fraction of milk, eggs, butter, and cod liver oil reversed the process [38–42] McCollum termed the critical factor "Fat Soluble A "[43] McCollum[44,45] and Goldschmidt[42] equated these diet-dependent ocular changes in animals with human xerophthalmia

Bloch, a Danish pediatrician, confirmed McCollum's suppositions In a series of papers that represent classics of clinical epidemiology, he described the full spectrum of human disease and its etiology, prevention and cure [4–6,46,47] Seeking an explanation of why children living in one dormitory of a Danish orphanage failed to thrive compared with children housed in other wards, he discovered the latter received more generous helpings of butterfat and whole milk Extending from 1912 through at least 1919, Bloch's studies convinced him that, in humans as in McCollum's animals, the earliest manifestations of progressive vitamin A deficiency were growth retardation accompanied by reduced resistance to infection (particularly of the respiratory and urinary tracts and the middle ear), and only subsequently by the appearance of the ocular changes of xerophthalmia Hence the title of one of his seminal publications contained the words

" xerophthalmia et dystrophia alipogenetica" (xerophthalmia and growth failure related to lack of a dietary lipid) [4] He found he could cure all these manifestations, including otherwise severe and seemingly intractable infections, with cod liver oil, butterfat or whole milk Upon learning of McCollum's description of fat-soluble A, Bloch declared that its deficiency was the sole factor responsible for the disease

Thus, by 1920 the clinical manifestations of vitamin A deficiency and their cause and cure were well established Not until many years later was vitamin A crystallized[48] or were xerophthalmic patients shown to have decreased levels in their blood [49]

Modern Perspective

> From that moment (December 21, 1917), everyone ate butter instead of margarine and since then there has been no xerophthalmia in Denmark it is impossible with certainty to throw any light on the extent to which change of diet in 1918 and 1919 affected the two other conditions associated with xerophthalmia in young children, viz dystrophy and the reduced power of resistance towards infection
>
> —C E Bloch, 1921[4]

> Recent research seems to have crystallized into the belief that vitamin A is concerned with raising the resistance of the animal body as a whole to various bacterial invasions The comparative scarcity of human investigations on this subject may perhaps be attributed to the fact it is not easy to point to any clearly defined group of symptoms associated with a deficiency of this factor in man in any way analogous to the syndromes of rickets and scurvy There is, however, good evidence for the belief that concentrates rich in this vitamin are valuable as prophylactics against infections
>
> —J B Ellison, 1932[50]

In retrospect, a profound amnesia appears to have settled over the broader context of vitamin A deficiency once it ceased to be a major concern of wealthier countries Although animal studies and clinical observations had seemingly delineated the wide spectrum of disease associated with vitamin A deficiency, particularly growth retardation and reduced resistance to infection, clinical interest soon fixated on the ocular manifestations (xerophthalmia and keratomalacia) To what can we ascribe this relative indifference to the systemic consequences of vitamin A deficiency and its potentially profound impact on the health and survival of young children? No clear answers exist, though a constellation of factors were probably responsible

1 Despite periodic but scattered reports attesting to the potential relationship between vitamin A status and infection,[36 50–52] it is unclear whether

this thesis was ever widely accepted or altered the practices of the medical profession (in the absence of xerophthalmia)

2 Scientific curiosity reached its peak in Europe, Japan, and the United States when vitamin A deficiency was a readily recognized local phenomenon As these countries succeeded in eliminating severe deficiency (xerophthalmia) through alterations in diet, the problem became less apparent and urgent Scientific and public health interest turned elsewhere

3 In contrast to xerophthalmia, which is associated with a dramatic and readily recognized constellation of signs and symptoms, the systemic consequences of vitamin A deficiency are more protean, insidious and, less specific As the quotation from Bloch makes clear, even he was unsure whether improved child health and survival in Denmark was a direct consequence of the marked and abrupt increase in vitamin A intake Too many factors seemingly influenced childhood morbidity and mortality to ascribe a major role to a single nutrient

4 The poverty and deprivation afflicting children in the developing world have long presented complex, overwhelming challenges to their health Given contaminated water supplies, poor hygiene, and multiple nutritional deficiencies, many scientists, including a panel of the National Academy of Sciences,[53] doubted that vitamin A deficiency alone could account for a significant proportion of severe childhood infections and death Nor were they convinced that improving vitamin A nutriture by itself would improve health and reduce mortality

The significance of "mild" vitamin A deficiency as an important determinant of child survival was overlooked until the chance observation that mild xerophthalmia (or, as we now recognize, *moderate* vitamin A deficiency) was closely associated, in a dose-dependent relationship, to mortality among children who seemed otherwise healthy and well nourished [2] That was more than ten years ago, it took another decade of observational studies and controlled clinical trials to convince ourselves and the broader scientific and public health communities that improving the vitamin A status of deficient children could, by itself, dramatically reduce their morbidity and mortality [10,54–64]

Vitamin A

"Vitamin A" is a generic term for a variety of related compounds Briefly, they include retinol, its esters (e g , retinyl palmitate), and retinoic acid The biochemistry and cellular and molecular biology of vitamin A are detailed in Chapter 8,[65–70] but a short overview will help orient the reader

Vitamin A is a fat-soluble substance found in ester form (usually as retinyl palmitate) in animal and dairy products It is hydrolyzed in cells of the small

intestine to alcohol (retinol) Carotene, the naturally occurring progenitor of vitamin A found in certain vegetables and fruits, is split in the intestinal cells to retinaldehyde, most of which is promptly reduced to retinol All-trans beta-carotene is the most active of the provitamin A carotenoids The bioavailability of provitamin A carotenes varies widely with the way in which food is prepared and consumed

Dietary lipids, pancreatic enzymes, and bile salts play an important role in the digestion and assimilation of these agents

Most of the absorbed retinol is re-esterified, enters chylomicra and makes its way to the bloodstream via the lymphatics Under normal conditions, over 90% of vitamin A is stored in the liver, mostly as retinyl palmitate This is why fish and animal livers (and their oils, like cod liver oil) are such potent sources of vitamin A

Liver stores are drained to maintain serum levels and the delivery of adequate amounts of retinol to target tissues When released from the liver, retinol is bound to a specific transport protein, RBP (retinol-binding protein) By itself the protein is known as apo-RBP Bound to retinol, the complex is called holo-RBP Interference with protein metabolism can depress RBP synthesis and holo-RBP release Conversely, pure vitamin A deficiency results in the accumulation of apo-RBP in the liver, administration of a bolus of retinyl ester will cause a sudden outpouring of holo-RBP This response is the basis for indirect assessment of liver stores (the relative dose-response [RDR], see Chapter 11)

A variety of intracellular retinol binding proteins have been identified Some in the intestines participate in the absorption of vitamin A, others promote its storage in the liver's stellate cells Retinol's role in the visual cycle of photoreceptors, remarkably well worked out, only begins to tell the story Exciting new molecular studies indicate that vitamin A influences the expression of over 300 genes, in most instances after oxidation to retinoic acid which then binds to nuclear receptors (RAR) The retinoic acid-RAR complex is thought to bind to DNA sequences (retinoic acid-responsive elements [RARE]) in the vicinity of target genes and thereby regulate their expression [66 69–74]

While retinoic acid-responsive genes are involved in a host of activities, including production of structural proteins, they clearly play a major role in cellular differentiation [75 76] Vitamin A's impact on cellular differentiation and morphogenesis affects (among other things) the epithelial lining of a number of organs, including the eye and the respiratory and genitourinary tract, and the differentiation of cells related to the immune response (Chapter 9)

Indices of vitamin A status and their interpretation are detailed in Chapter 11 For purposes of this overview, suffice it to say that no single measure alone provides a complete story of vitamin A nutriture at the cellular and organismic level

A variety of body tissues and biochemical techniques have been used to assess vitamin A "status" Serum "vitamin A" has a certain historical pride of

Table 1–1 Serum Vitamin A Classification of Vitamin A "Status"

μg/dl[a]	μmol/liter	Vitamin A "Status"
≥ 20	≥ 0 7	Normal
10–20	0 35–0 69	Low
< 10	< 0 35	Deficient

[a]Serum levels were traditionally expressed as μg/dl They are increasingly reported as μmol/liter (28 57 μg/dl = 1 μmol/liter) In still older literature, "international units" were used The reader is cautioned to pay close attention to the unit of measurement

place, having been first measured and correlated with clinical status (xerophthalmia) in the 1930s [49] Despite a number of well-recognized limitations, it has been the most common biochemical measure of vitamin A "status" and the basis upon which much of our knowledge about vitamin A status and its clinical correlates rests

Vitamin A levels have traditionally been categorized (Table 1–1)

Not surprisingly, interpretation of serum levels in any one individual is fraught with problems Aside from laboratory artifact, biologic variation dictates that an "abnormal" level in one individual is perfectly "normal" in another Hence "normal" levels of vitamin A are best considered a "range" with considerable overlap

While serum retinol levels above 20 μg/dl (0 7 μmol/liter) are generally considered normal, there are important caveats

- Carefully monitored vitamin A depletion of otherwise healthy adults demonstrates impaired dark adaptation at serum vitamin A levels between 20 μg/dl–30 μg/dl or higher [77 78]
- Non-xerophthalmic children with serum levels above 20 μg/dl have evidence of subclinical functional disturbances, like conjunctival metaplasia, which are directly associated with other health consequences (Chapters 2, 11)
- A significant proportion of children with clinical xerophthalmia have serum levels above 20 μg/dl, while an even larger proportion with seemingly normal eyes have levels below 20 μg/dl (Table 1–3)

Direct measures of various vitamin A-dependent functions (impression cytology, dark adaptation, etc) provide a snapshot of that particular function, but little about the adequacy of other functions or vitamin A reserves

Xerophthalmia's Relationship to Vitamin A Status

As the pathognomonic clinical sign of vitamin A deficiency, the presence and severity of xerophthalmia has classically served as a surrogate for "vitamin A status" in studies investigating the relationship between vitamin A deficiency

and factors with which it might interact A detailed description of the clinical entity appears in Chapter 4 and elsewhere [1]

The term "xerophthalmia" was coined nearly 200 years ago to describe dry eyes (Greek *xeros,* dryness), unrelated to the nutritional condition under discussion (cited in MacKenzie[16]) While a number of pathologic processes interfering with mucus or tear production may give rise to "dry eye," these are exceedingly rare among children in developing countries "Xerophthalmia" has since become synonymous with the ocular manifestations of vitamin A deficiency [5] Historically, the signs and symptoms covered by the term have varied from excluding nightblindness to including a variety of permutations and combinations of conjunctival and corneal abnormalities [79-84]

In 1974, participants at an international conference[85] proposed following the inclusive practice of Oomen and McLaren[86,87] "Xerophthalmia" includes all ocular signs and symptoms of vitamin A deficiency, from nightblindness to keratomalacia A standardized classification (modified after ten Doesschate[88]) has since been refined following clinical studies in Indonesia (Table 1–2) [1,89]

Nightblindness (XN), conjunctival xerosis (X1A), Bitot's spots (X1B), and corneal xerosis (X2) are entirely reversible Loss of corneal stroma (X3) will result in permanent sequelae (XS) a circumscribed ulcer (Latin *ulcus*) will leave a scar, "keratomalacia" (Greek *keras,* horn, *malakos,* soft), full thickness, often limbal-to-limbal necrosis of the entire cornea, commonly results in a blind, shrunken globe

Despite limitations of serum vitamin A as a guide to all aspects of vitamin A status, particularly among individual subjects, it is a useful basis for assessing the relative status between groups of individuals Indonesian studies documented a direct, dose-dependent relationship between serum vitamin A levels and the prevalence and severity of xerophthalmia (Table 1–3) [1]

While a significant proportion of preschool children with mild xerophthalmia had serum levels above 20 μg/dl, the prevalence and severity of disease increased markedly as levels fell below 20 μg/dl, particularly below 10 μg/dl–15 μg/dl Mean serum vitamin A among children with keratomalacia was well below 10 μg/dl (0 35 μmol/liter) [90,91]

Table 1–2 Clinical Classification of Xerophthalmia (WHO, 1981 Revision)

Nightblindness (XN)
Conjunctival xerosis (X1A)
Bitot's spots (X1B)
Corneal xerosis (X2)
Corneal ulceration/keratomalacia <1/3 corneal surface (X3A)
Corneal ulceration/keratomalacia ≥1/3 corneal surface (X3B)
Corneal scar (XS)
Xerophthalmia fundus (XF)

Table 1-3[a] Association Between Xerophthalmia Status and Serum Vitamin A

A[a]						
	Distribution of Serum Retinol Levels					
Clinical Status	*Deficient (<10 μg/dl)*	*Low (10–20 μg/dl)*	*Adequate (>20 μg/dl)*	*Total*	*Mean (μg/dl)*	*Number of Cases*
XN (+), X1B (−)	27%	55%	18%	100%	13 9	174
XN (−), X1B (+)	31%	57%	12%	100%	13 4	51
XN (+), X1B (+)	38%	53%	9%	100%	12 1	79
Neighborhood controls	N/A	N/A	N/A	N/A	17 7	282
Random sample	8%	37%	55%	100%	20 0	268
B[b]						
X2	62%	34%	4%	100%	8 2	26
X3A (ulcer)	69%	31%	0%	100%	7 2	32
X3A (necrosis)	92%	8%	0%	100%	5 4	25
X3B	80%	20%	0%	100%	5 2	15

[a]Modified from Sommer et al [90]—community based study

[b]Modified from Sommer et al [91]—hospital-based study

XN = nightblindness X1B = Bitot s spots, controls = neighborhood peers of same age and sex random sample = peers from throughout the six villages studied X2 = corneal xerosis X3A = ulcers and localized keratomalacia, X3B = widespread corneal necrosis (keratomalacia), "random sample ' distribution of serum retinol for XN(−)/X1B(−)

Data on XN, X1B, controls and random sample from a six village field study outside Bandung, Indonesia Data on corneal cases were children seen at the Cicendo Eye Hospital, Bandung

Serum levels among children with X1B appeared only slightly lower than among children with isolated XN, this may partially reflect inclusion of children with Bitot's spots that persisted despite a more adequate (if not necessarily normal) vitamin A nutriture (Chapter 4)

Correlation between children with "normal" eyes and serum vitamin A levels reveals an interesting aspect of vitamin A status among children without evident xerophthalmia Seemingly normal children living in the vicinity of an active case of xerophthalmia ("neighborhood controls") had serum levels higher than the neighboring case, but lower than those of children of the same age and sex living farther from the explicit case ("random sample") This finding indicates the influence of local, shared community and environmental factors on vitamin A status (e g , dietary practices, sanitation, educational levels) [92]

NOTE In Indonesia in the late 1970s, the mean serum vitamin A level of control children *without* xerophthalmia was at, or *below,* 20 μg/dl (0 70 μmol/liter) In other words, roughly half the population of *seemingly normal,* free-

living, rural Indonesian children had serum vitamin A levels classified as "low", serum levels in the remaining "normal" children were not much higher

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II

Consequences of Vitamin A Deficiency

2

Child Survival

> Our results suggest that the existence of even mild xerophthalmia in a community justifies vigorous intervention measures, as much to reduce childhood mortality as to prevent blindness
>
> —A Sommer et al., 1983[1]

Where it is the limiting nutrient, vitamin A deficiency causes anemia, growth retardation and xerophthalmia; increases the incidence and/or severity of infectious episodes; and reduces childhood survival. Reduced survival is the most severe and potentially the most widespread consequence of vitamin A deficiency, and the one that has generated the most interest recently.[2] The international Child Survival Initiative, a program directed at reducing the 14 million childhood deaths each year, has helped focus attention on mortality. Improvement in vitamin A status is now viewed as one of the most cost-effective measures to achieve the Initiative's goal,[3] joining immunization, breast-feeding and related strategies.[2,4]

Vitamin A-deficient animals die much earlier and at a far higher rate than vitamin-A sufficient controls. Under experimental conditions of gradual, progressive deficiency, mortality begins to take its toll even before the appearance of xerophthalmia (Fig. 2–1).[5] Indeed, one of the problems in creating an experimental animal model to study xerophthalmia has been keeping such animals alive long enough to develop ocular changes.

The situation in humans is far more complex. Vitamin A deficiency rarely occurs as an isolated disturbance; when it does, it is rarely recognized in the absence of severe xerophthalmia, a condition long associated with increased mortality.

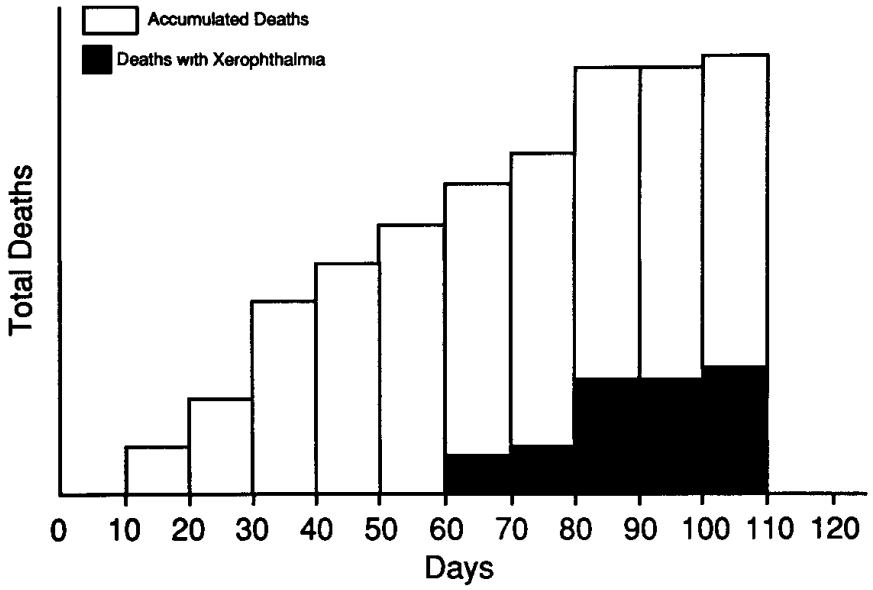

Fig. 2-1. Cumulative deaths of increasingly vitamin A-deficient rats. Most died before ever developing xerophthalmia. (From C.M. Stephenson.[5])

Mortality Associated with Moderate to Severe Deficiency (Xerophthalmia)

Severe xerophthalmia, particularly keratomalacia, has traditionally been associated with extraordinary mortality. This is hardly surprising since early reports were generally confined to children who went blind during chronic, debilitating illnesses like encephalitis and tuberculosis, complicated by severe pneumonia, diarrhea and emaciation—children like those recalled by Elliot (Chapter 4).[6] In 1868, Hirschberg reported mortality rates of 100%[7], and authors early in this century cited rates of 50%–80%.[8,9] Given the lack of understanding and means to treat kwashiorkor and underlying infections at that time, these rates probably approximate the natural, untreated outcome of keratomalacia today.

It seems reasonable to suspect these extraordinary mortality rates were determined, at least in part, by concomitant infections and protein-energy malnutrition (PEM). Indonesian children presenting with severe (e.g., corneal) xerophthalmia to the Cicendo Eye Hospital in Bandung received the recommended course of vitamin therapy (200,000 IU on two successive days and again one month later), as well as protein-rich diets and antibiotics appropriate for systemic infections. Cumulative mortality over the fourteen months of follow-up was 12.6%,[10] most of the deaths occurring within two months of initial presentation. PEM status at admission was the single most important determinant of mortality in this treated group of subjects: children with severe PEM (edema, serum albumin $\leq$ 2.5 μg/dl or weight for height < 70% of standard) died at almost seven times the rate of those who were better nourished (25.7% versus 3.8% respectively).

Years before, 30%–35% of severely malnourished children treated in the central hospital in Jakarta died whether or not they had accompanying xerophthalmia.[11] Only 8% of xerophthalmic children (most but not all of whom had PEM) treated at a nutrition rehabilitation center in India died, versus 10% of non-xerophthalmic, but more malnourished, children.[12] Children hospitalized for xerophthalmia in El Salvador had an in-hospital mortality of 21%; among those most severely malnourished, mortality was 25%.[13]

On the other hand, several reports suggest that all else being equal, xerophthalmia (e.g., severe vitamin A deficiency) can increase the risk of mortality among malnourished, hospitalized children. Of severely malnourished children hospitalized in El Salvador, 16%with xerophthalmia died; that rate was only 11% among those without xerophthalmia.[14] In South Africa, overall hospital mortality for severely malnourished children (25%) rose to 30% for those with ulcerated corneal lesions.[15] In Bangladesh, children hospitalized with severe malnutrition died at a 50% higher rate if they were xerophthalmic (10% versus 15%).[16] This difference was not statistically significant, however, and closer examination revealed that the xerophthalmic children were even more undernourished and hypoproteinemic than their non-xerophthalmic peers.

McLaren long ago argued that xerophthalmia increased mortality among malnourished children. Mortality among hospitalized xerophthalmic children was 56%–64%, four times the rate among non-xerophthalmic children of similar PEM status (judged by broad classifications).[17] Pereira et al.[18] reported similar though less dramatic results in Vellore, South India: mortality among children with both keratomalacia and kwashiorkor was 28%, twice the mortality rate among children with kwashiorkor alone. Given the observations in Bangladesh[16] that xerophthalmic children were generally more malnourished than non-xerophthalmic children, even when confined to the same broad nutritional categories, more detailed examination of nutritional status in McLaren's and Periera's studies would have been valuable.

Among girls in El Salvador hospitalized for severe malnutrition, the mortality rate of those with xerophthalmia was twice that of girls without xerophthalmia; mortality rates among xerophthalmic and non-xerophthalmic boys, however, were similar.[14] This was interpreted as evidence that admission of females with xerophthalmia was delayed, by which time they had become more malnourished. Scragg and Rubidge[19] reported in-hospital mortality of 52% among 1565 severely malnourished African children (over 90% with edema). In general, those who died had lower serum protein values than those who survived. Fourteen had rapidly progressive corneal perforation in eyes that had appeared normal the previous day. The mortality in this subgroup was an astounding 93% (thirteen of fourteen). Five had been admitted "in extremis," dying within a few hours.

Before 1980, therefore, evidence had linked xerophthalmia to mortality; unfortunately, it was primarily limited to children hospitalized with concomitant,

severe malnutrition. The data suggested that *severe* PEM (a strong predictor of mortality[20–22]) was a greater determinant of survival than was xerophthalmia, but also indicated, tentatively, that severe xerophthalmia (vitamin A deficiency) might further exacerbate the underlying risk of death.

The contribution of vitamin A deficiency to child mortality, isolated from the confounding effects of severe PEM, was identified in a prospective, longitudinal observational study of generally healthy, free-living rural Indonesian children.[1,23,24] Approximately 3500 preschool-age children up to six years of age were followed for eighteen months (Figure 2–2).[1,10,23] After baseline examination, the children were reexamined by the same team every three months, resulting in seven examinations encompassing six intervals. Serum samples were obtained at baseline from children with mild xerophthalmia (XN, X1B), from age, sex, neighborhood matched controls, and from a representative subsample of the study population (Table 1–3). Any child with advanced xerophthalmia (i.e., X2, X3) was immediately given 200,000 IU vitamin A by mouth and hospitalized for treatment.

Each child was assigned the ocular status present at the interval-initiating examination. Mortality for that interval was ascertained at the follow-up examination three months later. The process was repeated and summed across all six intervals. The data relate solely to children with mild xerophthalmia (XN, X1B) compared with peers with normal eyes (but not necessarily normal vitamin A status).

The incidence of mild xerophthalmia rose dramatically during the first four years of life, to 7% (5% XN, 2% X1B). No child under one year of age had evidence of xerophthalmia. Of the 132 deaths, 24 occurred in children within the three-month interval after the exam at which they were diagnosed with xerophthalmia (Table 2–1). Age-specific mortality among these mildly xerophthalmic children was four to twelve times the rate among their non-xerophthalmic peers.

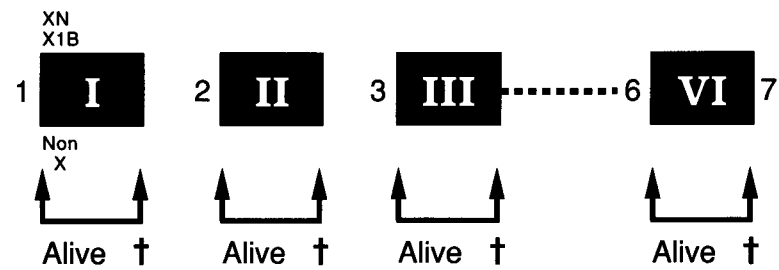

Fig. 2–2. Approximately 3500 preschool-age Indonesian children in Purwakarta District, Indonesia, were reexamined in their village every three months for eighteen months. Vitamin A status was based on the presence or absence of xerophthalmia at the examination initiating each three-month interval. Mortality rates were calculated for each interval, by xerophthalmia status at the start of the interval, and summed over the six intervals. (From A. Sommer et al.[1])

Table 2–1. Mortality in Relation to Xerophthalmia (Vitamin A) Status

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

From A Sommer et al [1]

Refer to Table 1–3 for relationship between serum vitamin A status and clinical xerophthalmia at baseline examination

It is important to note that the association between vitamin A status and mortality was dose-dependent: the greater the degree of vitamin A deficiency, the greater the mortality. Children with only nightblindness died at almost three times the rate of their non-xerophthalmic peers; children with Bitot's spots, at almost seven times the rate; and children with both nightblindness and Bitot's spots (a cleaner group than XN (−) X1B (+), in whom Bitot's spots were more likely to be residua of past deficiency), at almost nine times the rate (test for trend, $p < .05$).

At the interval-initiating examinations, respiratory infection (diagnosed by the pediatrician on the basis of wheezing, rhonchi, or rales) was more prevalent at the interval-initiating examinations among xerophthalmic than among non-xerophthalmic children (by 40%–90%). Since it was uncertain whether respiratory infection precipitated the xerophthalmia or xerophthalmia the infection (Chapters 3, 7), and respiratory infection could have contributed to the increased mortality seemingly associated with xerophthalmia, mortality rates were stratified for respiratory status at each interval-initiating examination (Table 2–2).

Even among children without respiratory disease at baseline, mortality increased in direct association with the severity of vitamin A deficiency. Though the numbers are much smaller, the same relationship is evident among children with respiratory disease at the start of the interval.

To simultaneously account for potential associations between age, mortality and respiratory disease, age-specific mortality was plotted for each degree of xerophthalmia (vitamin A deficiency) (Table 1–3) *excluding* children with respiratory disease at the interval-initiating round (Fig. 2–3). Each age group exhibited a dose-response relationship between the severity of vitamin A deficiency and subsequent mortality.

Wasting (as percent of median weight-for-height) was equally prevalent among children with and without xerophthalmia. Increased mortality was associated with xerophthalmia in the same monotonic, dose-dependent manner whether or not wasting was present (Table 2–3).[25] At every age, xerophthalmic

Table 2–2. Respiratory Disease, Xerophthalmia and Mortality

Respiratory Infection at Initiating Exam	*Ocular Status at Initiating Exam*	*Child Intervals*	*Deaths*	*Mortality (per 1000)*
ABSENT				
	Normal	18,321	93	5.1
	XN (+), X1B (−)	486	6	12.4
	XN (−), X1B (+)	235	6	25 5
	XN (+), X1B (+)	183	7	38 3
PRESENT				
	Normal	1568	15	9 6
	XN (+), X1B (−)	61	2	32.8
	XN (−), X1B (+)	34	0	—
	XN (+), X1B (+)	32	3	93.8

From A Sommer et al [123]

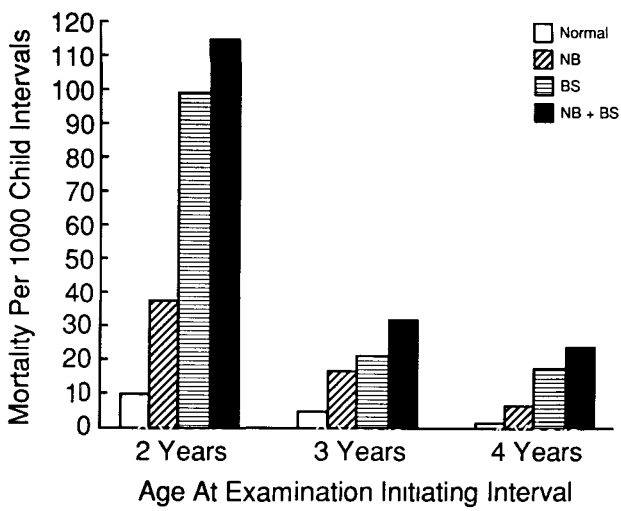

Fig. 2–3. Mortality per 1000 child intervals among children who were 2 to 4 years of age (at the first of their interval-initiating rounds). All children with respiratory disease at an interval-initiating round have been excluded. (From A. Sommer et al.[1])

Table 2–3. Relationship Between Weight-for-Height, Xerophthalmia and Mortality

Weight-for-Height[a]	*Ocular Status*	*Number Examined*	*Deaths*	*Mortality (per 1000)*
< 90%[b]				
	Normal	6079	58	9 5
	XN (+) X1B (−)	111	2	18 0
	XN (−) X1B (+)	94	5	53.2
	XN (+) X1B (+)	68	8	117.7
≥ 90%				
	Normal	9107	38	4 2
	XN (+) X1B (−)	283	4	14.1
	XN (−) X1B (+)	107	1	9.3
	XN (+) X1B (+)	76	2	26.3

[a]Based on the median of the "Harvard Standard " From D B Jelliffe et al [25]

[b]The number of children with severe wasting (< 80%) was negligible

From A Sommer et al [1,23]

children who were *not wasted* suffered a higher mortality than mildly wasted children who were *not xerophthalmic.* In this population at least, moderately severe vitamin A deficiency (mild xerophthalmia—XN, X1B) was a more important predictor (determinant?) of mortality than was mild PEM (Fig. 2–4),[1,23] even though weight-for-height was a powerful predictor of mortality.[26] The same results were obtained when the population was stratified by height-for-age.[23]

If anything, this study underestimates the increased risk of mortality associated with moderate vitamin A deficiency (xerophthalmia)[1,23].

- Children with severe deficiency (corneal xerophthalmia—X2, X3) were hospitalized, treated, and excluded from the study, removing those xerophthalmic children having the highest mortality.
- Although children were reexamined and their ocular status reclassified at the start of each three-month interval, ocular status could vary within the interval—some xerophthalmic children exhibited improved vitamin A status and developed normal eyes, and vice versa.[10,27] This dilutes the distinction between non-xerophthalmic and xerophthalmic (mild and more severely deficient) children, hence the relationship between vitamin A status and subsequent mortality. A similar time-related leveling is observed in the association between wasting PEM and mortality.[20]
- Treatment and referral of children with severe systemic disease or malnu-

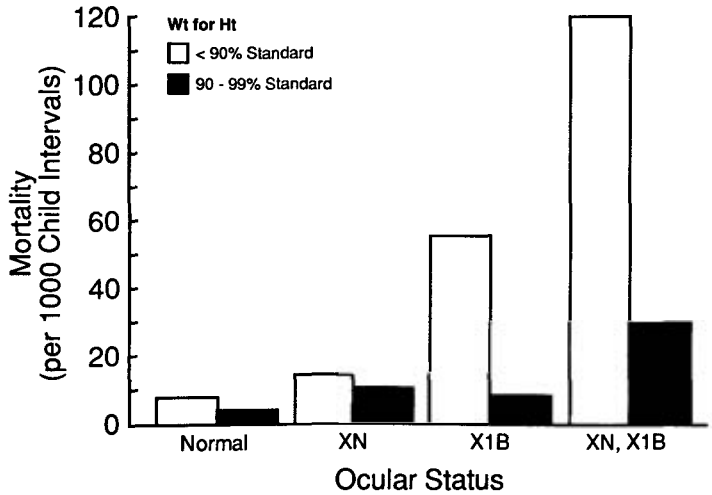

Fig. 2–4. At every degree of xerophthalmia, wasted children (< 90% weight-for-height; open bars) died at a higher rate than children of better anthropometric status (black bars). Within each anthropometric stratum, children with xerophthalmia were less likely to survive than were children without xerophthalmia. Wasted children (< 90% weight-for-height) without xerophthalmia (open bar, "normal" ocular status) had a lower mortality than nonwasted children with mild xerophthalmia (black bars, "XN" through "XN,X1B"). (From A. Sommer et al.[1,23])

trition undoubtedly reduced mortality. Since these conditions were more common among xerophthalmic children, treatment and referral further reduced the apparent risk.

- As we've seen (Table 1–3), normal eyes do not necessarily mean normal vitamin A status. At least half the "non-xerophthalmic" children had serum levels considered "low" (below 20 μg/dl); and levels in the other half were not much higher. Given the dose-dependent relationship between mild xerophthalmia and increased mortality, this monotonic trend might well be expected to extend downward to non-xerophthalmic, deficient children. Their mortality was two to three times higher than it might have been had their vitamin A status been truly normal (exactly what was observed in the vitamin A supplementation trials). A lower mortality among vitamin A-sufficient, non-xerophthalmic children would have increased the relative risk of death associated with mild xerophthalmia at least twofold (see Table 2–13).

Despite these factors, which individually and collectively drove results toward a conservative estimate of the increased mortality associated with xerophthalmia, the attributable risk was still substantial. Eliminating excess mortality associated solely with identifiable (mild) xerophthalmia in this population would reduce

overall mortality by *at least* 16%.[1,23] Given the conservative estimate of relative risk associated with mild xerophthalmia, the estimates of attributable risk and excess mortality are undoubtedly conservative as well (see Table 2–13).

The high relative risks, statistical significance, dose-dependent relationships, and internal consistency suggest a direct and probably causal relationship between vitamin A status and risk of childhood death, and provide a quantitative estimate of its potential magnitude.

Observational data collected in subsequent community intervention trials support these conclusions. Vijayaraghavan and colleagues[28] found that mortality among mildly xerophthalmic children in Hyderabad was more than twice as high as among their less deficient (i.e., non-xerophthalmic) peers.

In Tamil Nadu,[29] mortality was 50% higher among xerophthalmic compared with nonxerophthalmic children (10.6 versus 7.2 per 1000), even though they reportedly received supplemental vitamin A. The absence of comparative data stratified or adjusted for potential differences in age and other variables limit the ability to fully interpret the significance of these observations.

Herrera and colleagues[30] (who treated mildly xerophthalmic children and therefore could not directly study the connection between xerophthalmia and mortality) noted an inverse relationship between mortality and dietary vitamin A intake among Sudanese children. This relationship persisted after adjusting for a number of potentially confounding variables. Greater intake was particularly protective among children with chronic protein energy malnutrition or diarrhea.[31] Those in the highest quintiles of vitamin A intake (at or above the Recommended Daily Allowance [RDA]) died at only half the rate of those in the lowest quintile.

Vitamin A Supplementation Reduces Preschool-Age Mortality

> A further strong reason to promote programmes intended to control vitamin A deficiency and associated blindness is the now conclusive evidence that they can be expected to also significantly reduce mortality in young children and late infancy .
>
> —United Nations Administrative Committee on Coordination/Subcommittee on Nutrition, 1993[32]

The observation that mild xerophthalmia might be responsible for a substantial proportion of deaths in preschool-age children[1,23] resulted in a series of community-based controlled mortality trials to:

- determine whether intervention programs that improved vitamin A status of deficient populations would, by themselves, reduce overall childhood mortality;

- quantify the magnitude of reduction in mortality that might be expected in different cultures and environments;
- identify factors that might account for variations in the level of impact.

Eight major controlled community mortality prevention trials (CPTs) have been conducted. In each trial, one group of children received vitamin A supplements (the simplest means of improving vitamin A status for the purposes of these studies) and a comparable group did not. These studies indicate that any approach that significantly improves vitamin A status of deficient populations has a high likelihood of materially reducing preschool (six months to six years) childhood mortality.[32–39] Younger children (up to five months) may represent a more complex situation.[40]

Six studies were carried out in Asia (two each in Indonesia, India and Nepal) and two in sub-Saharan Africa (the Sudan and Ghana) (Table 2–4). Six employed periodic distribution (every four to sixmonths) of large-dose vitamin A (i.e., 200,000 IU for children over twelve months of age; smaller amounts for those younger); one distributed smaller amounts (equivalent to a week's RDA) on a weekly basis; and one fortified a commonly consumed condiment (monosodium glutamate [MSG]) with vitamin A.

The first of these studies, conducted in the Aceh province of North Sumatra[41] served as the stimulus and model upon which the subsequent trials were built and refined. Half of the 25,000 preschool children in 450 villages were randomized, by village, to receive high-dose vitamin A from a local resident twice in one year. The first dose was administered one to three months after the baseline

Table 2–4. Major Community Mortality Prevention Trials

Study	*Country*	*Vitamin A Supplement*	*Reported Mortality Reduction*[a]	*Primary Reference*
Aceh	Indonesia	Large dose every 6 mo	34%[b]	Sommer et al.[41]
Bogor	Indonesia	Vitamin A fortified MSG	45%	Muhilal et al.[42]
NNIPS	Nepal	Large-dose every 4 mo	30%	West et al[44]
Jumla	Nepal	One large dose follow-up at 5 mo	29%	Daulaire et al[45]
Tamil Nadu	India	Weekly RDA	54%	Rahmathullah et al[29]
Hyderabad	India	Large dose every 6 mo	6% (not SS)[c]	Vijayaraghavan et al[28]
Khartoum	Sudan	Large dose every 6 mo	(+6%, not SS)	Herrera et al[30]
VAST	Ghana	Large dose every 4 mo	19%	Ghana VAST Study Team[47]

[a]Six months and older at baseline (one year or older if younger children not reported separately)

[b]Alternative analyses suggest at least 40% to > 50%[53]

[c]As calculated from data in their publication but not reported as such[33,35]

examination, and the second dose six months later. The second Indonesian study, among 11,000 children, was conducted in Bogor, Java, 1200 miles away.[42,43] MSG bound for markets serving one set of villages was fortified to a level of ~ 810 μg RE/g MSG (MSG-A) calculated to provide children with half their RDA of vitamin A. MSG sold in an adjacent, control area was unfortified.

The larger and more comprehensive of the two Nepal trials (Nepal Nutrition Intervention Project, Sarlahi [NNIPS]) randomized 28,000 children (by ward) to receive large-dose vitamin A or placebo every four months.[44] It was carried out in the southern Terai, a region ecologically, culturally, and demographically contiguous with the Gangetic flood plain of South Asia. The other[45] was conducted in Jumla, a mountainous region in the far west with extraordinarily high childhood mortality. Unique to this intervention trial was that data collection and vital registration had been in place for several years as part of a community-based pneumonia case management trial. When it ended, a brief vitamin A intervention study was randomly implemented in eight of sixteen subdistricts: a large dose was provided at baseline in half the subdistricts, and mortality assessed five months later.

The more detailed of the two Indian trials was conducted outside Madurai, in Tamil Nadu.[29] Over 15,000 children in 206 clusters were randomized by cluster to receive a weekly supplement consisting of 8133 IU (8.7 μmol) vitamin A (one week's RDA) or placebo. The second Indian study, outside Hyderabad, randomized 15,000 children in 84 villages, by village, to either large-dose vitamin A or placebo every six months,[28] with tri-monthly morbidity monitoring and almost monthly contacts at the household level by trained health workers.[46]

Two studies were conducted in Africa: one in the Sudan and one in Ghana. In the Sudan, over 28,000 children living outside the capital of Khartoum were randomized by *household* (the only study to employ this logistically difficult approach) to receive either large-dose vitamin A or placebo every six months.[30] The Ghana VAST (Vitamin A Supplementation Trial) mortality study randomized 22,000 children in 185 clusters, by compound, to receive a large dose or placebo every four months.[47] It was conducted adjacent to a detailed morbidity trial.[47,48]

All studies reported their results on an "intent-to-treat" basis: mortality was compared for all enrolled children, whether or not they actually received the prescribed supplement. In six of the eight studies, the supplemented group had a significantly lower mortality, ranging from 46%–81% of the control group; two of the studies (Hyderabad and Sudan) recorded no discernable impact (Fig. 2–5).

Cumulative mortality curves following baseline enumeration for Aceh, Jumla and NNIPS all revealed reduced mortality among program children shortly after administration of the first large vitamin A dose (Figs. 2–6 to 2–8). Tamil Nadu and Bogor, trials in which smaller supplements were provided more frequently, produced comparable results after a brief delay (Figs. 2–9, 2–10). Smaller doses probably resulted in superior absorption and retention.[49,50] In addition, more

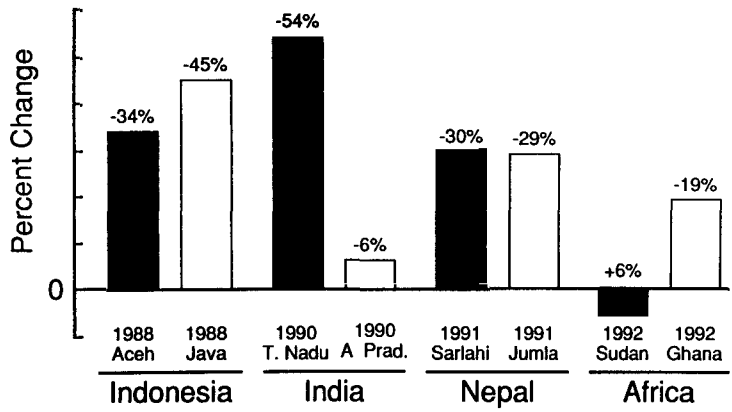

Fig. 2–5. Difference in preschool mortality (6 months or 12 months to 6 years of age) in eight major community-based vitamin A prophylaxis trials. Individual trials identified in Table 2–4. All figures as reported on an "intent-to-treat" basis showing proportional differences in mortality for the vitamin A group. There was a net reduction in mortality among the vitamin A group in all trials but the Sudan.

frequent supplementation, as in Tamil Nadu, probably achieved a higher "effective" compliance: since children were sought out individually for supplementation each week, they were more likely to receive at least some of their extra vitamin A. When dosing occurs only once every four to six months, however, one or two consecutive noncompliant contacts greatly reduces the potential impact.

The two Nepal studies continued to follow all children after the formal study was completed, and vitamin A was administered to the control groups. In both instances, mortality among the former control children dropped precipitously,

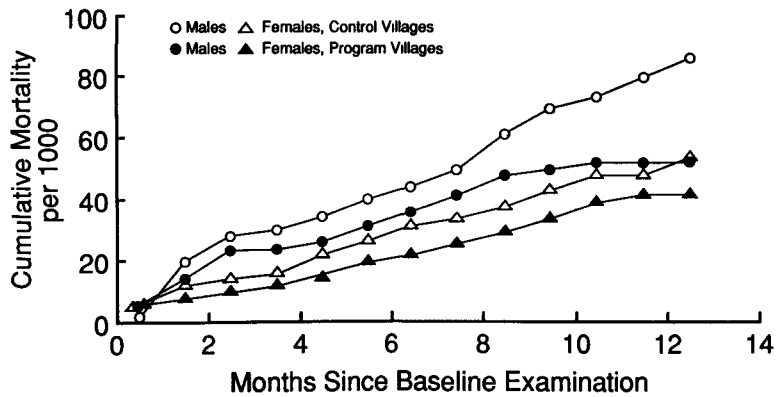

Fig. 2–6. Cumulative mortality, by sex, of preschool-age children in Aceh (Indonesia) trial. Vitamin A capsules were first distributed by local government teams zero to two months after baseline enumeration. The second distribution took place six months later (~ months 6–8). (From A. Sommer et al.[44])

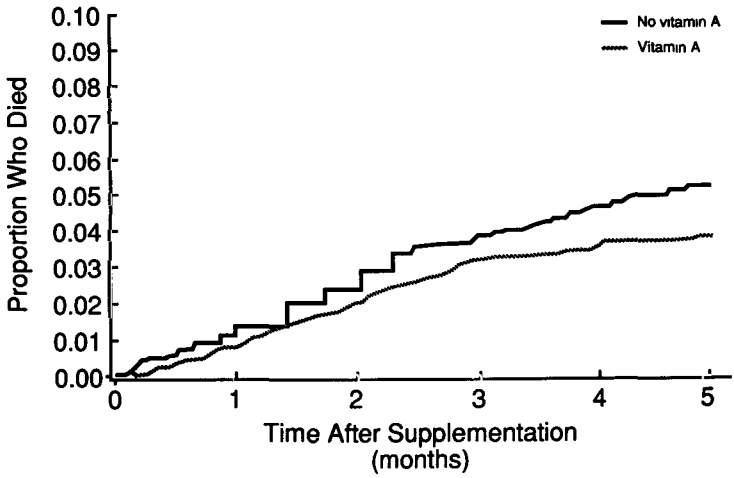

Fig. 2–7. Cumulative mortality of preschool-age children in Jumla (Nepal) trial. Only a single distribution took place, at baseline. (Figure drawn from data in N. Daulaire et al.[45])

approximating mortality rates of those assigned to receive vitamin A from the start (Fig. 2–11) (Nils Daulaire, personal communication, February 1993).[51]

At least four independent meta-analyses[33–35,37–39,52] addressed the crucial question: Would improvement in vitamin A status of deficient populations similar to those enrolled in the controlled community-based supplementation trials reduce overall preschool-age child mortality? All four unequivocally concluded that it

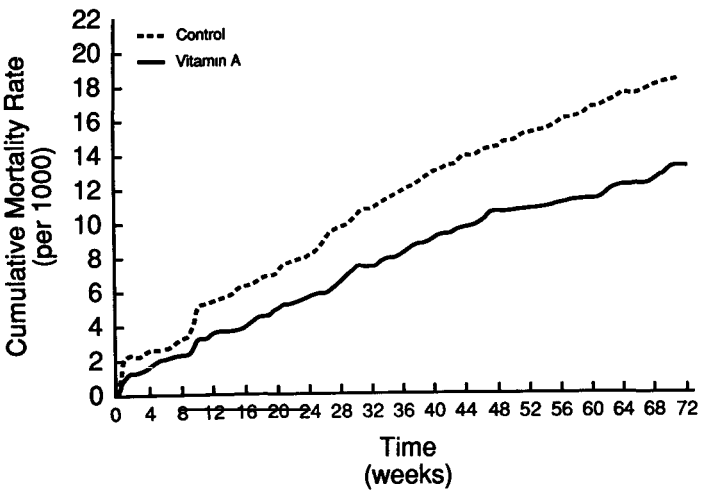

Fig. 2–8. Cumulative mortality of preschool-age children in NNIPS (Nepal) trial. Vitamin A (and placebo) distributed at baseline and every four months thereafter. (Drawn from data in K. West et al.[44])

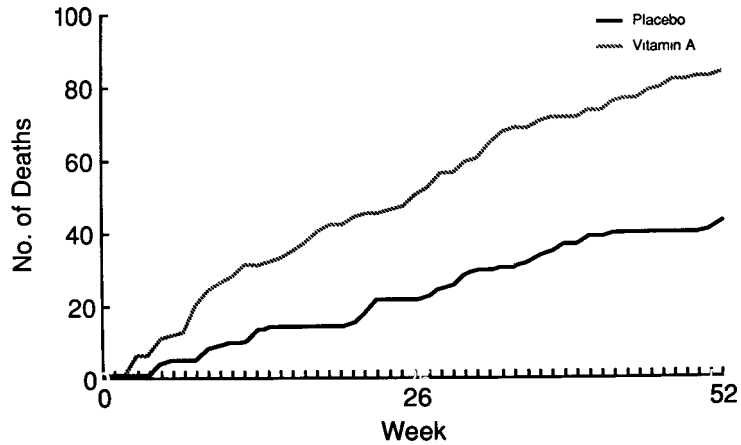

Fig. 2–9. Cumulative deaths of preschool-age children as reported for Tamil Nadu (India) trial. Recalculated (using denominator) as mortality, instead of absolute deaths, yields similar curves. (From L. Rahmathullah et al.[29])

would. Each suggested a similar level of anticipated impact, but these should be viewed only as rough orders of magnitude; the actual size of the anticipated impact will vary with local conditions.

The first of the meta-analyses, prepared by Dr. James Tonascia for the Bellagio meeting,[34–36] covered the six Asian trials (a more homogeneous group and the only trials published at that time) using "child-intervals at risk" (Fig. 2–12). The 34% estimated reduction in mortality attributed to vitamin A supplementation of children six months and older was exceedingly robust: eliminating any one of the trials, weighting factors that adjust a trial's contribution to the esti-

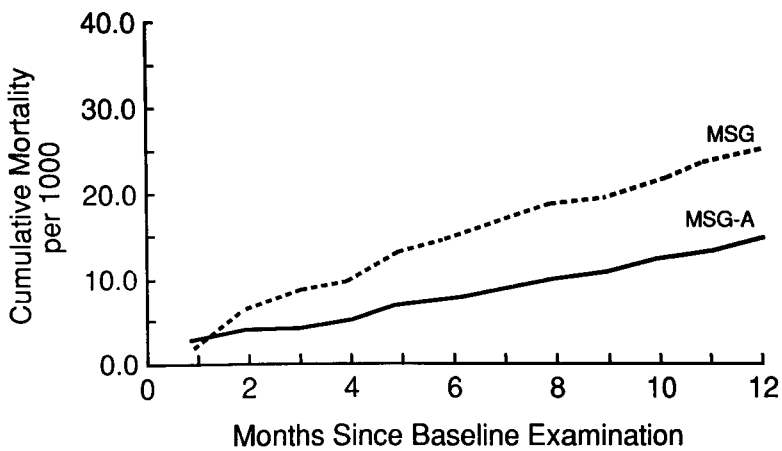

Fig. 2–10. Cumulative mortality of preschool-age children in Bogor (Indonesia) MSG fortification trial. (Drawn from data provided by the authors.[42])

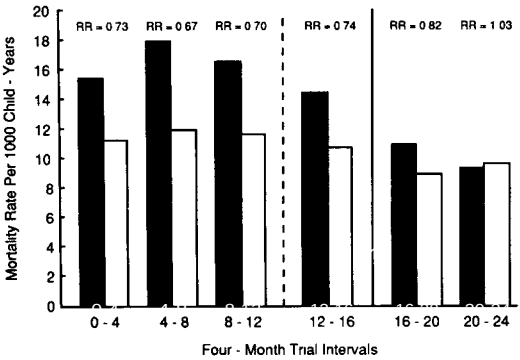

Fig. 2–11. Preschool-age mortality in vitamin A-supplemented (white bars) and -unsupplemented (black bars) villages in NNIPS trial. Vitamin A capsule distribution every four months was extended to control wards during round 4 (months 12–16) onward, when the trial ceased. By the sixth interval (months 20–24), mortality in the previously control (placebo-recipient) wards had fallen to the same level as in the treatment (vitamin A-recipient) wards. RR = relative risk. (From R.P. Pokhrel et al.[51])

mated impact in relation to the trial's variance, or substituting a random-effects for a fixed-effects model failed to materially alter the results.

The meta-analysis of Beaton et al.,[33] commissioned by the Canadian government at the request of the United Nations Administrative Committee on Coordination/Subcommittee on Nutrition (ACC/SCN), included all eight major studies ultimately undertaken (the six Asian and two subsequent African trials). The estimated overall reduction in mortality was a robust 23% (CL_{95} 0.12–0.32) (Fig. 2–13). When Beaton and colleagues limited their analysis to the six Asian trials, their estimated impact was 30%, remarkably similar to Tonascia's 34% using different analytic techniques and a different age range. The other meta-analyses arrived at much the same conclusions.

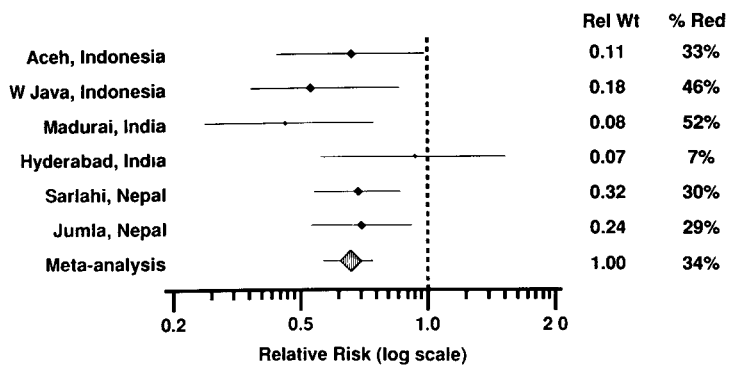

Fig. 2–12. Meta-analysis of six Asian community preschool-age mortality intervention trials. The results accorded each trial were weighted by its relative variance. (Prepared by J. Tonascia.[34,36])

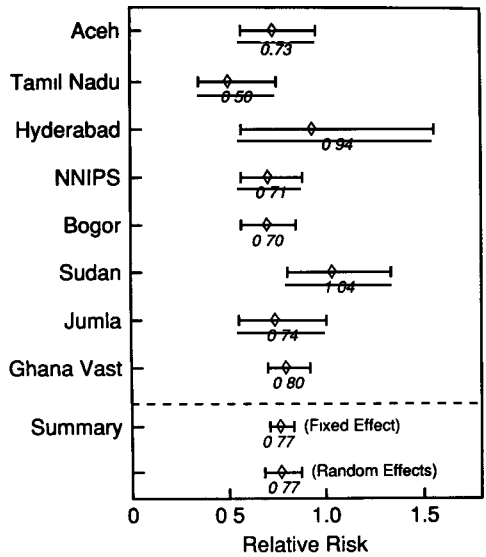

Fig. 2–13. Meta-analysis of the eight major community mortality intervention trials. A slightly different methodology was employed from that of Fig. 2–12 (see text). (From G. Beaton et al.[33])

The meta-analyses confirm that the reduction in preschool-age mortality associated with community-based improvement in vitamin A status is unlikely to have been due to chance ($p < .00000002$).[33] These results are remarkably consistent considering the enormous heterogeneity across populations that differed in their culture, ecology, xerophthalmia prevalence and underlying mortality; and the wide variations in trial design, mode of supplementation, number and size of clusters, dosing intervals, size of the vitamin A dose, duration of supplementation, and type and degree of study supervision and quality control.

All eight trials were conducted in populations in which children were considerably vitamin A-deficient. In all but Ghana, xerophthalmia rates surpassed World Health Organization (WHO) criteria for a public health problem; in Ghana the prevalence of severely depressed serum retinol levels was high.

Age-Specific Effects and Anthropometric Status

The Beaton meta-analysis concluded that the level of impact on child mortality did not predictably vary with either age or anthropometric status.[33] Subsequent data from the NNIPS study, and a reassessment of the other trials, suggest this may not, in fact, be entirely the case.

Age-specific mortality data, reported for six of the studies, are shown in Tables 2–5 to 2–11. In all six studies, vitamin A supplementation substantially reduced mortality among children six months and older. Except for Tamil Nadu, there was a distinct tendency for the benefit to increase with age. There was

Table 2–5. Age-Specific Mortality—Aceh

Age at Baseline (years)	*Program Villages*			*Control*			*Program Relative Risk*
	Deaths	*Children*	*Mortality (per 1000)*	*Deaths*	*Children*	*Mortality (per 1000)*[a]	
1	19	1979	9.6	22	1941	11.3	0.84
2	14	2086	6 7	25	2072	12 1	0.55
3	11	2274	4.8	8	2016	4 0	1.20
4	5	1887	2 6	7	1724	4.1	0.63
5	4	2686	1.5	13	2465	5 3	0 28
Total	53	10,917	4 9	75	10,230	7.4	0.66*

[a]Mortality per 1000 children

*p < 05

From A Sommer et al [41]

Table 2–6. Age-Specific Mortality—Bogor, 22 Months after MSG-A Marketed

Age at Baseline (months)	*Program*			*Control*			*Program Relative Risk*
	Deaths	*Children*	*Mortality*[a]	*Deaths*	*Children*	*Mortality*[a]	
< 12	109	1199	91	116	1134	102	0.89
12–60	77	4556	17	134	4311	31	0.55
Total	186	5775	32	250	5445	46	0 70*

[a]Mortality per 1000 children

*p < 05

From Muhilal et al [42]

Table 2–7. Age-Specific Mortality, June–October 1989, Jumla

Age (months)	*Program*		*Control*		*Program Relative Risk*
	Deaths	*Mortality*[a]	*Deaths*	*Mortality*[a]	
1–5	20	166	19	168	0 99
6–11	24	133	41	260	0 51
12–23	62	179	71	221	0.81
24–35	19	62	22	81	0.77
36–47	11	39	11	46	0.85
48–59	2	8	3	13	0.59
1–59	138	93	167	126	0.74*

[a]Mortality per 1000 child-years at risk

*p < 05

From N Daulaire et al [45]

Table 2–8 Age-Specific Mortality—NNIPS

Age at Dosing (months)	*Vitamin A*		*Control*		*Relative Risk (Vitamin A)*
	Deaths	*Mortality*[a]	*Deaths*	*Mortality*[a]	
0–5	150	57 1	130	51 6	1 11
6–11	39	28.0	47	35.8	0 78
12–23	53	19.0	75	27.5	0 69
24–35	27	9 9	31	12.0	0.83
36–47	18	6.8	28	10.6	0 64
48–59	13	4 9	25	9 7	0.51
60–72	2	2.1	4	4.2	0.50
Total 6–72	152	11.5	210	16 4	0.70*

[a]per 1000 child-years

*p < 01

Data on 0–5 months from extended, twenty-four-month trial[40] (West et al), all other ages from twelve-month trial[44] (K P West et al)

little if any effect on overall mortality during the first six months of life. The overall reduction in mortality for Aceh children (one through five years of age at baseline) was 34%. However, as vitamin A was not administered until one to three months after the baseline examination, a more appropriate estimate would exclude deaths, in program and control groups, for the initial period. This scenario raises mortality reduction to at least 41%.[53]

Overall mortality in program villages in the Bogor MSG study was 30% lower than in the program villages; among children one year and older it was 45%.[42] Jumla children in vitamin A villages died at only 74% (CL_{95} 0.55–0.99) the rate of control children, a reduction of 26%.[45] The effect was seen solely among children six months and older, for whom the reduction in mortality averaged 29%. Mortality rates in control areas remained consistent with those of prior years. Mortality in NNIPS program children six months and older was 70% that of their controls, a reduction of 30%.[44] There was a consistent drop in mortality with age.

Table 2–9. Age-Specific Mortality—Tamil Nadu

Age (months)	*Vitamin A*			*Control*			*Relative Risk (Vitamin A)*
	Children	*Deaths*	*Mortality*[a]	*Children*	*Deaths*	*Mortality*[a]	
6–11	689	4	16	678	14	21	0 28
12–35	3179	24	8	3185	52	16	0.46
≥ 36	3896	9	2	3792	14	4	0 63
Total	7764	37	5	7655	80	10	0 46*

[a]Mortality per 1000 children

*p < 05

Accidental deaths excluded

From L Rahmathullah et al [29]

Table 2–10 Age-Specific Mortality (0 to 5 Months)—NNIPS

Age at Dosing (months)[a]	*Vitamin A*		*Control*		*Relative Risk (Vitamin A)*
	Deaths	*Mortality*[b]	*Deaths*	*Mortality*[b]	
0	38	142	34	132	1 07
1	46	90	32	65	1 38
2	22	49	20	45	1 09
3	15	33	11	27	1 26
1–3	83	59	63	47	1 27
4	14	31	15	34	0 92
5	15	30	18	39	0 78
4–5	29	31	33	36	0.84
0–5	150	57	130	52	1.11[c]

[a]50,000 IU at < 1 month of life, 100,000 IU dose for children 1–5 months of age

[b]per 1000 child-years

[c]CL_{95} (0 86–1 42)

From K West et al [40]

In Tamil Nadu, mortality of children six months and older in program villages was only 46% (CL_{95} 0.29–0.71) of the mortality in control villages, a 54% reduction.[29] Tamil Nadu is the only trial in which the benefit clearly declined with age. It is uncertain whether this represents chance variation or a real difference, natural or imposed by study design (Tamil Nadu had weekly contacts).

In Ghana, the vitamin A-supplemented clusters experienced mortality rates 19% lower than that of control clusters (RR 0.81; P = .03).[47] The relative risk of dying was reduced for five of the seven age groups, but in no consistent pattern.

The concomitant but separate morbidity trial conducted in an adjacent area of Ghana also recorded fewer deaths in the vitamin A group (6 out of ~ 730) than in the placebo supplemented arm (20 out of ~720 children), a potential reduction in mortality of ~70%.[47] The impact on mortality occurred despite vitamin A treatment and subsequent exclusion of all cases of clinical xerophthal-

Table 2–11. Age-Specific Mortality (0–12 Months) after 50,000 IU Vitamin A or Placebo at Birth

	Vitamin A Recipients			*Placebo Recipients*			
Birth Weight	*Child-Years*	*Deaths*	*Mortality (%)*	*Child-Years*	*Deaths*	*Mortality (%)*	*Relative Risk (Vitamin A)*
Low (< 2500 gm)	88	6	68.3	86	8	92 5	0.74 (0 27, 2.03)
Normal (≥ 2500 gm)	882	1	1 1	871	11	12.6	0 09 (0.01, 0 70)
Total	970	7	7.2	957	19	19.9	0.36 (0.15, 0 85)

From J Humphrey et al [54 63]

mia and measles diagnosed at weekly visits, and referral of particularly sick children to clinics and hospitals.

The internal consistency of results within and between studies is remarkable. Despite the instability that small sample size imposes on age-specific estimates of mortality, in almost all of these individual trials the supplemented group (six months and older) had a lower mortality at every age.

Only NNIPS was designed to fully assess mortality under six months of age.[40] As in the Jumla trial,[45] which had many fewer children, overall mortality for children up to five months old at dosing was similar in program and control groups (Table 2–8). NNIPS data suggest that a very large dose (100,000 IU in a single bolus) may be detrimental during the first few months of life, becoming protective only at the fourth or fifth month (Table 2–10). Children initially dosed under one month of age (50,000 IU) died at the same rate as their controls. Children initially dosed at one to three months old (100,000 IU) appeared to die at a *higher* rate than their controls.

The apparent absence of a protective effect before four months may reflect special circumstances of the newborn, breast-fed child, who will have inherited considerable immunity from its mother and obtained additional passive immunity and vitamin A from breast milk. As xerophthalmia is rare among the youngest children (under two years of age), so, presumably, is moderate to severe vitamin A deficiency. However, a hospital-based study to be discussed later suggests 50,000 IU nearer birth is not only safe but offers considerable survival advantage.[54]

Less clear is the reason why the youngest children (one to three months) given large doses of vitamin A (100,000 IU) may have suffered increased mortality. Very young infants probably metabolize vitamin A differently than older infants with more mature metabolic systems. Premature infants, for example, are born with negligible vitamin A stores that generally decline during the first few weeks and months of life.[55–60] Although not the case with older children (Chapter 10), parenteral supplementation is more effective than oral in raising serum retinol in newborns, at least for the first one to four weeks of life.[61] Even more surprisingly, the apparent (but not statistically significant) excess mortality is predominantly confined to the best-nourished (i.e., least wasted) children (Fig. 2–14) (West KP, Katz J, Sommer A, unpublished data).[40]

The foregoing observations are consistent with preliminary reports from one morbidity trial suggesting that an apparent increase in acute lower respiratory infection (ALRI) is most pronounced among better-nourished children receiving high-dose vitamin A.[62] These unexpected findings await confirmation and a biologic explanation. They do suggest, however, that in determining whether to establish a *high dose* supplementation program to reduce mortality among very young infants, it may be prudent to consider both the vitamin A status and the anthropometric status of the population. The potential risk must be kept in perspective: in the Nepalese population studied, high-dose supplementation of children six months and older prevented over 150 deaths for each death to which

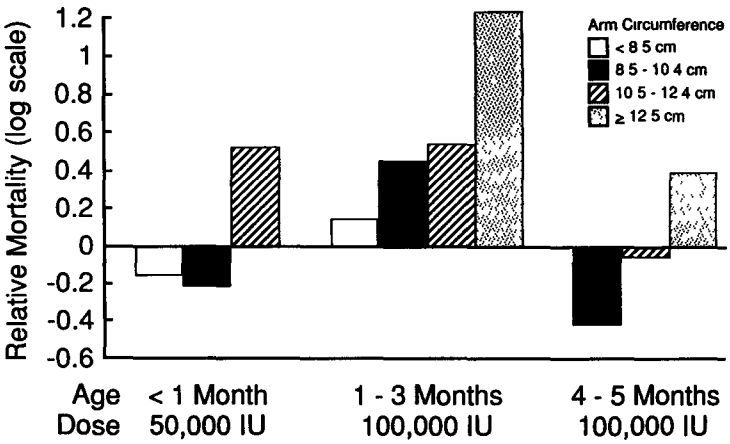

Fig. 2–14. Relative mortality among Nepalese children supplemented under 6 months of age with large-dose vitamin A. Children with the largest arm circumference (presumably least PEM) generally gained less advantage; indeed, better-nourished vitamin A recipients sometimes fared less well than placebo recipients, especially children receiving 100,000 IU vitamin A during the second through fourth months of life. (From K. West et al.[40] and unpublished data.)

it may have contributed among younger children. A smaller dose (i.e., 50,000 IU) may be entirely safe, as it appears to have been in children less than one month of age, or even protective, as was found in an Indonesian hospital-based trial.

The safety and value of directly dosing the youngest children (newborns) is supported by data from a sophisticated clinical trial recently completed in Indonesia. Newborn infants in Bandung received either 50,000 IU vitamin A or placebo in randomized masked fashion, within one to three days of birth. Over the succeeding twelve months, vitamin A recipients died at only 36% the rate of their controls (although associated confidence limits were wide). The benefit was most apparent during the second through fourth months of life (after the initial, high mortality associated with congenital malformations and neonatal tetanus, the intrinsic high death rate associated with low birth weight, and other causes of death during the first month unlikely to be affected by vitamin A status) (Fig. 2–15).[54,63] However, the impact of supplementation was considerably less positive in low-birth-weight infants (< 2500 gm) (Table 2–11).

A Bangladesh study of complex design and potentially confounding variables compared infant mortality among children born to mothers supplemented within three months after giving birth (300,000 IU). The findings suggest a sizeable reduction in mortality (≥ 30%) attributable to the maternal dose supplementation.[64] These observations close the loop. Previous reports have demonstrated that maternal supplementation raises maternal stores, vitamin A levels of breast

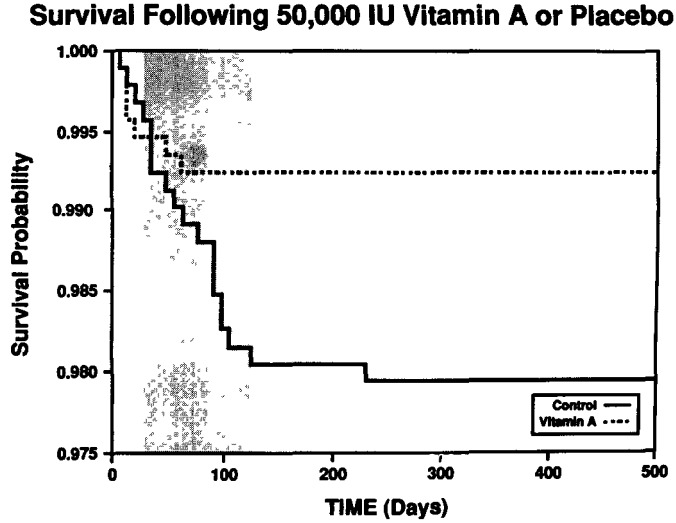

Fig. 2–15. Survival curves for children in Bandung, Indonesia, randomized to receive either 50,000 IU vitamin A or placebo at birth. The marked reduction in mortality observed among vitamin A recipients occurred almost exclusively during the 2d through 4th months of life. (From J. Humphrey et al.[54,63])

milk, and serum retinol of breast-fed children.[65,66] We now have evidence of the anticipated, beneficial impact upon infant mortality.

The implications of these potentially complex interrelationships among the youngest infants will remain uncertain until further study, though it seems likely that 50,000 IU supplements to infants and newborns is a safe and potentially effective prophylaxis against early childhood mortality (Chapter 15).

The relationship between various baseline anthropometric indices and the impact of vitamin A supplementation is variable. The meta-analysis by Beaton et al. failed to establish a consistent pattern.[33] This is hardly surprising. The interpretation of various indices and their relationship to subsequent mortality is far more complex than was once assumed; indeed, the importance of individual indices on subsequent mortality varies with age.[26]

Further, a child's anthropometric status can result from a number of interrelated factors that may or may not bear on the importance of vitamin A status. For example, the Tamil Nadu study[29] found that stunted children were more likely to benefit from supplementation than were wasted children. The authors concluded that this link was probably related to protein deficiency—which indeed may be the case (Chapter 7). However, vitamin A deficiency itself may contribute to stunting (Chapter 6), as do repeated infections. It is possible that the authors' observation reflects the fact that from the start, children at greatest risk of severe, fatal infectious episodes (and offering the greatest opportunity for positive impact) are those who are most vitamin A-deficient, suffering the highest exposure to infections, or both.

Cause-Specific Mortality

In the four trials in which cause-specific mortality was studied (NNIPS, Jumla, Tamil Nadu, and Ghana), vitamin A had its most profound and consistent effects on diarrhea/dysentery and measles. Results are summarized in Table 2–12.

Measles

All four studies show a consistent reduction in measles mortality, ranging from 18%–76%, which is particularly remarkable given the generally small numbers of cases involved. The average impact is almost identical to results of the measles treatment trials, to be discussed later in this chapter.

Diarrhea/Dysentery

All four studies reported a consistent reduction in diarrheal mortality of about 35%–50%. The number of diarrheal deaths were sufficiently numerous that the difference reached statistical significance in each of the individual trials. The Ghana trial reported that the reduction in mortality associated with chronic diarrhea or malnutrition (22 versus 36, RR = 0.67) was as sizeable as that associated with acute gastroenteritis.

Table 2–12. Cause-Specific Mortality, Vitamin A Supplementation Community Prevention Trials

	Symptoms/Diseases					
	Measles		*Diarrhea*		*Respiratory*	
Study	*Vitamin A*	*Control*	*Vitamin A*	*Control*	*Vitamin A*	*Control*
TAMIL NADU[29]						
Deaths (n)	7	12	16	33	2	3
RR[a]	0 58		0 48		0 67	
NNIPS[44]						
Deaths (n)	3	12	39	62	36	27
RR	0 24		0 61		1 29/1.00[b]	
JUMLA[a45]						
Deaths (n)	3	4	94	129	18	17
RR	0 67		0 65		0.95[c]	
GHANA[47]						
Deaths (n)	61	72	69	111	47	45
RR	0.82		0.66[d]		1.00	

[a]RR (Relative Risk) Cause-specific mortality rate of vitamin A group divided by rate in control group

[b]Original published results[44] RR = 1 29, reanalysis as an associated cause that recognizes other underlying causes RR = 1 00 (K P West, unpublished data)

[c]Pneumonia case management program may have confounded results

[d]Defined as "acute gastroenteritis"

[e]Except for Jumla, findings relate to children already ≥ 6 months of age when supplemented

Respiratory Infections

Excluding the tiny number of respiratory deaths in Tamil Nadu (2 in the vitamin A group versus 3 among controls), there was little evidence that vitamin A supplementation had any impact on the rate of respiratory deaths. A special WHO working group and related meta-analyses reached the same conclusion.[67,68]

Other Causes

Three of the studies recorded a significant reduction in mortality from less certain causes:

- Tamil Nadu—"convulsions" were reduced by 75% ($p < .05$), as were "other (infections)";
- NNIPS—"uncertain (infections)" were reduced by 48% ($p < .02$ by Z—test—unpublished data);
- Ghana—"meningitis" was reduced by 34%, "other" by 27%, and "not known" by 19% (individually, none of these differences were statistically significant).

The results of these four trials are particularly striking. None was designed with sufficient power to detect cause-specific mortality; each was hampered by difficulties in assigning cause of death from often complex, multi-system derangements,[69] frequently using data collected weeks to months after the event from medically unsophisticated relatives of the deceased child. Even trained health workers present at the time of death can have difficulty accurately designating *the* cause of death in children with multiple illnesses. An autopsy study at the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), in Dhaka, revealed that pneumonia was the underlying cause of death in 40% of children hospitalized with severe diarrhea.[70]

Magnitude of Mortality Reduction

> Vitamin A status probably modulates the incidence and severity of disease caused by a variety of pathogenic organisms. The impact that vitamin A supplementation will have on mortality will therefore depend upon a constellation of factors, including the prevalence and severity of vitamin A deficiency, the frequency of exposure to pathogenic organisms, size of the inoculum, and their virulence; and the presence and degree of other adverse influences with which the young child must contend (e.g., malnutrition, parasitic load)
>
> —A. Sommer et al , 1986[41]

The observational studies and community prevention trials provide sufficient data to assure policy makers (with 98% confidence) that improving the vitamin A status of their own populations, if similar to those studied, will almost certainly reduce preschool age mortality.[33] What remains unclear is the size of the reduction. Two major reasons account for this uncertainty: the precise extent of the impact in the studies already undertaken; and local factors that might modify the outcomes.

The published meta-analyses provide rough estimates of potential impact, but these were recognized to be "ballpark" figures related, in part, to the ways in which these analyses were prepared. The Tonascia analysis[34–36] (Fig. 2–12) employed results reported by the individual investigators of the six Asian trials as, for the most part, "deaths per child-intervals at risk." Beaton (George Beaton, personal communication, XIV IVACG Meeting, Arusha, Tanzania, February 1993) agreed this was the preferred method; but since he was unable to gather sufficient data to incorporate all eight trials into this approach, he employed the simpler device of calculating the proportion of enrolled children who died, ignoring their duration at risk (Fig. 2–13). These pooled estimates, ranging from a 23%[33] to a 34%[35] reduction in all-cause mortality, should not be taken at face value. They reflect *reported* reductions in all-cause mortality ranging from 0%[30] to 54%,[29] results that themselves are likely to underestimate the potential impact:

1. Several studies reported overly conservative estimates. The initial Aceh publication (34% reduction) evaluated the reduction in preschool (one to six years) deaths by including *all* deaths, even though large-dose supplements were first administered one to three months *after* baseline enumeration.[41] Simple exclusion of all deaths during the first three months raises the apparent impact to 41%.[53]
2. The intent-to-treat analysis reported by each of the studies provides an estimate of the impact likely to be observed in a particularly well-sustained supplementation program. But it underestimates efficacy: the impact potentially achieved if all target children received the intervention (perhaps through fortification of an item consumed by all children, full coverage with supplements, or a universal change in dietary behavior among those who were most deficient). In most public health intervention programs, those who fail to participate (noncompliers) are generally those who would benefit most.[71] In Aceh, for example (Fig. 2–16), children assigned to the first vitamin A dose who didn't receive it died during the ensuing six months at three times the rate of *controls.*[72] After the first distribution, almost all deaths in "vitamin A villages" were among children who had not received a vitamin A capsule: fifteen deaths among 2256 children who did not receive their capsule versus three deaths among 9573 children who did. Higher mortality among nonrecipients than among controls reflects selective, nonrandom participation. It is unlikely the Hawthorne

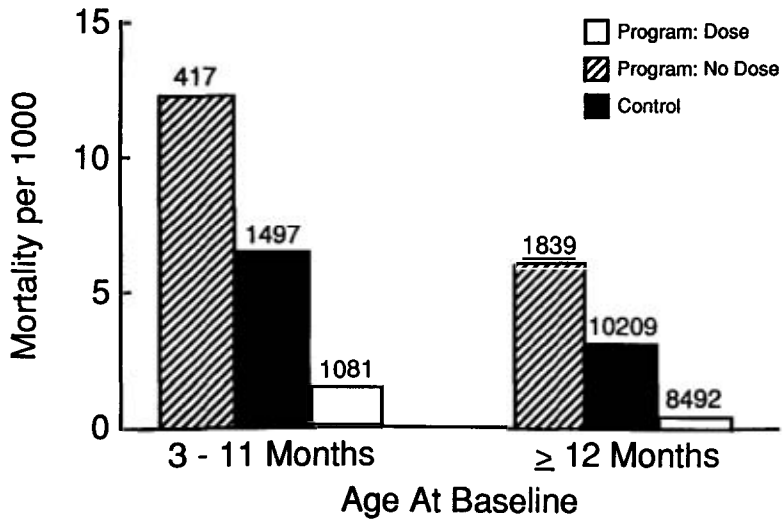

Fig. 2–16. Mortality in the Aceh trial among children in treatment villages who did and did not receive their intended vitamin A dose, compared with children in control villages. Mortality was higher among children assigned to receive vitamin A but didn't get it than for any other group, including children in control villages. (Drawn from I. Tarwotjo et al.[72])

Effect played a significant role in this disparity since contacts in the Aceh study were rare and infrequent. Noncompliance is therefore an important impediment to any intervention, and its impact on outcome disproportionate to the percentage of target children reached.

The actual level of impact among *participants* (efficacy) can be approximated directly by comparing mortality among only those who actually received their assigned supplement, or indirectly through a statistical approach if a placebo was not used.[53,72] In Aceh, the *efficacy* was estimated at 72% (CL_{95} 41%, 87%).[53] This does not mean that the reduction in mortality in Aceh would have been 72% if all children assigned to participate actually did. We have no way of knowing the degree to which dosing would reduce mortality among children who failed to participate: it might be more or less than the 72% estimated for those who did participate. It would surely be greater than *zero,* which is the assumption inherent in the intent-to-treat analysis; and the overall impact would therefore certainly have exceeded the estimated 34%–41% reported. Small, frequent dosing in Madurai (Tamil Nadu), with its inherently higher effective compliance, might explain (at least in part) the greater impact observed in the Tamil Nadu trial (54%).[29]

3. In most studies, children with xerophthalmia were identified (at least at baseline and sometimes at interim visits) and treated. Thus, children at the highest risk of dying,[1] and therefore with the most to gain from vitamin A, were effectively removed from the controlled comparison.

4. Periodic large-dose vitamin A supplementation every four to six months may prevent xerophthalmia and blindness,[10] but provides less than optimal vitamin A nutriture.[73–76] In moderately to severely deficient populations, large-dose periodic administration may increase serum retinol for only two to four months,[75] and in mildly deficient populations it maintains a normal relative dose-response (RDR) (a surrogate for "safe" liver stores) for only four to six months.[74] More frequent administration, as in Tamil Nadu,[29] was probably more physiologic, causing greater vitamin A retention[49,50] and a more sustained improvement in vitamin A status. While weekly dosing requires a prodigious infrastructure, fortification, as in Bogor,[42,43] provides a relatively inexpensive mechanism for achieving the same end (Chapter 15).
5. The maximum measurable impact of isolated alteration in vitamin A status requires a sustained improvement in the vitamin A status of program children (optimally into the "normal" or "sufficient" range), with little or no change in the vitamin A status of control children or in the underlying pattern of childhood disease responsible for their preschool mortality. Few, if any, of the intervention trials achieved this ideal. The effect of even minor, transient alterations in any of these factors is readily apparent from the "spontaneous" variation in mortality observed among placebo recipients in NNIPS (Fig. 2–11).[51]

If a trial does not cause a meaningful difference in vitamin A status between the two comparison groups, or if it reduces the background level of disease and mortality upon which the vitamin A was to act, it becomes difficult if not impossible to demonstrate a measurable impact. These problems are thought to have interfered with the Hyderabad and Sudan studies, the two trials with the lowest (and nonstatistically significant) alterations in mortality.[33] They may in fact represent valuable exceptions that help prove the rule. Mortality rates in Hyderabad, even in the control arm, were *far* lower than anticipated, an outcome attributed by the authors to frequent home visits by trained health workers attached to the study.[46] Not only did this drastically reduce the power of the trial to detect a difference in mortality (as evidenced by its wide confidence limits [Figs. 2–12, 2–13]), but may have specifically reduced just those forms of mortality most susceptible to vitamin A status. Further, a potentially larger impact may have been obscured by differential loss to follow-up in the two study arms and the relatively low level of subject compliance.[33,77,78]

The Sudan trial may have created relatively little difference in the vitamin A status between the two groups studied,[33] as represented by the negligible impact of supplementation on subsequent rates of xerophthalmia (both groups appear to have had the same prevalence of xerophthalmia at the end of the trial [Table II in their original report])[30,79]. The population might not have been particularly deficient to begin with (reported xerophthalmia rates notwithstanding, almost half the children lived in houses with latrines and piped-in water

supplies, atypical of disadvantaged Third World children). Even if the remaining children in the Sudan trial were deficient, the effective sample size was halved and the power to detect a difference in outcome reduced accordingly.

The authors of the Sudan study suggest 200,000 IU every six months may have been inadequate supplementation for their population.[30,38] As Beaton et al. point out, while this may have been the case, there is no evidence to support it.[33] Indeed, this explanation seems highly unlikely. As seen from the cumulative mortality curves (Figs. 2–6 to 2–8), a single large dose affects mortality within a very short period, certainly before a second dose is given. While there is reason to suspect a large dose may not produce optimal vitamin A status over a sustained period,[10,73–75] 200,000 IU should have been adequate, even in the Sudan, to yield an effective response for at least half to two-thirds the inter-dose interval, thereby producing half to two-thirds the estimated impact (rather than none at all).

Quite apart from the individual trials to date, and their pooled (if imperfect) estimates of impact, one would expect outcomes of intervention programs to vary in relation to local population and ecological factors that determine the *potential* for impact; and in the nature and success of the programs themselves. Local variables include the severity and prevalence of underlying vitamin A status, and the impact it exerts on residual causes of childhood mortality. In both Ghana and Tamil Nadu, vitamin A supplementation reduced measles and diarrhea-related deaths to a similar degree. In Ghana, but not in Tamil Nadu, 23% of all preschool deaths were attributed to malaria, a cause of death seemingly unaffected by vitamin A supplementation.[47,80] Hence, all else being equal (which is rarely the case), improvement in vitamin A status would reduce all-cause mortality to a greater degree in Tamil Nadu than in Ghana—exactly what was observed.

Similarly, the dramatic reduction in measles mortality associated with vitamin A supplementation will have less impact on all-cause mortality where measles immunization rates are high than in populations where they are low and measles epidemics are therefore common. Comparable considerations may explain, in part, why vitamin A has limited potential for protection among some children less than five months of age, but a strong beneficial impact among older children. Pneumonia is a leading cause of death in children under five months, and seems relatively impervious to vitamin A status; diarrhea accounts for almost half the deaths among older children,[81,82] and is sensitive to vitamin A status.

The impact of intervention will also be determined by the degree to which it improves vitamin A status, particularly among those who are most deficient. Simply raising mean serum vitamin A levels to a normal range is not necessarily indicative of success. Raising levels among the most compliant children, who generally need help the least, may increase the overall average without ever impacting on more deficient, noncompliant children who need it most. Individuals who fail to comply with public health interventions are almost always at higher intrinsic risk than their peers seeking better health, whether the outcome is

mortality,[28,29,53,72] high cholesterol[83,84] or cholera.[71] As already noted, in Aceh the noncompliers died at twenty times the rate of vitamin A recipients and *three times the rate of controls.*[72] Similar results were reported from Hyderabad.[28,46]

Mortality Attributable to Mild Vitamin A Deficiency

Despite the many factors working to minimize the *apparent* impact of vitamin A supplementation on childhood mortality, these "underestimates" exceed the estimated excess mortality attributable to clinical xerophthalmia (moderate vitamin A deficiency). For example, the most conservative intent-to-treat analyses, for Aceh and Bogor, suggest a mortality reduction of 34%–41%. In contrast, the observational study in West Java[1,23] (having the same xerophthalmia prevalence observed in Aceh during the trial) predicted that prevention of all xerophthalmia would reduce mortality by only 16%.

It therefore seems likely that the vast majority of preschool children with mild or marginal vitamin A deficiency, but not xerophthalmia, suffer excess mortality as well.[36,85] This should come as no surprise, as xerophthalmia is a relatively late manifestation of slow depletion of vitamin A stores. It generally follows keratinizing metaplasia of the respiratory and genitourinary tracts; altered impression cytology (and other histologic manifestations of abnormal cellular differentiation) (Chapters 4, 11); reduction in immune system response (Chapter 9); anemia (Chapter 5); and perhaps growth failure (Chapter 6) and other evidence of impaired physiology. As has been shown in vitamin A-deprived animals, mortality rises sharply long before the appearance of xerophthalmia (Fig. 2–1).

Hence, while much of the mortality reduction associated with improved vitamin A status may be related to preventing severe deficiency associated with xerophthalmia, a significant proportion, perhaps a majority, of deaths averted may represent reduced mortality among the larger number of children who are less deficient.[1,23] The 16% of childhood deaths attributable to xerophthalmia in Indonesia was calculated from the comparison in mortality between mildly xerophthalmic (XN and/or X1B) children and their non-xerophthalmic (but apparently deficient) controls (RR = 1). Improving the vitamin A status of the mildly deficient, non-xerophthalmic controls by raising their serum vitamin A from 18 μg/dl–20 μg/dl to 30 μg/dl would conservatively be expected to reduce their mortality rate by 30%–50%, as suggested by the CPTs. Accordingly, Table 2–13 revises the estimated excess mortality associated with inadequate vitamin A nutriture of varying severity. If improving the vitamin A status of the deficient but non-xerophthalmic controls halves their mortality, it doubles the relative mortality of all the groups followed in the Indonesian observational study, and correspondingly contributes to excess deaths. While the assumptions employed are approximations, they provide a useful order of magnitude.

Because decreased survival is directly related to the severity of deficiency, xerophthalmic children (with the most severe deficiency) will have the highest

Table 2–13. Vitamin A Status and Extrapolated Excess Mortality—Indonesia

Vitamin A Status	Extrapolated Mortality (as Relative Risk)		Proportion of all Children[d]	Extrapolated Proportion of "Excess" Deaths[e]
	Observed in Observational Study[a]	Extrapolation[b]		
Adequate (supplemented)	(intervention trials) (~1/2)	1		
Deficient (non-xerophthalmic) "controls"	1	2	40.0%	56%
XN+, X1B−	3	6	3 3%	14%
XN−, X1B+	6	12	0.9%	8%
XN+, X1B+	9	18	1 4%	18%
X2	—	50	< 1%	2%
X3[c]	—	100+	< 1%	3%

[a]See Table 2–1

[b]RR rescaled from preceding column based on CPTs, field epidemiologic data and hospital experience (X2/X3)

[c]Untreated X3 probably has an absolute mortality rate approaching 95% [10]

[d]Among "controls" ("random sample" Table 1–3) 45% had serum levels < 20μg/dl, of whom 5% had xerophthalmia Assume X2 + X3 = 0 08% [27]

[e]Proportion of excess deaths = excess deaths in that stratum of deficiency − total excess deaths among all "deficient" children

relative mortality; but children with less severe vitamin A deficiency (and proportionately lower excess mortality) represent a larger segment of the population, and therefore a significant proportion of all unnecessary deaths.

Calculations suggest vitamin A deficiency may be responsible for as many as 1 million to 2.5 million deaths annually worldwide.[86]

Vitamin A Therapy Reduces Measles Mortality

Measles represents a special case of vitamin A deficiency-related morbidity, mortality and blindness (Chapters 3, 4, 7). Measles not only accounts for a large proportion of preventable childhood blindness, particularly in Africa (Chapter 4), but acute and delayed mortality as well.[87–89] Measles-associated mortality was consistently reduced by ~50% in the four CPTs in which cause-specific mortality was investigated (Table 2–12).

In a recent observational study, Markowitz[90] and co-workers studied 283 children up to five years of age hospitalized in Zaire with moderate to severe measles. Overall case fatality was an astounding 26%. In univariate and multivariate analyses (adjusting for wasting, among other variables), depressed serum vitamin A (< 5 μg/dl) was associated with a threefold risk of mortality (RR = 2.9 [CL_{95} 2.3, 6.8]), but only among children less than twenty-four months old.

Depressed vitamin A levels were also associated with younger age, wasting, and the presence of pneumonia at the time of admission. Similar observations link severity of measles illness with lower serum retinol in the United States.[91]

Three controlled measles treatment trials (MTTs) have demonstrated that vitamin A treatment, *after* the onset of measles, reduces associated mortality.[92–94] A fourth study by Coutsoudis et al. carefully assessed the impact of treatment on morbidity (Chapter 3).[95,96] The first of the most recent trials was conducted in Tanzania.

Tanzanian Trial

Soon after recognizing the relationship between vitamin A status and all-cause mortality[1] (indeed, before the report had even appeared), a hospital-based study was designed to test the hypothesis that vitamin A deficiency contributed to measles mortality in Africa; and that vitamin A supplementation might reduce such mortality, even if administered after the onset of disease.

Children admitted to a Tanzanian missionary hospital with moderate to severe measles were randomly assigned to receive either standard therapy alone (including antibiotics where indicated) or standard therapy in combination with 200,000 IU vitamin A administered orally on admission and again the following day.[92] Children who presented with xerophthalmia or corneal ulcers were immediately treated with vitamin A and excluded from the trial. Only 9% of subjects had serum vitamin A levels above 0.35 μmol/liter ("low"), despite the exclusion of cases with clinical xerophthalmia.

Nineteen children died: six of the eighty-eight vitamin A recipients; and twelve of the ninety-two controls (Table 2–14). The vitamin A group suffered only half the mortality of the controls. All of the benefit was confined to children below two years of age ($p < .05$). Vitamin A recipients fared better than controls in every category of weight-for-age ($p < .05$) (Table 2–15).[92] Complications accompanying measles, usually present on admission, were common in both groups. In every instance, mortality among vitamin A recipients was lower than among controls, particularly for croup/laryngotracheobronchitis (LTB) (Table 2–16).

By the time these data were published, results of the Indonesian observational study[1,23] and Aceh intervention trial[41] were widely known. So, too, were the increased risks of respiratory and diarrheal disease in vitamin A deficient children,[24] and the increased risk of xerophthalmia among those with systemic infections[98]—particularly measles.[1c] Within four months of publication of the Tanzanian vitamin A treatment trial, WHO and UNICEF issued a Joint Statement recommending immediate high dose supplementation of all children with measles from communities where vitamin A deficiency was a "recognized problem" or where measles case-fatality rates were 1% or greater.[99,100] This recommendation was meant as much to reduce mortality as to prevent blindness. WHO and

Table 2–14. Measles Mortality—Tanzanian Trial

	Vitamin A			*Controls*			*Relative Risk*
Age (months)	*Children*	*Deaths*	*Mortality (%)*	*Children*	*Deaths*	*Mortality (%)*	*Controls: Vitamin A*
< 9	14	0	—	9	2	22	
9–11	12	0	—	10	2	20	
12–23	20	1	5	23	3	13	
0–23	46	1	2.1	42	7	16 7	8:1*
24–35	11	3	27	16	2	13	
36–47	11	1	9	13	1	8	
48–59	8	1	13	6	0	—	
≥ 60	12	0	—	15	2	13	
24– ≥ 60	42	5	11.9	50	5	10	0 84 1
0– ≥ 60	88	6	7	92	12	13	1 9 1

*p < 05

From A Barclay et al [92]

UNICEF also suggested serious consideration be given to instituting programs to prevent vitamin A deficiency and its exacerbation by measles (with its attendant increase in morbidity and mortality) by initiating routine high-dose supplementation of all preschool-age children. As was pointed out in an invited commentary, the latter approach has the potential for greater impact.[101] Attempting to boost vitamin A reserves only among children who present with measles "is like putting out a raging fire: some homes will be saved but a substantial proportion will burn down before aid arrives."[101]

Table 2–15. Measles Mortality by Nutritional Status—Tanzania

	Vitamin A			*Controls*			*Relative Risk*
Weight-for-Age[a]	*Children*	*Deaths*	*Mortality (%)*	*Children*	*Deaths*	*Mortality (%)*	*Controls Vitamin A*
> 80%	24	1	4.0	27	3	11.0	2 8.1
60–80%	47	1	4 0	57	6	11.0	2.8 1
< 60%	17	4	24 0	8	3	38.0	1.6:1
Total	88	6	6.8%	92	12	13.0%	1.9:1

[a]Percent median NCHS standards [97]

From A Barclay et al [92]

Table 2–16. Measles Mortality by Complications—Tanzania

	Vitamin A			Controls			Relative Risk
Complications	Number With Complications	Deaths	Mortality (%)	Number With Complications	Deaths	Mortality (%)	Controls. Vitamin A
Pneumonia	38	3	8.0	47	7	15.0	1.9·1
Otitis media	19	1	5.0	20	3	15.0	3.0.1
Croup/LTB	8	0	—	13	4	31.0	(31 0)
Dysentery	2	1	50.0	6	3	50.0	1.0·1
Hemorrhagic rash	28	1	4.0	34	4	12.0	3.0:1
Oral candida	9	1	11.0	5	1	20.0	1.8.1

From A Barclay et al [92]

Cape Town Trial

A superbly conducted hospital-based trial, similar in design to the Tanzanian study, was reported from Cape Town, South Africa, in 1990.[94] Children with measles requiring admission for treatment of complications were randomly assigned to receive 200,000 IU water-miscible vitamin A or placebo orally on admission, and again the following day. Children who had already received vitamin A, suffered from xerophthalmia (none in this instance), or had a rash more than four days old were excluded. All subjects received oxygen, intravenous fluids, and antibiotics as appropriate. Two-thirds of the subjects were below twelve months of age and 85% were under two years. Serum retinol was below 20 μg/dl (0.7 μmol/liter) in 92% of the children; almost 50% had levels below 10 μg/dl (0.35 μmol/liter).

Mortality was markedly reduced in the vitamin A group (Table 2–17).

Hussey and Klein subsequently compared the results of their controlled trial with outcomes in a much larger number of children followed in uncontrolled fashion. They examined their hospital records before (1985–1986) and after (1989–1990) vitamin A supplementation (200,000 IU for two successive days) had become routine therapy.[102] The latter group, which received vitamin A as a matter of course, experienced lower mortality (1.6% versus 5%, $p < .001$), a shorter hospital stay (10 versus 13 days, $p < .001$), and less frequent need for intensive care (4.3% versus 10.5%, $p < .001$).

London Trial

Ellison carried out a long overlooked hospital-based clinical trial in London fifty years before the Cape Town trial took place.[93] Understandably, it employed a somewhat cruder design.[33] Children admitted to the Grove Hospital were assigned

Table 2–17. Measles Mortality—Cape Town Trial

Age (months)	Vitamin A			Controls			Relative Risk
	Children	Deaths	Mortality (%)	Children	Deaths	Mortality (%)	Controls Vitamin A
< 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

From G Hussey et al [94]

routine therapy or routine therapy plus 1 ounce of cod liver oil (high in vitamins A and D) daily. Results were virtually identical to those of the Tanzanian and Cape Town studies. Mortality among controls under two years old was 16%; mortality among cod liver oil recipients was half that of the controls (Table 2–18). As in Tanzania, mortality was reduced among cases already complicated by pneumonia.

To bolster his conclusions, Ellison compared the mortality rates of controls with nearly 5,000 previous measles admissions (October 1929–July 1930); age-specific and overall mortality (8.1%) were virtually identical.[93] Ellison concluded the value of cod liver oil came from its vitamin A, since previous studies[103] had failed to demonstrate any association between vitamin D and infection.

Overview

Mortality among control children up to 23 months of age was similar in all three treatment trials, as was the reduction in mortality among their vitamin A-recipient

Table 2–18. Measles Mortality Trial—London

Age (years)	Treated			Controls			Relative Risk
	Children	Deaths	Mortality (%)	Children	Deaths	Mortality (%)	Control Treated
0–1	31	1	3 0	32	4	13.0	4.3 1
1–2	101	8	8 0	90	15	17 0	2 1 1
2–3	55	1	2 0	60	4	7 0	3 5·1
3–4	61	1	2.0	61	2	3 0	1.5.1
4–5	52	0	—	57	0	—	0 0
Total	300	11	3 7	300	25	8.3	2 4·1

From J Ellison [93]

peers. The great bulk of all deaths (among controls) was in children under two years.

Given the small sample sizes and great differences in geography, population, time, age distribution, and case management, overall reductions in mortality in the three studies are remarkably consistent (Fig. 2–17).

Reduction in measles mortality by *treatment* with vitamin A suggests that a change in vitamin A status can rapidly alter basic physiologic functions concerned with cellular repair and resistance to infection; a conclusion for which there is growing evidence at the molecular level. It also suggests these changes should be reflected in a reduction in the incidence and/or severity of measles complications, phenomena well documented by Hussey and Klein,[94] Coutsoudis,[95,96] and others (Chapter 3).

However, unpublished trials conducted in three hospitals in the Philippines (personal communications, L.Lucero, 1993), terminated prior to reaching statistical significance, suggest the issues may sometimes be more complex. The Philippines studies used only half the vitamin A dose of the two large hospital-based MTTs (and Coutsoudis' morbidity study) in Africa. Two of the Philippine hospitals recorded reduced case fatality among vitamin A recipients; one reported increased mortality. The latter, a tertiary pneumonia referral hospital, differed from the other two hospitals (and the African treatment trials) by requiring the presence of severe pneumonia on admission, and by enrolling children long past the onset of their disease (as long as eighteen days, versus less than five days post-onset of rash). In addition, the children were all severely ill and severely malnourished; but only a third had low or deficient serum vitamin A levels. The significance of these results is difficult to ascertain, though they suggest vitamin

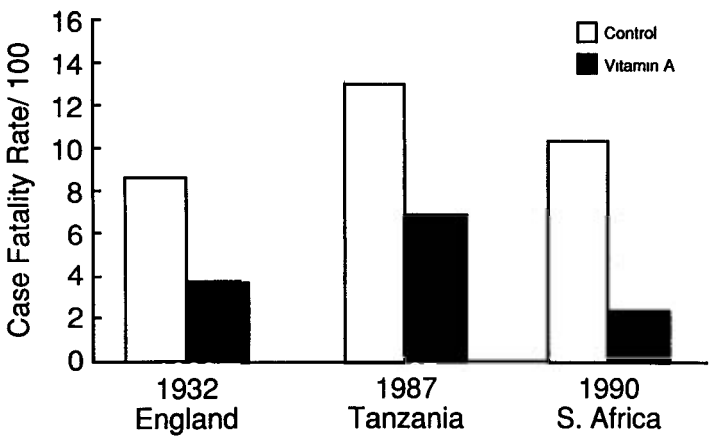

Fig. 2–17. Measles case-fatality rates among hospitalized patients randomized to receive high-dose vitamin A (cod liver oil in the London trial) compared with those of their controls. Vitamin A supplementation reduced mortality by ~ 50% in all three trials.[92,93,94]

A supplementation may be more effective earlier in the course of complicated measles. The principal investigator of this study, Dr. Luluth Lucero, undertook a meta-analysis of extant trials and concluded that even in the Philippines, children hospitalized with measles should receive large-dose supplemental vitamin A regardless of the severity of illness.

To explain the lack of clinically recognized xerophthalmia in their Cape Town population, Hussey and others proposed that vitamin A was not reaching target tissues (hyporetinemia) in children with otherwise adequate vitamin A stores.[94,104] The suggestion is consistent with the claim that administering a very large dose of vitamin A at a critical moment overcomes a measles-induced block of vitamin A metabolism, rather than addressing underlying measles-induced exacerbation of vitamin A deficiency.[29,105] However, there is little evidence that this is the case. Quite the contrary. Measles mortality was reduced to the same degree in the CPTs (Table 2–12, Fig. 2–18) even though large doses were never used (Tamil Nadu) or were not timed to an acute measles episode. It seems more likely that measles mortality was reduced secondary to the improvement of vitamin A status of deficient children. For the same reasons it is highly doubtful that the impact observed in the hospital treatment trials represents a nonspecific "adjuvant" effect of a large-dose of vitamin A.[106]

Most likely, children studied by Hussey and Klein had suboptimal vitamin A stores to begin with and their status was further eroded by their severe measles. This would help account for other observations, including recent associations between measles morbidity and mortality and low serum levels in the United States.[91,107–109]

These considerations again raise the issue of treating measles versus prophylactically improving the vitamin A status of all children. Both approaches appear

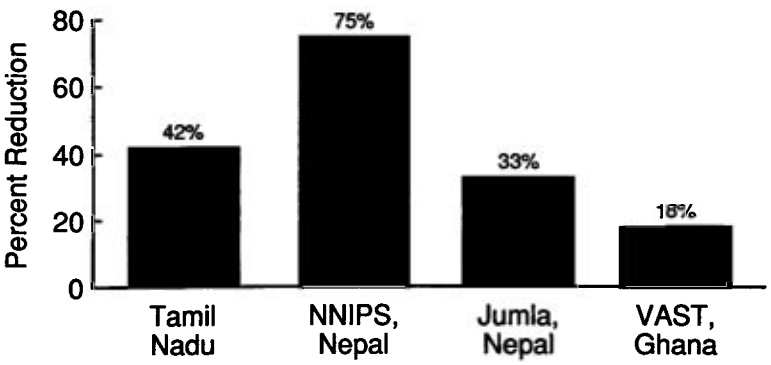

Fig. 2–18. Reduction in measles-related mortality among children in vitamin A-recipient villages compared with measled-related mortality among children in control villages. Results are comparable to those of the hospital-based measles treatment trials (Fig. 2–17). Only four of the eight major community mortality prophylaxis trials investigated cause-specific mortality.

to have the same level of impact among recipients. Even from the narrow perspective of measles, improving the vitamin A status of all children will increase their ability to withstand a measles attack and thereby protect them from developing serious complications in the first place, from which they might well die or suffer long-term sequelae before ever reaching a hospital. It is also the only way to help those who haven't access to urgent care.

On the other hand, this strategy will take considerable time to accomplish, particularly since the long-term goal is normal, not merely improved, vitamin A status. Therefore, treatment with vitamin A of all cases of measles, as has been repeatedly urged, makes enormous good sense.[69,99,100] It not only provides vitamin A to those who may still be deficient, but also increases their chances of maintaining an adequate vitamin A status afterward. As Berggren insightfully points out,[110] widespread measles coverage under the WHO Expanded Program on Immunization (EPI) should not alter this policy. Since many children develop measles before they are old enough to be successfully vaccinated,[88,89,111-113] and with vaccine efficacy considerably less than 100% among current recipients in Third World countries,[89] a million children die of measles annually despite EPI efforts.[114] Vitamin A therapy offers a safe, simple, inexpensive, accessible and effective means for further reducing this number. Even the American Academy of Pediatrics recommends vitamin A treatment of measles in selected patients in the United States.[115]

It should be noted that the two African measles mortality trials (and Coutsoudis' important morbidity study [Chapter 3]) all administered vitamin A on two successive days (in the South African studies, water-miscible, not oil-miscible, vitamin A was used; the Tanzanian trial used standard, oil-miscible preparations). Until studies demonstrate that a single dose is just as effective as a dose on two successive days, it is probably prudent to follow the double-dose schedule already proven rather than the single-dose treatment recommended by WHO and UNICEF.[99,100] Indeed, EPI has recently expanded the indications for use of vitamin A in measles to "all cases of severe measles," not just to children from populations where vitamin A deficiency is known to exist or where measle case fatality exceeds 1%.[116]

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3

Infectious Morbidity

> It is, in fact, difficult to avoid the conclusion that an important, and probably the chief, function of vitamin A from a practical standpoint is as an anti-infective agent, and that a large number of common infective conditions are due to a deficiency of this substance in the diet of many people
>
> —Green and Mellanby 1928[1]

> no nutritional deficiency is more consistently synergistic with infectious disease than that of vitamin A
>
> —Scrimshaw et al., 1968[2]

The strong impact of vitamin A status on mortality (Chapter 2) must be mediated, in large part, by its effect on the incidence and/or severity of life-threatening infections. Vitamin A prophylaxis and treatment have their clearest efficacy in reducing mortality associated with diarrhea and measles. An understanding of the relationship(s) between vitamin A status and infection is important in identifying the mechanisms that account for altered mortality and in quantifying the burden of infectious morbidity associated with vitamin A deficiency. These relationships are being examined with growing interest [2–5].

As already noted, animals develop infections relatively early in the course of vitamin A deficiency,[1,2,6–10] often dying of overwhelming sepsis before developing xerophthalmia [1,11]. In 1924, Webster reported that mice raised on "McCollum's complete diet" were much more resistant to the lethal consequences of an oral challenge with mouse typhoid bacillus than were apparently equally healthy mice raised on the standard Rockefeller Institute diet [12]. By 1930, Green and Mellanby had demonstrated a dose-dependent relationship between dietary carotene intake and the risk and severity of infection in rats [13]. Vitamin A-deficient animals raised in a germ-free environment grow better and live longer than animals

raised in conventional facilities [14,15] Survival of conventionally reared, deficient animals can be dramatically extended by the use of broad spectrum antibiotics [16] Organs particularly susceptible to infection in deficient animals include the respiratory, genitourinary, and gastrointestinal tracks Massive infection involving the respiratory tract is often a terminal event, though sometimes more apparent at autopsy than antemortem [1]

Early clinical reports, largely anecdotal, suggested similar relationships existed between vitamin A status and human infection Thus, blinding xerophthalmia (severe vitamin A deficiency) has been associated with respiratory tract infections,[17–24] diarrhea,[18,20,25–30] tuberculosis,[17,21,31,32] childhood exanthems (chickenpox and measles),[18,20,24,33–39] pertussis,[17,40] scarlet fever,[18,30,41] typhoid,[18,42] malaria,[31] encephalitis,[30] and urinary tract[18,43,44] and other infections It is no surprise that severe, chronic infections leading to debilitating malnutrition and dehydration are complicated by severe vitamin A deficiency and xerophthalmia [18,43] Also well known is that decompensation of borderline vitamin A status by infections that have a particularly deleterious effect on vitamin A metabolism (such as measles) result in acutely severe vitamin A deficiency At issue is whether vitamin A deficiency, per se, increases the incidence and/or severity and outcome of infectious episodes Interpretation of most cross-sectional associations, whether clinical reports or population-based surveys, is complicated by

- the bidirectional relationship between vitamin A status and infection—each appears to increase the risk of the other[2,3,45,46] (Chapter 7 deals with evidence that systemic infections increase the risk of subsequent vitamin A deficiency and xerophthalmia[47,48]),
- the frequency with which xerophthalmia and diseases with which it has long been associated occur in developing countries (turn-of-the-century Europe as much as today's Third World) where the risks of infection, malnutrition, and poverty are deeply entwined,
- the varying nature of the studies, populations, instruments, and definitions employed,
- differences in the clinical recognition and significance of infectious episodes

Some of the earliest clinical reports suggested the possibility that vitamin A deficiency, by itself, might specifically increase susceptibility to infection Bloch noted that among children on vitamin A–poor diets, growth failure was commonly followed by infections, particularly of the respiratory tract, urinary tract, and middle ear [43] These infections, which had otherwise been resistant to conventional therapy, disappeared after treatment with vitamin A (as cod liver oil, butter, cream, or whole milk)

By 1934, Mellanby had concluded that humans became more susceptible to infection long before vitamin A reserves were depleted or xerophthalmia was

Table 3–1 Cross-Sectional Associations Vitamin A Status and Infections

Report	Origin of Subjects[a]	Vitamin A Status	Morbidity[b]				
			Cough/LRI	Diarrhea/GI	Measles	TB	UTI
BANGLADESH							
Brown et al [44]	C/H	XN-X3	—	—		—	+
Mahalanabis et al [59]	C/H	XN/X1B/X2/X3	+	+	+		
Stanton et al [56]	Pop	XN	—	+	—		
INDIA							
Gujral et al [62]	Pop	XN/X1B	—	+	+		
NEPAL							
Brilliant[57] Brilliant et al [58]	Pop	X1B	—	+	+/−		
THAILAND							
Bloem et al [65]	Pop	serum retinol	+	+			
PHILIPPINES							
Solon et al [40]	Pop	XN/X1B	+	—	—	+	
INDONESIA							
Sommer[45]	Pop, C/H	X1B		+	—		
Sommer[45]	C/H	X2/X3	+	+	+		
JORDAN							
Patwardhan[60]	Pop	X1	—	+			
GUATEMALA							
Arroyave et al [63]	Pop	serum retinol	+	+			
PERU							
Salazar-Lindo et al [66]	H/Pop	serum retinol		+			
MALAWI							
Tielsch et al [109]	Pop	XN/X1B	+	—	+		
ETHIOPIA							
DeSole et al [61]	Pop	XN-X3	+	+	+		
MICRONESIA							
Lloyd-Puryear et al [64]	Pop	CIC	+	+			

[a]Pop Population-based data

C/H Clinic or hospital-based data

CIC = Conjunctival impression cytology

[b]UTI = Urinary tract infection

LRI = Lower respiratory infection

GI = Gastroenteritis

TB = Tuberculosis

evident [49] Clausen found that children with low levels of plasma "carotenoids" ran a higher risk of repeated respiratory infection, and that children receiving supplemental cod liver oil or carotene-rich vegetables experienced a lower incidence and severity of infection [50] He further reported that carrots caused rapid improvement of severe otitis in children seemingly destined to suffer mastoiditis [51]

Ramalingaswami observed twenty children two to nine years of age who presented with watery diarrhea, usually of one to two months duration [52] All were emaciated with varying degrees of xerophthalmia (mostly nightblindness or Bitot's spots) Serum vitamin A was severely depressed (< 10μg/dl) in all cases in which it was measured, and fever and bilateral rhonchi were present in twelve of the children When the diet of fifteen of the severest cases was supplemented with vitamin A concentrate (72,000 IU per day), there was a dramatic reduction in their diarrhea within forty-eight hours In five of the remaining mildest cases (Bitot's spots and diarrhea), the diarrhea persisted until vitamin A was added to the treatment regimen

Henning and co-workers found vitamin A treatment did not alter the course of acute, watery diarrhea.[53] On the other hand, the average episode lasted only two days, not a particularly long window in which potential benefits could be observed, particularly if these were likely to require replacement of damaged intestinal mucosa [54] Further, those treated with vitamin A had a smaller rise in serum vitamin A and a lower level at twenty-four hours than those given placebo! The potential failure of vitamin A treatment to relieve acute, self-limited diarrhea is in contrast to its apparent beneficial effect on persistent diarrhea [52] This finding is consistent with vitamin A deficiency's stronger association with persistent diarrhea (≥ 14 days) than with acute diarrhea [55,56]

In the 1930s, Blackfan and Wolbach found early keratinizing metaplasia (evidence of mild to moderate vitamin A deficiency) in infants who died of a variety of infections without clinically apparent xerophthalmia [19]

A vast array of relevant data have become available over the past two decades These include cross-sectional associations based on clinic or hospital patients and population surveys (Table 3–1), population-based longitudinal observational studies, and controlled intervention trials These data provide overwhelming evidence that vitamin A status alters the incidence and/or severity of a variety of infections, particularly diarrhea, measles, urinary tract infection, and probably some forms of respiratory disease

Diarrhea

Vitamin A supplementation has been shown to reduce diarrhea-specific mortality (Table 2–12), thus, vitamin A status must affect the frequency or outcome of clinically significant gastrointestinal disease, as Ramalingaswami's early clinical study suggested [52]

At least twelve cross-sectional studies have found a strong association between impaired vitamin A status (usually defined by mild xerophthalmia) and the presence, or a recent history, of diarrhea (Table 3–1) Diarrhea was often the strongest risk factor associated with xerophthalmia in these studies in Nepal[57,58] the odds ratio was 20, in Indonesia, 12 (diarrhea during the past week) to 23 (diarrhea during the past month) [45] The associations were often strongest for "persistent," "chronic," or "severe" diarrhea or dysentery [56,59–62] Gastroenteritis was present in three times as many cases of severe xerophthalmia (X2, X3) as in cases of milder disease (X1B) presenting to the Cicendo Eye Hospital in Indonesia, and twice as common in corneal cases as in their controls [45] A Guatemalan study found that gastroenteritis was more frequent among children with serum retinol levels < 10 μg/dl [63] In Truk, Micronesia, diarrhea was almost three times more common among children with abnormal conjunctival impression cytology (CIC) [64] In Thailand, a 1 μmol/liter increase in serum retinol was associated with a 50% reduction in the prevalence of diarrhea [65] Children in Lima, Peru, hospitalized with dehydrating diarrhea had serum retinol levels half that of matched controls, 75% versus 14% had levels below 20 μg/dl [66] In the Sudan, children with three watery stools during the past 24 hours were 20% more likely to have Bitot's spots, even after multivariate analyses controlled for other contributory factors [67]

Despite the consistent association between indices of vitamin A deficiency and evidence of recent or existing diarrhea (especially protracted or severe diarrhea), it is not always clear which occurs first (Chapter 7)

The Indonesian observational study[68] suggests that children with preexisting, mild xerophthalmia (closely correlated in this population with serum retinol)[69] (Table 3–1) are more likely to develop subsequent diarrhea (Table 3–2) [70] Since

Table 3–2 Age-Specific Incidence of Diarrheal Disease among Children With and Without Xerophthalmia[a]

Age	*Child-Intervals*		*Cases of Diarrhea*		*Rate (per 1000)*		*Relative Risk*	
(years)	*N–N*	*X–X*	*N–N*	*X–X*	*N–N*	*X–X*	*N–N*	*X–X*
≤ 1	5425	36	421	9	78	250	1	3 2*
2	3014	135	202	31	67	230	1	3 4*
3	3018	160	151	27	50	169	1	3 4*
4	2958	147	93	14	31	95	1	3 1*
≥ 5	3624	183	87	14	24	77	1	3 2*
Total	18,039	661	954	95	53	144	1	2 7*

[a]Without xerophthalmia (N–N)—children with normal eyes at both the start and end of the three-month observational interval, with xerophthalmia (X–X)—children with mild xerophthalmia (nightblindness and/or Bitot's spots) at both the start and end of the interval

*$p < 001$

From A Sommer et al [70]

vitamin A status fluctuates over time,[71] a child's xerophthalmia status was reclassified at each interval-initiating examination Given the lag between a change in vitamin A status and a change in signs and symptoms of xerophthalmia, vitamin A status was defined as follows

- **N–N:** no evidence of xerophthalmia at either the interval-initiating or interval-ending examination (best vitamin A status),
- **X–X:** xerophthalmia at start and end of the interval (poorest vitamin A status),
- **X–N, N–X:** vitamin A status in transition, improving in the former, declining in the latter

Diarrhea was three times were common among X–X than N–N children at every age (Table 3–2) The excess risk of diarrhea, at each age, was consistent across nutritional strata (height-for-age and weight-for-height) (Fig 3–1) As might be expected, the rate of diarrheal disease among children whose vitamin

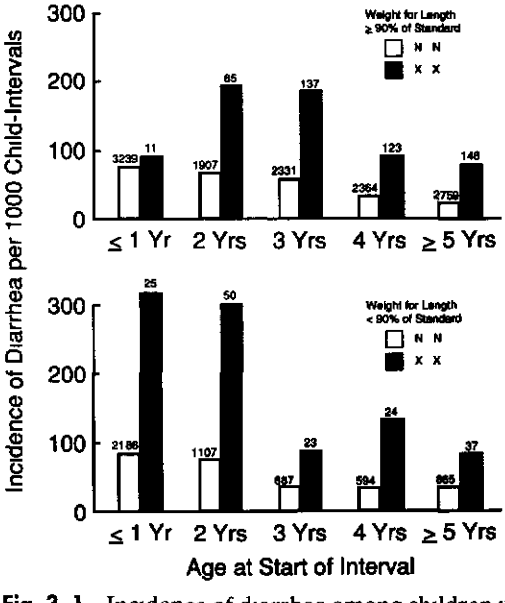

Fig. 3–1. Incidence of diarrhea among children with normal (N-N) or xerophthalmic eyes (X-X) at the examination initiating and ending the same three-month interval, summed over all six intervals The upper graph includes only children with superior nutritional status (weight-for-length/height 90% of standard) The lower graph indicates that the same relationship exists for wasted children (< 90% weight-for-length/height) In both anthropometric strata, diarrhea was associated with the presence of xerophthalmia At every age, better-nourished xerophthalmic children (upper graph) had higher rates of diarrhea than less-well-nourished, non-xerophthalmic children (lower graph) (From A Sommer et al [70])

A status was improving (X–N) was similar to the rate among children with normal eyes (N–N)

Further, vitamin A status seemed even more important than general nutritional status (weight-for-height or height-for-age) as a determinant of diarrheal incidence well-nourished xerophthalmic children were at greater risk of diarrhea than poorly nourished children with normal eyes (127 versus 61 per 1000, $p < 001$), which is consistent with prior observations that the risk of developing diarrhea is independent of anthropometric status [72–74]

Following publication of the Indonesian study, investigators at the Indian National Institute of Nutrition reanalyzed data from one of their earlier studies in a comparable manner [75] Over 1500 slum-dwelling, preschool-age children were examined at baseline, six months, and one year, morbidity data were collected at weekly home visits [76] The study differed in several regards from Indonesia children were classified by their ocular status only at the interval-initiating round, a milder definition of diarrhea was used (three or more loose stools in one day), and more frequent (weekly) home visits collected additional data, but also had a far greater likelihood of inducing a Hawthorne Effect

The risk of diarrhea was no greater among 'xerophthalmic" than "normal" children However, the subsequent vitamin A mortality prevention trial (Chapter 2) conducted by the same institution[77] studied this issue prospectively Children were examined for xerophthalmia at baseline and again six months later Field workers visited homes every three months to collect information about diarrhea (three or more loose stools a day) and other morbidity Children were classified, as in the original Indonesian study, by their ocular status at the examinations initiating and ending a six-month interval (Table 3–3) It is unclear whether the analysis was limited (as it should have been) to children in the placebo arm All three xerophthalmia categories registered increased rates of subsequent diarrhea (relative risks ranging from 1 13 to 1 31), the greatest risk (as for respiratory disease) was among children who *developed* xerophthalmia during the six-month interval (the clearest evidence for active vitamin A deficiency in a population prone to persistent X1A and X1B)

Table 3–3 Incidence of Infections in Relation to Vitamin A Status—India

Clinical Vitamin A Status		*Diarrhea*		*Respiratory Infection*	
Baseline	*After 6 Months*	*Incidence (%)*	*Relative Risk*	*Incidence (%)*	*Relative Risk*
Mild xerophthalmia	Mild xerophthalmia	8 6	1 19	16 6	1 29*
Mild xerophthalmia	Normal	8 2	1 13	17 5	1 35*
Normal	Mild xerophthalmia	9 5	1 31	22 5	1 74**
Normal	Normal	7 3	1 0	12 9	1 0

*$p < 05$

**$p < 01$

From Vijayaraghavan et al [7]

The weaker association between xerophthalmia and diarrhea observed in India compared with Indonesia may reflect a real difference between the two populations Alternatively, it may reflect the dilutional effect within the Indian study from use of X1A, widely regarded as unreliable evidence of vitamin A deficiency[78], a longer (six months) interval that leads to less precise temporal classification of vitamin A status, and the milder definition of diarrhea, which may have recorded more trivial, less clinically significant, life-threatening events

In a similar vein, a small study (n = 146) of Thai children failed to demonstrate a relationship between baseline serum retinol and the incidence of subsequent diarrhea[61] (Table 3–11) [65]

The impact of vitamin A supplementation on subsequent diarrheal morbidity was studied in several of the mortality trials discussed in Chapter 2 (usually as incident or point-prevalent events at the completion of the study), and in controlled trials specifically designed to assess morbidity With the exception of two small trials,[65,79] these uniformly failed to find any statistically significant difference in the "incidence" of mild diarrhea (usually defined as $\geq$ 3 or 4 loose stools a day) [80–85] Nor should they necessarily have been expected to Given a conservative estimate of relative risk (RR) of diarrhea among mildly xerophthalmic (XN, X1B) compared with non-xerophthalmic children of 2 0, a 2% prevalence of mild xerophthalmia, and an expected four such diarrheal episodes annually for all children (Madurai recorded an average of 5 6[82]), elimination of xerophthalmia and its excess risk of diarrhea would reduce the overall incidence of diarrhea in the study population by a mere 2% [86] Even if xerophthalmia were twice as prevalent, the reduction would still be only 4% If, in addition, the relative risk of more mildly deficient children was elevated proportionately (e g , RR ~ 1 2), these children constituted 35% of the study population, and the intervention was 100% effective, the maximum potential reduction in the overall incidence of mild diarrhea would be less than 8% By comparison, mortality trials were launched with an anticipated reduction of at least 20%–25% The difference lies in the higher relative risk of vitamin A deficiency for death than for incident (presumably trivial) episodes of diarrhea

Since vitamin A supplementation caused a consistent reduction in diarrhea-related deaths, it should also reduce the incidence of severe diarrhea There is growing evidence that this indeed is the case In northeastern Brazil 1240 non-xerophthalmic children six to forty-eight months of age received large-dose vitamin A supplements or placebo every four months for one year [84] The vitamin A-supplemented group was at lower risk of subsequent diarrhea. The more severe the diarrhea, the greater the benefit (Table 3–4) The protection afforded by the vitamin A dose lasted only three to four months Despite the striking difference in rates of "severe diarrhea" as defined by the frequency of movements, there was no difference in "severity" as defined by either blood or mucous in the stool This finding is consistent with observations that most diarrheal deaths in young children are secondary to dehydration Of particular interest,

Table 3–4 Diarrheal Episodes by Frequency of Movements—Brazil

Number of Loose Movements	*Relative Risk (Vitamin A Group)*
3	0 92
4	0 90*
5	0 80**
6	0 77**

*p < 05

**p < 01

From M Barreto et al [84]

the Brazilian population is considered only mildly vitamin A-deficient and free of xerophthalmia

A smaller study in Calcutta randomized 174 preschool-age children to 200,000 IU or placebo The subsequent rate of mild diarrheal episodes was similar (1 35 versus 1 73 respectively), though the difference in the mean duration of each episode (2 05 days versus 3 03 days) and mean diarrheal days per child (5 29 versus 8 42) during the six month follow-up period were highly significant [85]

A controlled morbidity intervention trial specifically designed to assess severity was conducted in Ghana[83,87] in proximity to the VAST mortality trial Almost 1500 children six months to fifty-nine months of age were randomized to either high-dose vitamin A or placebo every four months, and were followed weekly All children with measles or xerophthalmia received vitamin A and were excluded from further analyses Ill children were referred to clinics and severely ill children were hospitalized Careful records were maintained of all such referrals and hospitalizations While there was no difference in the apparent incidence or prevalence of trivial diarrhea between the two groups, supplementation had a marked impact on the severity of illness The vitamin A group experienced fewer diarrheal episodes with high stool frequency (> 6 movements per day, RR = 0 92) or signs of dehydration (sunken eyes, RR = 0 90, p < 04, drowsiness, RR = 0 70, p < 01), findings that are consistent with the results from Brazil [84] In addition, all-cause clinic attendance was reduced by 12% and hospitalization by 38% (Tables 3–5, 3–6) Diarrhea-specific clinic attendance of the vitamin A group was reduced by 17% (p < 02) and hospitalization by 32% The vitamin A group also had fewer clinic revisits (p < 02), suggesting less severe disease among those who attended clinic

Table 3–5 Clinic and Hospital Attendance—Ghana VAST Health Study Trial

	Vitamin A (N)	*Placebo (N)*	*Relative Risk (CL_{95} %)*
Clinic attendance	1193	1341	0 88 (0 81, 0 95)*
Hospital admissions	36	57	0 62 (0 42, 0 93)**

*p < 001

**p < 02

From Ghana VAST Study Team [83]

Table 3–6 Hospital Discharge Diagnoses—Ghana VAST Health Study Trial

	Vitamin A (N)	*Placebo (N)*	*Relative Risk (CL_{95} %)*
Diarrhea	12	18	0 68 (0 33, 1 41)
ALRI	5	10	0 48 (0 16, 1 45)
AURI	9	15	0 61 (0 27, 1 38)
Malaria	17	25	0 70 (0 38, 1 29)
Malnutrition	5	7	0 69 (0 22, 2 15)
Measles	3	6	0 48 (0 12, 1 92)
Anemia	5	15	0 33 (0 25, 3 94)

From Ghana VAST Study Team [8]

In summary, it appears that improvement in the vitamin A status of deficient populations protects preschool-age children from severe, dehydrating, life-threatening diarrhea, but may have little if any impact on the frequency of trivial diarrheal episodes

Measles

Measles bears a striking relationship to vitamin A status Measles precipitates a large proportion of X3 (Chapters 4, 7), which partially accounts for the strong association between a history (or presence) of measles and corneal destruction But vitamin A status also modifies the severity and outcome of measles and its systemic complications, as amply demonstrated in all four measles treatment trials[88-91] and by reductions in measles-related deaths in the four community-based vitamin A prophylaxis studies (CPTs) that examined cause-specific mortality (Table 2–12) Measles is the only specific pathogen for which we have direct, overwhelming evidence that vitamin A status influences morbidity (severity) and mortality

Vitamin A deficiency clearly increases the severity of measles Whether vitamin A supplements are administered prophylactically on a community-wide basis before children have measles, or as treatment for moderately severe, hospitalized cases of measles, mortality is reduced by roughly half The striking similarity in the magnitude of the reduction of measles-associated mortality under these two very different conditions strongly suggests that

- vitamin A supplementation, even as high-dose treatment, influences outcome by correcting underlying vitamin A deficiency rather than by a nonspecific, adjuvancy effect,
- it is unlikely that vitamin A status materially affects the *incidence* of measles, since the entire impact of supplementation can be explained by the reduction in case fatality

The dramatic and consistent reduction in case fatality associated with vitamin A supplementation is mirrored by a corresponding reduction in the severity of measles and its complications These issues were carefully evaluated by Hussey and Klein[89] in their Cape Town mortality trial, and by Coutsoudis et al [91 92] in a measles morbidity trial in Durban

In the Cape Town trial, placebo recipients fared far worse than children given high-dose vitamin A (Table 3–7) pneumonia lasted longer ($p < 001$) and chronic pneumonia ($\geq$ 10 days) was more frequent ($p < 01$), diarrhea lasted longer ($p < 001$) and chronic diarrhea was more frequent ($p < 05$), post-measles croup was twice as common ($p < 05$) and post measles croup requiring airway intervention, three times as common, herpes stomatitis was almost five times as frequent, the need for intensive care was three times as common, and surviving children were hospitalized 50% longer ($p < 01$) Most adverse outcomes (75 of 77) occurred in children less than two years of age ($p < 01$)

The detailed hospital-based measles treatment trial conducted in Durban evaluated the impact of vitamin A therapy on both short- and long-term measles-associated morbidity and the related immune response [91 92] Sixty African children four months to twenty-four months of age with measles rash less than five days complicated by concomitant pneumonia and diarrhea were randomized to high-dose, water-miscible vitamin A (100,000 IU if < 1 year, 200,000 IU if $\geq$ 1 year) or placebo on admission, and again on days 2, 8, and 42 (a post-hospitalization return visit) The children were also asked to return at six months

As with the shorter follow-up in Cape Town, vitamin A recipients responded rapidly to treatment (Table 3–8) They recovered more quickly than placebo recipients from pneumonia ($p < 05$), diarrhea, and fever Almost all vitamin A recipients, but only two-thirds of placebo recipients, recovered fully by day 7 ($p < 002$) The beneficial effects of vitamin A supplementation persisted beyond the initial hospitalization An "integrated morbidity score" (IMS) was developed by pooling the presence and severity of clinical complications (diarrhea, upper respiratory tract infection, pneumonia, laryngotracheobronchitis [LTB], herpes,

Table 3–7 Measles Complications—Cape Town Vitamin A Controlled Treatment Trial

			Relative Risk
Complication	*Vitamin A (N)*	*Controls (N)*	*Vitamin A Controls*
Pneumonia $\geq$ 10 days	12	29	0 44 (0 24, 0 80)
Diarrhea $\leq$ 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	$p < 004$

From G Hussey et al [89]

Table 3–8 Hospital Outcome—Durban Measles Vitamin A Controlled Treatment Trial

Outcome	*Vitamin A (N = 29)*	*Placebo (N = 31)*
Duration (days)—pneumonia	3 8 ± 0 4	5 7 ± 0 8
Duration (days)—diarrhea	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[a]	0 24 ± 0 15	1 37 ± 0 40

[a] Integrated Morbidity Score

From A Coutsoudis et al[91,92]

and x-ray evidence of pulmonary involvement) Vitamin A recipients did better at every IMS assessment, including the six-month visit (Fig 3–2)

The 61% reduction in IMS at six weeks and 85% reduction at six months largely reflect differences in the subsequent frequency and severity of pneumonia (Table 3–9) Since measles commonly precipitates severe deterioration of general nutritional status, which subsequent and recurrent complications exacerbate, it's not surprising that vitamin A recipients gained considerably more weight by six weeks ($p < 05$) and that this advantage continued for at least six months

The persistent benefits conferred by repeated vitamin A supplementation (a fourth dose at day 42) are consistent with improved vitamin A status during the prolonged period it often takes children with severe measles to regain their pre-morbid health and nutritional status, and also with the high rate of post-measles

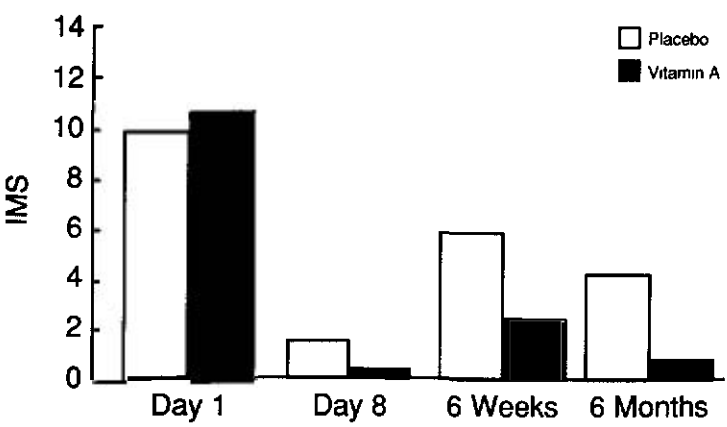

Fig. 3–2. Integrated morbidity scores (IMS) on follow-up of children admitted with acute measles to hospital in Durban, South Africa, and randomized to large-dose vitamin A or placebo (IMS described in text) Follow-up lasted six months post-baseline, when children still seemed to benefit from earlier vitamin A supplementation (From A Coutsoudis et al[91,92])

Table 3–9 Post-Hospital Outcome—Durban Measles Vitamin A Controlled Treatment Trial

	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
Diarrhea episodes	6	12	3	6
Score/episode—diarrhea	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

From A Coutsoudis et al [91,92]

illness, malnutrition, and delayed mortality that commonly follow an acute measles episode [93–95]

Less detailed intervention trials support the results of Hussey et al and Coutsoudis A single large oral dose of vitamin A administered to Kenyan children hospitalized with measles reduced the risk of severe, progressive LTB and otitis media, and the duration and severity of diarrhea [96] Finally, a small, village-based vitamin A prophylactic trial conducted in West Bengal, India, in 1972 on 153 preschool children (100,000 IU, or a placebo every four months for one year) yielded forty cases of measles [97–99] While there was no difference in the incidence of measles between the two groups, "associated complications such as pneumonia, diarrhea, and eye discharge were significantly higher in the placebo group "

Results of the community- and hospital-based intervention trials indicate the powerful influence vitamin A status exerts in reducing the severity and complications of measles There is relatively little evidence to suggest any impact on incidence Most of the hospital and population-based cross-sectional associations noted between xerophthalmia and measles relate to severe corneal disease (X3), for which measles is a common precipitating event (Chapters 4, 7), or less frequently, acute decompensation of chronic, underlying vitamin A deficiency exacerbated by recurrent complications (pneumonia, diarrhea, protein-energy malnutrition), and changes in dietary patterns that commonly follow measles

Since measles[100,101] and vitamin A deficiency both impair immune competence, it is reasonable to expect the combined impact is devastating to host defenses Depressed measles antibodies and lymphopenia are associated with more severe measles and a fatal outcome [92,102,103] Measles antibody titers are lower in children with lower serum vitamin A,[104] while African children with measles treated with

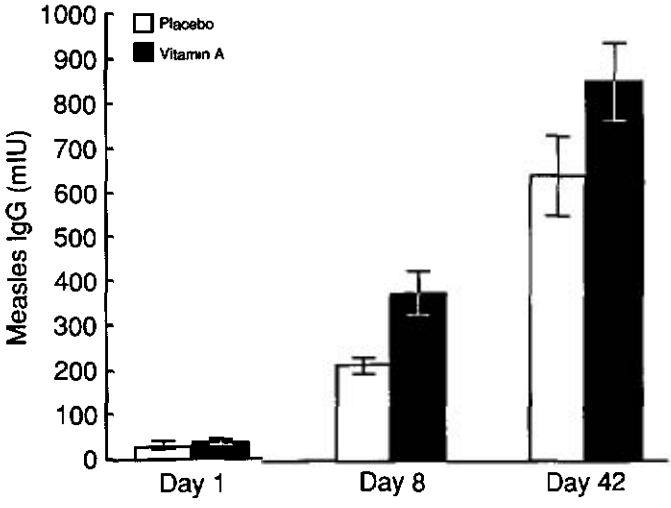

Fig. 3–3. Measles immune (IgG) response among children randomized to vitamin A or placebo supplementation upon hospitalization in Durban, South Africa, for acute measles Vitamin A-supplemented children achieved higher IgG titers (From A Coutsoudis et al [92])

a large dose of vitamin A mount a stronger measles IgG response to the natural disease than do unsupplemented children (Fig 3–3) [92]

Pasatiempo and colleagues[105] demonstrated that vitamin A supplementation of deficient rats as late as one day after an antigenic challenge increased the antibody response to near normal levels Semba et al [106] found a similar phenomenon among deficient children given a large dose of vitamin A (200,000 IU) two weeks prior to immunization with tetanus toxoid (Fig 3–4)

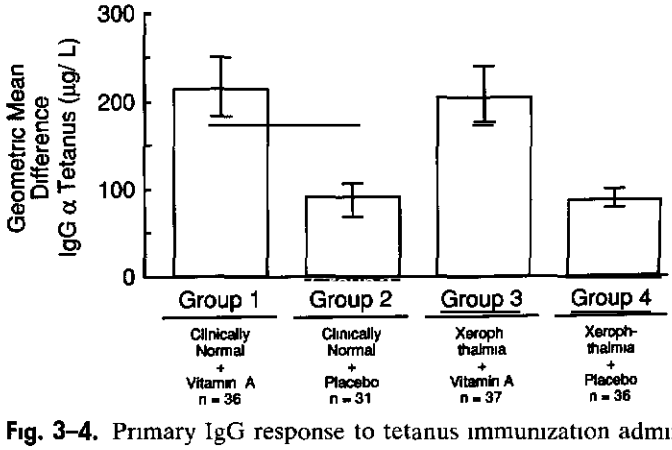

Fig. 3–4. Primary IgG response to tetanus immunization administered two weeks after supplementation with large-dose vitamin A or placebo among Indonesian children (From R Semba et al [106])

A preliminary but intriguing report suggests large-dose vitamin A supplementation simultaneous with measles immunization may inhibit immune conversion among six-month-olds with preexisting high levels of maternal blocking antibody, further suggesting that vitamin A might be interfering with replication of the live vaccine virus If so, it would help explain vitamin A's beneficial effect on morbidity and mortality associated with infection with the wild strain virus [107]

One small study in Ndola, Zambia, failed to elicit and enhance the measles antibody response among children receiving vitamin A during an acute measles episode [108] However, the children did not require hospitalization, received only a single vitamin A dose, and already had mean serum retinol levels well within the normal range (> 40 μg/dl)

Respiratory Disease

The effect of vitamin A status on respiratory diease is clearly shown in animal models of vitamin A deficiency, clinical and autopsy studies in children, and observational incidence data in human populations However, population-based prophylaxis trials, whether for morbidity or mortality, imply the situation is more complex and uncertain than it first appears

Clinical and histopathologic studies of vitamin A-deficient animals reveal early metaplasia of the tracheobronchial tree [16 8–10] Clinical and histopathologic studies in deficient children parallel observations in deficient animals [19 43 49 50]

Lower respiratory disease was associated with vitamin A deficiency in eight of the cross-sectional clinic and population-based studies (Table 3–1) In Guatemala,[63] respiratory disease was more common among children with serum retinol < 10 μg/dl, in Thailand, an increase of 1 μmol/liter of serum vitamin A was associated with an 80% reduction in respiratory disease [65] In the Lower Shire Valley of Malawi, the prevalence of mild, active xerophthalmia (XN, X1B) was one-third more common among children with a history of cough and fever during the preceding week ($p < 05$) [109] In the mono-crop regions of Ethiopia,[61] severe respiratory disease (unable to walk and play for at least five days) was twice as common among cases of xerophthalmia as among controls ($p < 02$) Lower respiratory disease (fever plus rales) was the most common infection accompanying xeropthalmic children presenting to the Cicendo Eye Hospital [45] The prevalence increased with the severity of xerophthalmia/vitamin A deficiency ($p < 01$) (Fig 3–5) Lower respiratory disease was seven times more common among cases of X3 than among their controls (64% versus 9%, $p < 01$)

While these cross-sectional associations could, by themselves, be explained by the impact of respiratory disease on vitamin A status rather than the other way around (Chapter 7), natural history studies confirm the increased risk of respiratory disease in children with preexisting vitamin A deficiency

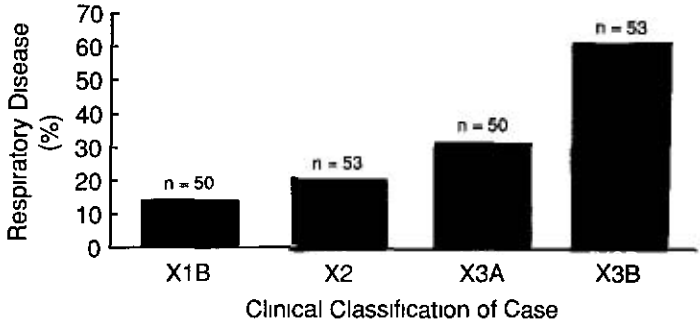

Fig. 3–5. The prevalence of respiratory disease among Indonesian children presenting to the Cicendo Eye Hospital increased with the severity of their xerophthalmia ($p < 01$ for linear trend) (From A Sommer [45])

In the Indonesian observational study (Chapter 2, Fig 2–2), respiratory disease was defined as "clinically significant cough, rhonchi or rales" leading the examining pediatrician to diagnose "bronchitis or pneumonia "[70] The incidence of respiratory disease was almost twice as common among X–X as N–N children (RR = 1 8, $p < 001$) This relationship was consistent across age and nutritional strata (Fig 3–6) As with diarrhea, the incidence of acute respiratory infection (ARI) among children whose vitamin A status was improving (X–N) was similar to that among children with normal eyes (N–N) Also, vitamin A status seemed a more important determinant of respiratory disease than did weight-for-height or height-for-age (Fig 3–7) Well-nourished xerophthalmic children were at greater risk of subsequently developing respiratory infections than were their more poorly nourished, non-xerophthalmic peers (92 versus 72 per 1000, $p < 05$)

The study from Hyderabad, India[76] yielded virtually identical results (RR = 2 0 p = 06) (Table 3–10) An excess rate of subsequent respiratory disease was associated with xerophthalmia at every age over twelve months

In their vitamin A mortality intervention trial, Vijayaraghavan et al confirmed the increased risk of respiratory disease among children with vitamin A deficiency [77] The risk was greatest among children who began the study with normal eyes but developed mild xerophthalmia by the time of the ocular follow-up, six months later, indicating deteriorating vitamin A status (Table 3–3)

The placebo arm of the MORVITA trial displayed a similar relationship between vitamin A deficiency (as determined by baseline serum retinol) and subsequent cough—acute lower respiratory infection (ALRI) [110]

The small Thai study[65] demonstrated that the risk of subsequent respiratory disease (a history of "clinically significant" respiratory complaints accompanied by fever) was related, in a dose-dependent manner, with vitamin A status at baseline (Table 3–11) The rate of respiratory disease among children with "deficient" serum retinol levels (< 0 35 μmol/liter) was nearly four times that among children with "adequate" levels (> 0 70 μmol/liter, $p < 01$)

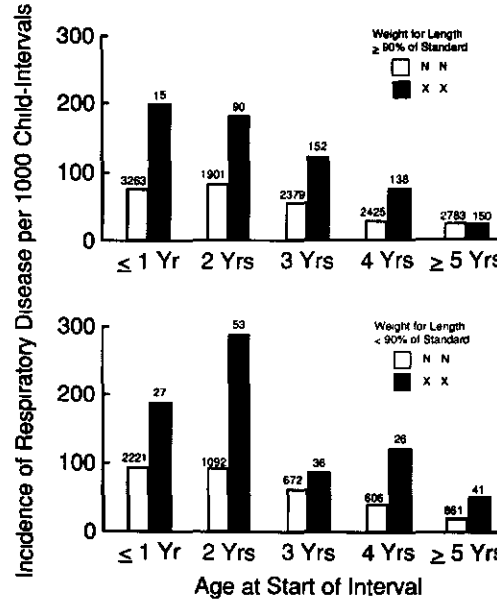

Fig. 3–6. As with diarrhea (Fig 3–1), the incidence of respiratory disease in the Indonesian observational study was greater at every age among children with xerophthalmia at the start and end of an interval (X-X), summed over all six intervals of observation A comparison of upper and lower graphs indicates that at each age, nonwasted children with xerophthalmia had a higher incidence of respiratory disease than wasted children without xerophthalmia (From A Sommer et al [70])

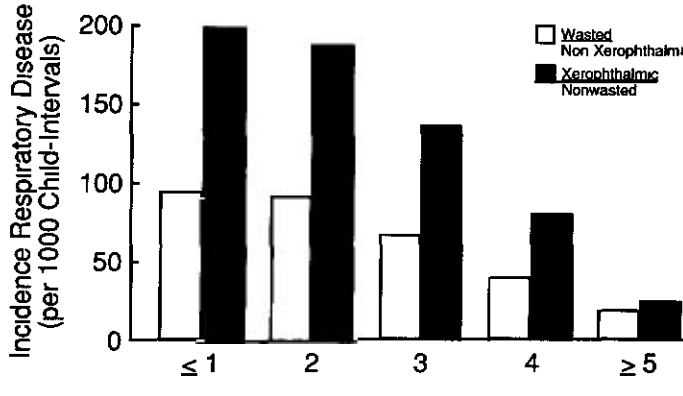

Fig. 3–7. The relationship between wasting, xerophthalmia, and the incidence of respiratory disease suggests that at every age (years), xerophthalmia is a more important determinant of subsequent respiratory infection than is moderate wasting (Drawn from A Sommer et al [70])

Table 3–10 Incidence of Respiratory Disease among Children Age < 5 Years With and Without Mild Xerophthalmia[a] in India

Age (years)	Child Intervals		Cases of Disease		Rate (per 1000)		Relative Risk[b]	
	N–N	X–X	N–N	X–X	N–N	X–X	N–N	X–X
≤ 1	1540	3	153	0	99	0	0	0
2	724	11	49	3	68	273	4	0
3	756	21	36	2	48	95	2	0
4	600	27	29	4	48	148	3	1
Total < 5	3620[c]	62	267	9	74	145	2	0*

[a]Without xerophthalmia (N–N) with xerophthalmia (X–X) See text for definitions

[b]Ratio of rate per 1000 child intervals with vitamin A deficiency to rate per 1000 child intervals without vitamin A deficiency

[c]Six-month intervals

*p = 06

From R Milton et al [76]

In an intriguing report from the United States, respiratory syncytial virus (RSV) infection was associated with a transient but dramatic decline in serum retinol Infants with the lowest serum retinol values ran the greatest risk of requiring respiratory assistance (6/7 versus 4/16, for serum retinol levels below and above 10 μg/dl, respectively, p < 02) [111] RSV, like measles, is a paramyxovirus that replicates in the respiratory track

In addition to the strong and consistent relationship between vitamin A deficiency, histopathologic changes, and subsequent risk of respiratory infection, vitamin A morbidity supplementation trials suggest a potential reduction in the severity of respiratory events (if not necessarily their frequency) [85] The detailed Ghana morbidity trial[83 87] recorded similar rates of respiratory illness among vitamin A and placebo groups, including the mean duration of "probable" ALRI, but the vitamin A group had less frequent episodes of wheeze, stridor, and other observed "respiratory noises" (RR = 0 86, p < 02), and of "rapid breathing"

Table 3–11 Relationship between Risk of Respiratory Disease and Serum Retinol—Thailand

Serum Retinol at T = 0	Incidence of Respiratory Disease (3 mo follow-up)		
	Number	%	95% CI[a]
Deficient (0–0 35 μmol/liter)	23	39 1[b]	21–61
Marginal (0 35–0 70 μmol/liter)	68	26 5	16–38
Adequate (> 0 70 μmol/liter)	55	10 9	4–24
Total	146	22 6	

[a]CI = confidence interval

[b]Significant difference (p < 01) between the group of children with "deficient" and that with "adequate" levels of serum retinol

From M Bloem et al [65]

with cough (RR = 0 92, p = 08) Pyrexia (axillary temperature ≥ 101°) was also less common in the vitamin A group (RR = 0 87, p = 07) The authors concluded that vitamin A reduced the severity of illness, and that the more acute the indicator of severity, the greater the impact In the Brazilian population,[84] in which xerophthalmia is rare, there were similar incidence rates of mild ALRI among vitamin A and placebo recipients, and a nonstatistically significant reduction in the incidence of pneumonia (RR = 0 94) among the vitamin A group

A meta-analysis of available data from mortality and morbidity intervention trials failed to indicate any consistent impact of vitamin A supplementation on the incidence of ALRI, a conclusion supported by a World Health Organization (WHO) ad hoc review panel [112 113] Results of individual studies however, varied The small but unique Thai trial[65] recorded three times more respiratory disease in the control group at two months (not statistically significant), an apparent protection that persisted for at least four months among one-to-two year olds (the group not attending day care centers, among whom the opportunity for transmission was probably lower [RR = 2 5, p < 05]) A small controlled clinical intervention trial (one large dose at six-month intervals) in Hebei, China[79] reported a marked reduction in the incidence and duration of respiratory illness in the vitamin A group, which was statistically significant at every age except infancy (six months to eleven months) Though the numbers were small, the difference in the rate of hospitalizations between the two groups was marked 4/78 controls (three with pneumonia) versus 0/98 vitamin A recipients In contrast, a preliminary report from the MORVITA morbidity trial in Central Java[110] reported an increase in cough (RR = 1 07) and ALRI (cough and rapid breathing, RR = 1 40) among vitamin A recipients, particularly among children less than one year of age The apparent increased risk associated with high-dose supplementation was largely confined to children with the best vitamin A and anthropometric indices at baseline

While meta-analyses[112 113] concluded there was no convincing evidence that large-dose vitamin A supplementation increased the risk of respiratory disease, particularly life-threatening pneumonia, the MORVITA report is consistent with the apparent increase in the risk of death observed in the best-nourished, youngest Nepalese infants supplemented with large-dose vitamin A (100,000 IU) during the second through fourth months of life (Chapter 2) [114] These unexpected, but potentially related, observations suggest prophylactic massive dose supplementation of *very* young children needs careful consideration—particularly the size of the dose, especially in populations where a substantial proportion of children are otherwise healthy, vitamin A-sufficient, and well nourished Nonetheless, improving vitamin A status of deficient children reduces the risk of severe respiratory disease, life-threatening diarrhea, and all-cause mortality There is no evidence that increasing retinol levels above 20 μg/dl is harmful indeed, levels of 30 μg/dl–40 μg/dl are the norm in well-nourished Western countries The concern, if any, centers on the appropriate size of a single large bolus administered to a

very young infant It should be recalled that 50,000 IU at birth can dramatically reduce subsequent mortality [115,116]

It is, of course, possible the two observations are unrelated, and increased cough in vitamin A-supplemented children simply reflects their recovered ability to generate a cough subsequent to the reversal of keratinizing metaplasia of the respiratory tract with return of mucous-producing epithelium When deficient Bangladeshi mothers received a 300,000 IU supplement within three months of delivering, it reduced subsequent infant mortality by 30%, while at the same time increasing the incidence of clinically detectable ALRI and diarrhea (but not dysentery) [117] The authors speculate that improved vitamin A status increased the infants' ability to respond to infections—making them more readily apparent—rather than increasing the risk of infection itself On the other hand, the Indonesian children supplemented with 50,000 IU at birth were less likely to seek medical attention for cough and fever (e g , suffer medically significant respiratory disease) during the first three months of life [115,116]

Unexplained is the discrepancy between the apparent increased susceptibility of vitamin A-deficient children to severe respiratory disease, and the general failure of vitamin A supplementation to reduce respiratory-related deaths (except in measles[89,91]) This stands in marked contrast to the dramatic reductions observed in measles and diarrheal deaths This difference may be real, accounting for the relative lack of impact of vitamin A supplementation on all-cause mortality during the first few months of life, when the predominant cause of infectious death is respiratory disease Alternatively, the impact may have been obscured by one or more limitations of study design, particularly the relatively blunt instruments utilized for identifying respiratory-related deaths A number of mortality intervention trials recorded significant reductions in death from "unspecified" or "infection-nonspecific" causes, categories that might have harbored respiratory disease There is no proof, however, that this was the case or would account for these seemingly incompatible phenomena Despite the value of vitamin A as treatment for measles and its respiratory complications, there is little evidence such treatment affects the outcome of non-measles associated ALRI [118]

While beyond the scope of this text two areas of investigation bear on other aspects of vitamin A status and respiratory function Pinnock and co-workers observed that otherwise healthy well-nourished, seemingly vitamin A-replete preschool-age Australian children with a history of frequent respiratory illness supplemented with one RDA of vitamin A daily experienced a 25% reduction in their rate of lower respiratory infection, despite the absence of any increase in serum retinol [119] In a follow-up study[120] of different design, vitamin A supplementation had no apparent impact on preschool children who had suffered from a prior episode of RSV in infancy The significance of these observations remains to be clarified

In a series of studies related to problems of low birth weight, Shenai and co-workers found such infants to be particularly vitamin A-deficient at birth and

for months thereafter [121] In a small controlled clinical trial, vitamin A supplementation at birth appeared to reduce the incidence of bronchopulmonary dysplasia (BPD) by 50%, though not "all-cause" mortality [122] A subsequent study in North Carolina failed to confirm these results,[123] but control infants received twice the vitamin A as did controls in Shenai's Vanderbilt study, and few had serum levels < 20 μg/dl Further, all subjects received more elaborate neonatal care, including steroids and surfactant [123 124] Given the growing number of low-birth-weight infants, their complex management needs, low survival, and high risk of BPD and blinding retinopathy, this is an important area in need of further work, particularly in the developed world

Urinary Tract Infection

Urinary tract infections are among the most consistently reported clinical accompaniments of vitamin A deficiency Bloch[43] repeatedly reported their presence in deficient children, as well as their response to vitamin A Fifty years later, Brown and co-workers[44] compared infections in children hospitalized for severe malnutrition Those with active xerophthalmia were even more malnourished and ill than those with normal eyes The most striking difference, however, was their rate of bacteruria 78% versus 17% The prevalence of bacteruria increased with the severity of xerophthalmia (hence vitamin A deficiency), from 40% in children with XN to 92% in those with X3 Little else is known about this clinical manifestation, not even whether it represents colonization or true tissue invasion More than likely, it represents both vitamin A-deficient animals[9] and (autopsied) infants[19] frequently display pyelonephritis

Otitis

Like bacteruria, otitis was one of the first infections to be associated with vitamin A deficiency in humans and reported to respond to vitamin A therapy [43 51] Animal studies confirm that squamous metaplasia of the middle ear occurs early in deficiency[125] and greatly increases the risk of otitis media [126]

In a recent clinic-based investigation in Truk, children with abnormal CIC indicative of mild, "subclinical" vitamin A deficiency were at three to four times greater risk of middle ear infection than children with normal CIC [127]

Only one of the community intervention trials (NNIPS) attempted to assess the impact of vitamin A supplementation on the prevalence of "drippy ear "Given the comparatively low relative risk suggested in Truk, and low incidence of the disease in the NNIPS trial, the absence of an apparent effect is not surprising or necessarily definitive

Otitis media is responsible for a good deal of hearing loss and disability in the developing world, and its relationship to vitamin A status deserves greater attention than it has received to date

Other (HIV)

AIDS is the only other important infectious entity whose relationship to vitamin A status has begun to receive significant attention Studies in this area are recent and often preliminary in nature, and their biologic implications uncertain Nevertheless, they may well be charting an important new course in understanding the pathogenesis of the pandemic, and in identifying potentially valuable approaches to treatment and control [128] Vitamin A levels are reportedly depressed in a significant proportion of asymptomatic individuals infected with HIV-1[129], high doses of beta-carotene increase CD4 levels in HIV-1 infected adults[130], cytokine production may be retinol-responsive[128], the more advanced and severe the disease, the lower the serum retinol level[128 131], subsequent mortality is higher among AIDS patients with lower retinol levels, even after adjusting for CD4 cell counts[132], and HIV-1 infected mothers who are vitamin A-deficient are (much) more likely to pass the infection to their offspring than are infected mothers with higher serum retinol values (women with levels < 20 μg/dl were four times more likely to infect their offspring than were mothers with levels ≥ 40 μg/dl (32 4% versus 7 2% respectively, p < 001) [133 134] Among HIV-infected mothers, infant mortality was an astounding 90+% if maternal serum retinol was below 20 μg/dl [133] While we must await supplementation trials to determine whether these relationships can be influenced, Coutsoudis et al have already shown that vitamin A supplementation of HIV-infected infants reduces the eighteen-month incidence of diarrhea, prolonged diarrhea, and thrush by 50%, and hospital admissions for diarrhea by 80% [135]

No doubt research interest and activity in this area will continue to accelerate in the years to come

Conclusions and Pathogenesis

> why is vitamin A so very important?" Dr McCollum smiled "The simplest way is to say this vitamin A builds fences that keep germs out "
>
> —McCall's magazine, 1935

It is clear from a wide range of evidence that vitamin A status and infection, particularly non-trivial, severe morbidity, are closely associated Difficulties in mounting scientifically definitive, ethically acceptable investigations preclude

quantification of the total burden of infectious morbidity suffered by vitamin A-deficient populations As a large proportion of mortality averted by vitamin A supplementation has been from unspecified causes,[83,136,137] it is likely these mirror a corresponding reduction in unrecognized infectious morbidity as well

In contrast with other investigations, one report claimed an increase in both diarrheal and respiratory morbidity among supplemented children [138] These results, however, were widely considered uninterpretable[139–142] given the high loss to follow-up and low compliance of study children (two-thirds of all children were lost to follow-up and only one-third received the three prescribed doses), the simultaneous introduction of other public health interventions which were neither detailed nor evaluated, disproportionate interview rates in the "two groups", and general weaknesses in study design (yielding for example, an estimated twenty-four or more diarrheal episodes annually per child, five times the rate reported from other developing countries)

There are a variety of mechanisms by which vitamin A status might influence the risk of life-threatening infections Their relative roles and importance are not well delineated, indeed, it is likely they vary with the particular pathogen and the state of the host [143] What is known of these complex relationships is detailed in Chapter 9

Vitamin A deficiency must either lower the body's ability to prevent a pathogen from invading its tissues (establishing "clinical" or "subclinical" infection) in the first place, or its ability to cope with such invasion once it occurs Both factors may be important alterations in the epithelial lining of vital organs involved in serious infections (e g , the respiratory and genitourinary tracts) occur early in vitamin A deficiency, suggesting a potentially important role for their "barrier function " The speed with which seriously ill measles patients respond to vitamin A indicates the ability for coping with established and sometimes disseminated infections after they have occurred The critical function vitamin A plays in regulating cellular differentiation provides a unique, "core" mechanism that would explain, at least in part, its influence on epithelial barriers, immune competence, and cellular/tissue/organismic functioning, healing, resistance and recovery [144,145]

The rapid and dramatic clinical response to vitamin A supplementation observed in severe measles mirrors the rapid and global biochemical response recognized at the molecular level Vitamin A influences the expression of over 300 genes (a number rising rapidly), vitamin A supplementation of deficient animals alters gene products within one to four hours [145–148]

Epithelial Metaplasia

The high rates of infection, particularly pulmonary infection, observed in vitamin A-depleted animals[6,149,150] led to a concerted search for its origins [149] Mori[8,151,152] was the first to systematically study histopathologic changes in the vitamin A-

deficient rat, describing keratinizing metaplasia of normally mucous-secreting ciliated epithelium of the tracheo-bronchial tree, with complete loss of goblet (mucous producing) cells followed by an "inflammatory process" that often terminated in bronchopneumonia, the cause of most deaths

Wolbach and Howe[9] extended these observations by systematically observing the sequence of histopathologic changes in the vitamin A-depleted rat They concluded the specific pathology was "wide-spread keratinization " Gross pathology revealed atrophy of a number of organs (e g , the submaxillary and Harderian glands, testes, and parotid) Desquamated keratinized cells partially filled the bladder, and ureteral blockage caused dilatation of the ureter and renal pelvis Keratinization was specifically noted in the respiratory tract (nares, sinuses, larynx, trachea, and bronchi), the alimentary tract (submaxillary, parotid and accessory salivary glands), the genitourinary tract (bladder, ureter, renal pelvis, uterus and oviducts, epididymis, prostate), and the eyes (conjunctiva, meibomian ducts, cornea, and the lacrimal, Harderian, and extra-orbital lacrimal glands) In some areas, particularly glandular ducts, atrophy and hypoplasia may have preceded the appearance of keratinizing epithelium Infection, which was common, was clearly secondary to and followed the metaplastic changes

Wolbach and Howe were able to follow the temporal sequence most readily in the respiratory tract it began as multiple foci that subsequently spread to adjacent columnar epithelium, infection, when it occurred, followed these changes As a rule, keratinization affected the respiratory tract before involving the genitourinary tract Bronchiectasis appeared to be secondary to occlusion of bronchi by desquamated keratinized cells The spleen became increasingly depleted of lymphoid and "erythrocyte-forming cells." Extreme atrophy of the thymus was *universal*, indeed, it "practically disappears!" (an important observation that will bear on the subsequent recognition of compromised immune status)

Wolbach and Howe[9] noted that histopathologic abnormalities of the eye followed those of the respiratory tract Tilden et al [153] made the same observation in the monkey nine of eleven animals had widespread keratinization of other organs without ever involving the eye

Although early investigators failed to recognize vitamin A-dependent metaplastic changes of the gut (indeed, Wolbach and Howe specifically noted their absence), more recent observations in the mouse reveal a substantial reduction in the number of goblet cells per duodenal villus when compared with control and pair-fed animals,[154] and a reduction in gastric mucosal and luminal mucous [155] Decreased cellular division precedes histologic abnormalities in mildly deficient animals [156]

The findings of Wolbach and Howe were confirmed in animal models in which the possibility of concomitant vitamin D deficiency was rigorously excluded [10]

Green and Mellanby[1] produced vitamin A-deficient animals supplemented with vitamin D Their animals were "uniformly attacked and ultimately killed by infective and pyogenic complications " They concluded that supplementation

with vitamin D actually hastened the onset of infection, conjecturing that growth stimulation (among animals initially deprived of both A and D) "made a greater call on the vitamin A stores of the body." This is exactly the same reasoning advanced to explain the sudden appearance of xerophthalmia among starved children fed high-protein supplements (unfortified by vitamin A),[45] and the seasonal variation of xerophthalmia, which often peaks during the spring growth spurt.[157]

Green and Mellanby[1] also found that xerophthalmia was a relatively late phenomenon: even advanced infections were common in its absence. Infections of the urinary tract were particularly prominent: 44% of animals had pyelonephritis or cystitis. They suggested that a "favorable medium," provided by obstruction and a keratinized surface, accounted for at least some of the reduced resistance to infection. Certainly the overgrowth of the xerosis bacillus on xerotic conjunctiva supports the concept that a keratinized surface provides a conducive substrate for bacterial replication.[158]

It has also been shown that replenishing vitamin A-deficient rats causes relatively prompt reversal of the metaplastic process and healing with little organ destruction.[159]

By 1937, the catalogue of animal species in which vitamin A deficiency induced typical keratinization of the epithelium included rats, mice, guinea pigs, monkeys, swine, dogs, rabbits, cattle, domestic fowl, and humans. Wolbach coined the process "keratinizing metaplasia."[160]

More recent studies have directly demonstrated the importance of vitamin A to the keratinizing process. Vitamin A-deficient rats display alterations in epithelial cytokeratin expression of the genitourinary tract, conjunctiva, and salivary glands before histologic evidence of metaplasia becomes apparent.[161] Huang and colleagues[162] have traced the impact of vitamin A-free cell cultivation of hamster trachea to increased keratin synthesis and the appearance of novel keratin species not normally present in mucociliary tracheal epithelium—an area of research of enormous current interest.[163,164] Earlier, Fell[165] had demonstrated the ability of vitamin A-enriched cell culture medium to transform normally keratinized epithelium into columnar, non-keratinized epithelium. Compared with pair-fed controls, marginally depleted guinea pigs suffer significant reductions in goblet cell density and secretory granules before any change in the number of ciliated epithelium of the tracheobronchial epithelium.[166]

The first detailed pathologic description of human vitamin A deficiency was provided by Leber,[167] 30 years before the existence of "fat-soluble A" was recognized or any pathology accounts of controlled animal depletion studies had been reported. He described marked keratinizing metaplasia of the renal pelvis, which he considered the same process that affected the cornea and conjunctiva.

Forty years later, Wilson described autopsy findings in a five-month-old infant[168] who presented with corneal xerophthalmia, irregular fever (as high as 104°F), and semistuporous condition. Eighteen days later the corneas perforated

and the child died The histopathologic changes mirrored those described in deficient animals The necropsy material was subsequently reexamined by Wolbach,[9] who considered keratinization of the lungs, uterus, and submaxillary glands, and severe atrophy of the thymus, identical to that of the rat The only thing Wilson and DuBois had overlooked was keratinization of the epithelium lining the renal pelvis The lungs were striking for the metaplasia of the bronchiolar epithelium, the presence of bronchiectatic abscesses, and purulent infiltration of alveoli Wilson's description was followed by additional, isolated reports [169]

Ten years later, Blackfan and Wolbach described an extensive series of cases from the same institution [19] These were particularly noteworthy for the absence of clinical xerophthalmia in seven of the eleven children (almost all were infants), the diagnosis of vitamin A deficiency having been based on keratinization of one or more epithelial-lined organs Keratinizing metaplasia of the trachea and bronchi was the earliest and most consistent finding Blackfan and Wolbach ascribed the frequently lethal pneumonia to "loss of protective powers of the epithelium due to diminished or absent mucous secretion and loss of ciliary motion " The organ next most frequently involved was the renal pelvis In fact, (keratinized) epithelial cells were found in the urine of at least four of the eleven cases Wolbach subsequently concluded that "In the human being, as in the rat, involvement of the eye occurs later "[160]

Sweet and K'ang[21] reviewed autopsy material available from cases of xerophthalmia and keratomalacia in Beijing In contrast to "experimental animals on well controlled diets, these patients were almost always suffering from multiple deficiencies " Yet the conditions were similar to those described previously, including Blackfan's seven cases that had not yet developed clinically detectable xerophthalmia In Sweet's series of more advanced deficiency, eight of seventeen had evident metaplasia of the trachea (in more severe cases, extending to the smaller branches of the bronchi), five had definite keratinization of the esophageal mucosa (in some it was "so marked that the mucosa closely resembled hyperkeratotic epidermis") Only three cases displayed metaplasia of the urinary tract In numerous instances systemic infections clearly complicated or followed the onset of ocular lesions The most common was respiratory disease, which Sweet believed was probably related to lowered resistance secondary to metaplastic changes

In summary, histopathologic alterations accompanying vitamin A deficiency in animals and humans indicate that keratinizing metaplasia affects a multiplicity of vital organs, most dramatically the respiratory and genitourinary tracts This may well affect the ability of organ surface linings to protect the tissue from bacterial (and viral) invasion Chandra[170] demonstrated that the number of bacteria adhering to nasopharyngeal cells obtained by saline lavage from Indian children was inversely related to their serum retinol levels The conjunctival surface of xerophthalmic children shows large numbers of bacteria, Bitot's spots sometimes

being a pure "culture" of the xerosis bacillus (Chapter 4) [45] In addition to attracting or serving as a supportive medium for bacteria and/or increasing the ability of pathogens to invade tissue layers, keratinizing metaplasia might (as suggested in both animals and humans) initiate locally destructive changes in the lung and elsewhere that then become secondarily infected Rotavirus infection of vitamin A-deficient mice causes almost complete destruction of the tips of intestinal villi, changes not seen in non-deficient animals [154]

While keratinizing metaplasia must, perforce, act locally, widespread distribution of these keratinizing changes provides one potential explanation for multiorgan involvement, in addition, serious infection in any organ can lead, systemically, to more generalized complications In an early experiment, Lassen[171] found that vitamin A-deficient rats were more susceptible to systemic infection and death from paratyphoid bacillus, whether it was given orally or subcutaneously Mellanby, on the other hand,[49] concluded from his own data that vitamin A played a greater role in "mucous membrane resistance" than in "general resistance" to infection (e g , the ability to deal with established septicemia) Eusterman[172] also favored the importance of the integrity of the epithelial linings, but acknowledged that too little was known about the role of vitamin A in systemic resistance

Immune Competence

It is unlikely that keratinizing metaplasia explains all the infectious phenomena associated with vitamin A deficiency The severe course of measles in vitamin A deficiency and its rapid response (therapeutic and protective) to vitamin A supplementation suggest more profound and rapidly reversible mechanisms that control resistance and cope with established, systemic infections The relationship between vitamin A status and immune competence is detailed in Chapter 9.

Clinically, persistent measles in malnourished children[173] and prolonged or disseminated herpes recrudescence,[174] blamed on especially severe immune depression accompanying some cases of measles,[175 176] might well have its genesis (at least in part) in vitamin A deficiency Treating measles with vitamin A may reduce the incidence (or speed healing) of herpes[89] and yield a more vigorous measles immune response [92]

In a classic experiment,[177] Bang demonstrated that vitamin A-deficient chicks become susceptible to influenza A infection, a virus to which they are normally resistant, and shed 100 times more Newcastle Disease Virus (NDV) than do normally fed controls In a follow-up study[178] it was shown that NDV greatly exaggerates lymphocyte depletion of the thymus and bursa caused by vitamin A deficiency, offering further evidence of the devastating interaction between vitamin A deficiency and infection Vitamin A deficiency not only increases the severity of disease caused by Newcastle Disease Virus, but NDV further depresses already marginal plasma levels of vitamin A through increased retinol utilization and by inhibition of retinol release from the liver [179 180] Thus, in this single animal

model we find vitamin A deficiency affecting the population of lymphoid structures and resistance to established infection (as seen in more severe disease and persistent viral shedding), while NDV infection further reduces vitamin A status and impairs already compromised immune competence

An enormous body of literature explores the relationship between vitamin A status and immunocompetence in animals Human data is far more limited and somewhat contradictory, reflecting problems in studying human subjects and the rarity with which modern techniques have been applied to deficient, remote, Third World populations With all its limitations, recent work suggests significantly impaired immune response in marginally vitamin A-deficient children and marked improvement following vitamin A supplementation [92 106 107 181]

It is worth recalling that early anatomic studies in vitamin A-deficient animals and humans[19 160] noted profound atrophy of both the thymus and spleen In previously deficient rats, the spleen begins to regenerate within six to eight days of vitamin A supplementation [160]

The complex and emerging relationship between vitamin A status and immune competence is addressed by Ross in Chapter 9

Other

No doubt there are other, as yet unidentified, mechanisms that modulate the severity of infectious episodes (as well as other clinical manifestations of vitamin A status) Filteau et al [182] report that children who benefitted most from vitamin A supplementation in the Ghana morbidity trial, primarily by experiencing less vomiting, severe diarrhea, or dehydration, had a tendency for increased levels of serum acute phase proteins (alpha$_1$-acid glycoprotein, C-reactive protein and amyloid A) compared with matched placebo recipients They suggest synthesis of these or other APP may be an important mechanism in conferring the beneficial effects of improved vitamin A status

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4

Xerophthalmia and Keratomalacia

> Never whilst memory lasts, can one obliterate the mental pictures of those pitiful little bundles of marasmic, apathetic humanity Their faint, feeble, fretful wails ring still in one's ears today—summoning up visions of wasted, stick-like limbs, of distended abdomens, of dry, inelastic, scurfy scaly skins, of hair scanty brittle and dry and of sightless desiccated eyes
>
> —R H Elliot, 1920[2]

> A carotenoid-free world would be drab to behold and a retinoid-free world would be swathed for us in eternal darkness
>
> —J A Olson, 1993[3]

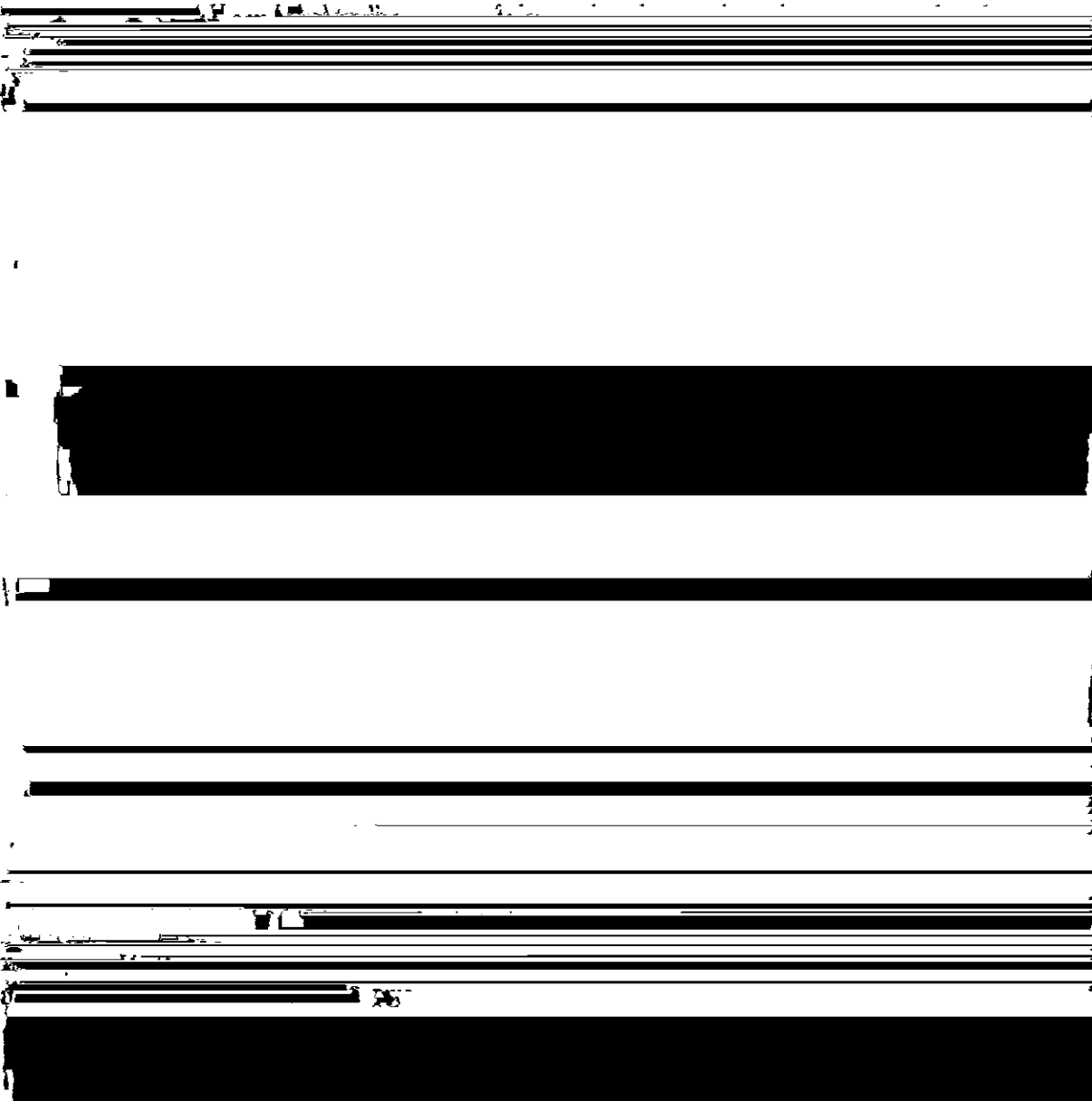
Xerophthalmia (*xeros*, dry, *ophthalmia*, inflamed eye) is a constellation of ocular manifestations long associated with vitamin A deficiency, representing the "classical" presentation and (with rare exceptions) pathognomonic signs and symptoms of this particular form of malnutrition In some ways, xerophthalmia has been too closely associated with vitamin A deficiency

- while xerophthalmia is the leading cause of acquired pediatric blindness in the world, responsible for 5 million–10 million cases of milder ocular disease (XN–X2) every year, it is sufficiently infrequent on a population-wide basis that health officials have often overlooked its existence and therefore have been misled into assuming that vitamin A deficiency was absent or not a significant health problem
- the more severe forms of xerophthalmia (X2–X3) are so often associated with severe malnutrition and life-threatening systemic disease that it is

Revised and condensed from A Sommer, *Nutritional Blindness*, 1982 [1]

these associated conditions that have been held solely responsible for the problem, masking the underlying effects of vitamin A deficiency

- the high mortality associated with severe, blinding disease (and its accompanying illnesses) means that the number of surviving, blinded children (XS) identified in population-based surveys is extremely small, minimizing the apparent size of the problem
- the clinical appearance is so dramatic that it has diverted attention from the more common and prevalent systemic consequences of vitamin A deficiency, as a result, xerophthalmia, rather than its underlying cause and associated systemic consequences, has received the lion's share of clinical attention and research



For a more extensive discussion of clinical, historical, histologic and epidemiologic data relevant to xerophthalmia, consult *Nutritional Blindness* [1]

Retina and Nightblindness (XN, XF)

Nightblindness (XN) and fundus specks (XF) were the first and last of the signs and symptoms of xerophthalmia to be described They both arise from faulty retinal metabolism associated with vitamin A deficiency Nightblindness is the earliest and most ubiquitous clinical sign and symptom of vitamin A deficiency, XF is largely a clinical oddity

While there are a number of causes of nightblindness (nyctalopia), they generally fall into two major categories hereditary abnormalities (that may not show up until later life) and acquired disease Among children of the developing world, acquired nightblindness is almost always a manifestation of vitamin A deficiency

Under normal conditions, retinal photoreceptors (rods and cones) produce photosensitive pigments that respond to light, triggering neural impulses that eventually make their way to the brain Rhodopsin, the photosensitive "visual purple" synthesized by the rods and responsible for vision under low levels of illumination (scotopic vision), contains vitamin A [4–6] Synthesis of rhodopsin, and therefore the ability to see under low levels of illumination, will depend upon the availability of vitamin A Under scotopic illumination, high levels of rhodopsin are generated in the rods and the sensitivity of the eye to light is greatly increased Full "dark adaptation" normally takes twenty to thirty minutes The cones, which dark adapt in five to seven minutes, are responsible for color and fine, reading vision, but never become sufficiently sensitive to low levels of illumination to provide scotopic vision It is scotopic vision that normally permits a child or adult to find his way about the house or village at dusk or dawn, or by the light of a weak flame

The ancient Egyptians and Greeks recognized nyctalopia and treated it with calf's or goat's liver (high in vitamin A content) [7] By the nineteenth century, nightblindness was known to occur primarily among the poorer strata of society, particularly during periods of dietary deprivation[2,8–10], was exacerbated by photic stress (which bleached so much rhodopsin that synthesis could not keep up with demand, causing borderline deficiency to become manifest as nightblindness)[11,12], and could be effectively treated with liver or liver oils [8,13,14] In fact, most other manifestations of xerophthalmia were first recognized by their association with nightblindness [8,12,13,15] Nightblindness (without evidence of xerophthalmia) was reported to have disabled Confederate soldiers between dawn and dusk,[16] and to have affected whole regiments during the Crimean War [17]

Hence, when vitamin A deficiency becomes a limiting factor, rhodopsin levels decline, rod thresholds rise, and nightblindness ensues [18] Treatment may quickly

restore adequate levels of rhodopsin, but animal work suggests full return of retinal sensitivity awaits regeneration of rod outer segments damaged from prolonged deficiency [19]

Despite the tremendous avidity of retinal pigment epithelium for vitamin A,[20–23] impaired dark adaptation is the earliest clinical manifestation of xerophthalmia In vitamin A-deprived rats[18,24] and human volunteers,[25–27] elevated rod thresholds and nightblindness precede other ocular manifestations of deficiency Impaired dark adaptation identifies previously unrecognized deficiency in older adults and children suffering from a variety of malabsorptive states [28–33] Clinically (and functionally) significant nightblindness characteristically occurs in the second, but principally the third, trimesters of pregnancy[34–36] in marginally deficient populations, usually in the absence of other signs of xerophthalmia

Deficiency initially results in slowing of rod adaptation followed by reduction in threshold sensitivity and, lastly, abnormal cone adaptation [37] As expected, these parameters respond to vitamin A in reverse order Standard electroretinographic abnormalities probably appear later, beginning with disappearance of the a-wave [37,38]

As befitting the earliest manifestation of deficiency, nightblindness is commonly the most prevalent form of xerophthalmia[39–43] and the ocular abnormality associated with the least depression in serum vitamin A levels (Table 1–3) [39] These associations have sometimes been obscured by complicating diagnostic issues The two most important are the sensitivity and specificity of a history of nightblindness as provided by the child's parent or guardian (Chapter 11), and the specificity of X1A and X1B as evidence of active vitamin A deficiency In Indonesia, careful assessment revealed that more than three-fourths of children with vitamin A-responsive conjunctival xerosis (X1B) were nightblind [44]

Although vitamin A levels of 20 μg/dl have traditionally been considered indicative of normal vitamin A status,[45] almost 20% of children with nightblindness (and 10% with nightblindness plus Bitot's spots) have higher levels [39] Carefully controlled depletion studies reveal impaired dark adaptation at vitamin A levels between 20 μg/dl to 30 μg/dl [26,27]

Nightblindness of nutritional origin responds rapidly (often within hours) to the administration of vitamin A [1,24,26,27,46–50] More sensitive indices of dark adaptation may take days or weeks to fully recover [37,51]

It is often unnecessary to measure dark adaptation (a cumbersome procedure, particularly in young children under field conditions) to determine whether or not a child is nightblind Many cultures have locally appropriate, specific terms that show high sensitivity and specificity for the condition [39,40,42,43,52–56] Presumably these are populations in which vitamin A deficiency has been endemic for many years Some communities may not have such terms [57,58] The critical issue is in identifying this specific, often extremely local term and an individual who can reliably report the status of the child (e g , a parent or older sibling) Nightblind-

ness generally goes unrecognized until the child begins to ambulate (one to two years of age) [39] The condition is exacerbated by photic stress, as when the child has flown a kite on a sunny day or watched too much television [1]

A new, simplified technique is showing promise for objectively assessing dark adaptation in young children using the consensual pupillary response to a graduated light stimulus (Chapter 11) [59]

Fine, white dots or mottling of the retina has been noted in children with xerophthalmia since the turn of the century [12,60–64] In 1928, Uyemura[64] reported their disappearance following vitamin A supplementation

On ophthalmoscopy, the retinal lesions appear to be small, discrete yellowish-white dots deep to the vessels and distributed about the equatorial region and periphery of both eyes (Plates 1–2) [47,64–68] Although they occasionally approach the disc,[47,67] the lesions remain outside the temporal vascular arcade Closer examination under magnification afforded by the slit lamp and contact lens, and by fluorescein angiography, suggest mottled depigmentation at the level of the pigment epithelium [47]

The number of lesions increases with the chronicity of deficiency In a case of self-imposed dietary deprivation, the lesions became more numerous over time [69] In Indonesia, fundus changes were more prevalent in older xerophthalmic children and in children with more severe vitamin A deficiency [1,70]

In most instances, the retinal lesions fade within one to three months of vitamin A therapy [47,64,65,67,68,70] They can also recur with considerable speed,[47] presumably representing either a predisposition to the retinopathy of vitamin A deficiency or, more likely, persistent pigmentary alterations that enhance visibility of new lesions or related retinal changes [1]

XF may represent disrupted outer segments of rods (and possibly cones) with accompanying alterations in the retinal pigment epithelium (RPE) [47] Impairment of dark adaptation in vitamin A-deprived animals is accompanied by decreased production of photoreceptor outer segments[71] and declining levels of the opsins of which they are composed[18,72], degeneration of both rods and cones, beginning with the distal portions of their outer segment[18,72–76], and alterations and loss of pigment epithelium [18,75] The latter appears related to the inanition and generalized interference with cellular metabolism that accompanies severe vitamin A deficiency, rather than specific interference in synthesis of visual pigment [46] Although there is a paucity of correlative histopathology,[77] one monkey that developed typical retinal lesions had degenerated rods, cones, and RPE [75] Retinal whitening (commotio) in owl monkeys suffering traumatic retinopathy, which can have a similar clinical and angiographic appearance, is related to disruption of outer segments of rods and cones, and their phagocytosis by cells originating in the RPE [78]

Degeneration of a large proportion of rods and cones should cause correlative alterations in the visual field Although some reports claim patients have normal fields, vitamin A-responsive constriction of the visual fields has been noted in a

number of deficient subjects,[37 38 79 80] including two carefully studied cases with fundus lesions [47 69] In one of these,[47] the defect was absolute and congruent with the area of retinal involvement Clinical recovery appears to proceed in three stages[47] nightblindness disappears within four days, presumably as vitamin A becomes available for regeneration of rhodopsin in outer segments outside the area of structural damage, visual fields return to normal one week later, perhaps as still-viable rods and cones in the affected area manufacture sufficient opsin (and terminal outersegments) to become functional, and retinal lesions fade within one to three months as the histologic alterations are reversed Functional and histologic deterioration and restoration follow a similar time course in vitamin A-deficient rats[18 46 74] and cats [19]

Conjunctiva (X1A, X1B)

The epithelial surface of the conjunctiva and cornea participate in the same process of keratinizing metaplasia induced by vitamin A deficiency in other organs [81] When sufficiently advanced and severe, they become clinically visible as conjunctival and corneal xerosis (*xeros,* dry)

Conjunctival xerosis (X1A) almost always precedes corneal xerosis (X2), an advanced (though still reversible) lesion representing more severe, prolonged vitamin A deficiency.

Histologic abnormalities are widespread prior to their clinical recognition Biopsy specimens of *clinically* normal conjunctiva from the inferonasal quadrant of children with temporal xerosis or Bitot's spots reveal early metaplastic changes [82] Keratinized cells and occasionally a prominent granular cell layer may be present [1,82]

More subtle involvement can be demonstrated in milder deficiency by conjunctival impression cytology (CIC) (Chapter 11) Epithelial surface layers removed by adhesion to a millipore filter and examined by light microscopy range from normal sheets of small, uniform, nonkeratinized epithelial cells with abundant mucous-containing goblet cells to abnormally irregular and fragmented sheets of large, often keratinized epithelial cells devoid of goblet cells (Plates 3–4) [83–86] In young children, there is ordinarily a direct correlation between the prevalence of abnormal CIC specimens and the severity of vitamin A deficiency (Tables 11–5, 11–6) [87] Almost half the children with "normal" eyes (void of clinical xerophthalmia) and serum retinol levels below 20 μg/dl had abnormal CIC, compared with 6% of children whose eyes looked the same but whose serum vitamin A was above 25 μg/dl Not all individuals with abnormal CIC will have depressed serum retinol (Chapter 11) [38]

When vitamin A deficiency is sufficiently severe and chronic, keratinization becomes apparent as a dry, corrugated, irregular surface The tear film or lacrimal lake may cover the surface and mask the irregularity Only after the tears have

been allowed to drain will the abnormal area appear "like sandbanks at receding tide" (Plate 5) [88]

The abnormal surface is almost always associated with some degree of overlying, white, foamy, or "cheesy" material This material, generally consisting of desquamated keratin and a heavy growth of bacteria (commonly the xerosis bacillus, a gram positive diphtheroid),[81,82,89] is easily wiped away, revealing the xerotic base below The overlying material reaccumulates within hours

For the most part, Bitot's spots (X1B) are the *sine qua non* of conjunctival xerosis "Pure" xerosis, in the absence of a fine or gross foamy/cheesy surface (X1A), is probably rare and overdiagnosed, as such, it is an unreliable sign of xerophthalmia and not acceptable evidence of vitamin A deficiency (Chapter 11) [45,90]

Bitot's spots first appear temporal to the limbus, in a characteristic oval or triangular shape This is not, however, invariable (Plates 6–11) With more serve deficiency the nasal, inferior, and lastly the superior quadrants become involved [1,91,92] By then, vitamin A deficiency is usually severe, corneal xerosis (X2) apparent, and the conjunctiva has a thickened, corrugated, skin-like appearance (Plates 20, 28) (Table 4–2) [1,8,92–95]

The histopathologic appearance of conjunctival xerosis/Bitot's spots is well described[47,82,95–105] a thickened, superficial layer of flattened cells commonly containing a keratinized surface, a prominent granular cell layer, acanthotic thickening accompanied by an irregular maturational sequence with mild to moderate disorganization of the basal layer and enlarged cells and nuclei with prominent nucleoli, complete absence of goblet cells (from areas in which they are usually dense[106], and in some instances, mild, chronic inflammatory infiltration of the substantia propria (Figs 4–1, 4–2) As noted, the surface is frequently covered by a frayed mass of keratin intermixed with gram-positive bacilli, other bacteria and even fungi In cheesy lesions, the surface debris sometimes reaches mammoth proportions [82]

Bitot's spots are so closely associated with vitamin A deficiency and xerophthalmia, and so misunderstood, that it is useful to review their character and significance The lesions were recognized in the early 1800s among patients with liver disease (presumably deficient from interference with intake, absorption and

Table 4–2 Geographic Extent of Conjunctival Involvement

Patient's Clinical Classification	*Number of Eyes*	*Quadrants with Conjunctival Xerosis (Percent of Eyes)*			
		0	*Temporal*	*Temporal + Nasal*	*≥ 180°*
X1[a]	100	5	37	51	7
X2[a]	106	4	9	40	47
X2/X3	167	8	12	35	45

[a]Difference in distribution between X1 and X2 (or X2/X3), $p < .01$

From A Sommer et al [92]

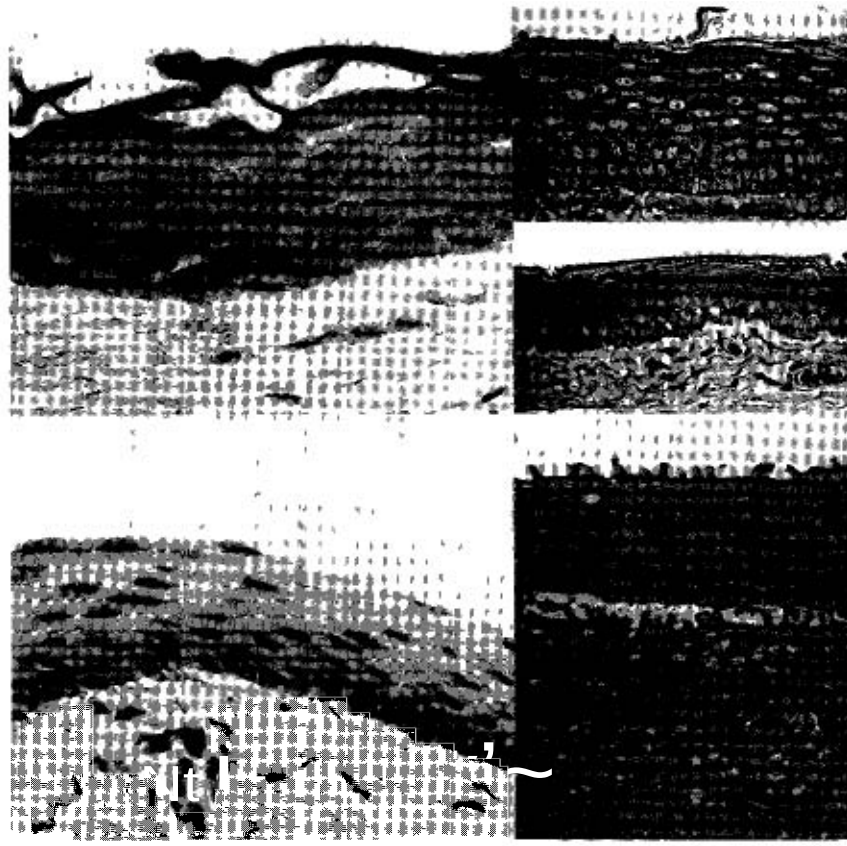


Fig 4-1. Top left By light microscopy, pretreatment temporal conjunctival biopsy (right eye) reveals a prominently keratinized surface (Dane s, × 528) Top right Phase-contrast microscopy of same area resolves surface keratinization kerathohyaline granules, acanthosis and dyskeratosis (paraphenylenediamine, × 480) Bottom left Biopsy of temporal conjunctiva (left eye) seven days following systemic vitamin A appears normal by light microscopy (hematoxylin-eosin, × 528) Middle right Phase contrast photomicrograph of same area shows decrease in epithelial thickness (cf top right, paraphenylenediamine, × 480) Bottom right TEM (transmission electron microscopy) of same area illustrates partial return of surface membrane infoldings and microvillae (× 10,700) (From A Sommer et al [82])

utilization of the vitamin) [107,108] These cases must have represented relatively late, chronic, severe deficiency [38] In 1860, Hubbenet described progressive epithelial dryness and scaling of the conjunctiva (and cornea) in undernourished nightblind soldiers, which responded to intake of beef liver [8] In 1863, Bitot described the same association between silvery-white, foamy conjunctival lesions in nightblind, but otherwise healthy, children [15] With rare disagreement,[109] the association be-

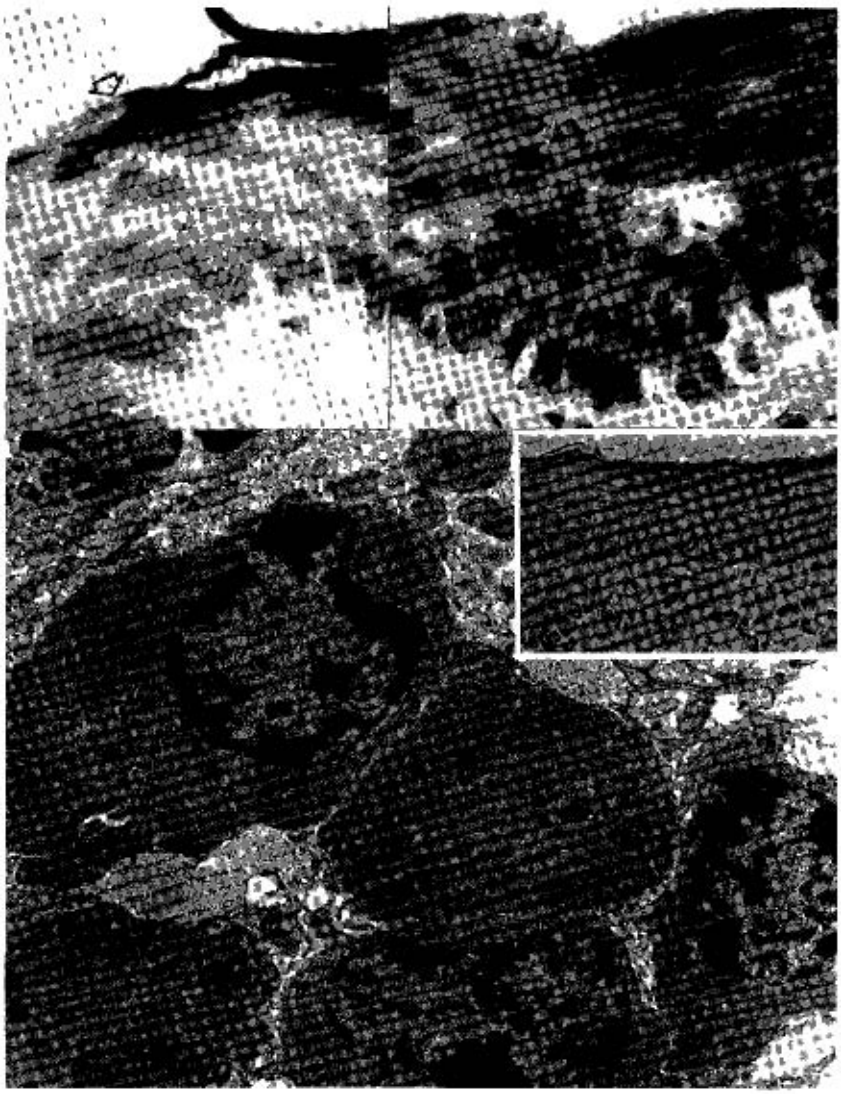


Fig. 4–2. A 13-year-old female with nonresponsive foamy Bitot's spots Top left Light microscopy of temporal conjunctival biopsy (right eye) exhibits abrupt transition (arrowhead) between the abnormal, heavily keratinized surface (right) and more normal adjacent conjunctiva (left) (Dane's, × 495) Top right Another section from the same specimen similarly demonstrates by light microscopy the abrupt transition between normal epithelium (left) and abnormal epithelium (right) with keratin, granular cell layer, and irregular maturational sequence (PAS, × 495) Bottom inset Phase contrast photomicrograph of inferonasal conjunctiva illustrates chronic inflammatory infiltrate of the stroma, predominantly composed of plasma cells (circled) (paraphenylenediamine, × 450) Bottom TEM of area circled in inset reveals clustered plasma cells having typical nuclear and cytoplasmic features (× 5700) (From A Sommer et al [82])

tween XN and X1B has been repeatedly confirmed [10 15 60 93 94 97 110–112] It is not, however, invariable

Graefe's[113] failure to recognize the connection between Bitot's "hemeralopic spot" and the corneal destruction he witnessed in the same children was credited as long ago as 1883[96] with fostering an artificial division between these two abnormalities, despite subsequent reports suggesting they arose from the same underlying condition[10 93 94 110 114] and that both could[13] respond to cod liver oil

What has confused investigators and clinicians alike is the observation that Bitot's spots do not always reflect active vitamin A deficiency or necessarily respond to vitamin A treatment [48 56 98–101 115–126] This apparent discrepancy is readily explained by studying the clinical and histopathologic response in children treated with vitamin A [44 82 91] Results separate subjects into two relatively distinct groups those in whom the Bitot's spots are a manifestation of active, systemic vitamin A deficiency, and those in whom they are not In the "active" group, the Bitot's spots contract or disappear entirely within two weeks, in the latter group, they persist largely unchanged The histologic characteristics of responsive and nonresponsive lesions are virtually identical, what distinguishes one from the other is the appearance of the surrounding conjunctiva [82] Nonresponsive lesions are sharply demarcated (Fig 4–2) In contrast, the histologic abnormalities of the responsive lesions merge with widespread but milder metaplasia of the rest of the conjunctiva, including the distantly located inferior nasal quadrant Animal models confirm the presence of generalized metaplasia of the conjunctiva in vitamin A deficiency, manifested as widespread disappearance of goblet cells [127] It would appear that responsive lesions are accompanied by active vitamin A deficiency, while nonresponsive lesions are not

Characteristics of children with responsive and nonresponsive lesions supports this thesis Children with nonresponsive lesions are older (almost 80% were ≥ 6 years, versus 11% of responsive children), have higher serum retinol levels (29 5 ± 4 7 μg/dl versus 13 5 ± 1 47 μg/dl), their temporal Bitot's spots (present in all subjects) are less likely to be accompanied by nasal spots (6% versus 66%), and concomitant nightblindness and punctate keratopathy (the earliest corneal manifestation of vitamin A deficiency)[128] are far less prevalent (punctate keratopathy, 13% versus 96%, nightblindness 0%–33% versus 78%–91%) [44] Recent studies confirm higher serum retinol values and lower rates of abnormal relative dose-response (RDR) (0% versus 32%) among nonresponsive cases [126]

The origin of the nonresponsive spots is uncertain and may involve a variety of factors Given the location and age of the population, it is conceivable they represent persistent sequelae of previous vitamin A deficiency, perhaps exacerbated by chronicity and by local factors Nonresponsive lesions were much more common in older than younger children and more likely to have been present longer [1] Most of the nonresponsive patients examined by Sie-Boen-Lian[99] and Metivier[124] claimed their lesions had first appeared in early childhood, suggesting

that their Bitot's spots (and/or their vitamin A deficiency) were more longstanding. Sinha made the interesting observation that treatment with high-dose vitamin A every four months prevented the development of Bitot's spots in preschool Indian children previously free of xerophthalmia, but had no impact on the seasonal reappearance of Bitot's spots among children in whom they had been previously present [56,129].

Although vitamin A induces keratinizing metaplasia throughout the bulbar conjunctiva, clinically visible involvement is ordinarily most frequent, most pronounced, and slowest to resolve temporally, and temporal lesions are usually the only ones to become nonresponsive. Vulnerability of the temporal perilimbal area may be related to the underlying conjunctival histology: it is the area most devoid of mucous secreting goblet cells [106]. Lying along the interpalpebral zone, it is also the area of bulbar conjunctiva most exposed to the outside environment and least wiped clean of bacteria and debris (which may be concentrated, by lid action, in this vicinity). Appelmans[130] reported a Bitot's spot located on the superior bulbar conjunctiva, in an area exposed by a coloboma of the upper lid. McLaren[131] reported a Bitot's spot in the inferior bulbar conjunctiva exposed by ectropion of the lower lid, Metivier[124] reported two instances of unilateral (nonresponsive) lesions, one in an eye proptosed by an orbital osteoma. We observed the disappearance of severe, plaquelike xerosis on the protuberant surface of a staphyloma (a consequence of keratomalacia) in a xerophthalmic child following systemic vitamin A [1]. Total excision of nonresponsive spots prevents their return, unless, of course, vitamin A deficiency recurs.

Since a child's vitamin A status may improve despite persistence of the Bitot's spots, the population prevalence of X1B is a poor basis by which to assess the impact of an intervention program [132,133]. Furthermore, given the greater frequency of nonresponsive lesions among older children, only X1B in children younger than six years is considered a suitably reliable index of vitamin A deficiency to serve as an acceptable prevalence criterion for establishing the vitamin A status of a community (Chapter 11).

The fact that human volunteers enrolled in depletion studies generally fail to develop Bitot's spots[25–27,134] merely confirms other evidence that these subjects never became particularly deficient. The previously healthy, well-nourished case reported by Bors and Fells was allegedly on a diet devoid of vitamin A for five years before developing significant ocular pathology [69]. Finally, the absence of typical Bitot's spots in some cases of classical corneal melting[69,89,121,135–139] speaks to the complexity of vitamin A-related corneal necrosis and the corresponding conjunctival response. As noted elsewhere, a precipitous "collapse" in vitamin A metabolism will result in corneal melting before metaplastic changes become clinically evident. In addition, conjunctival inflammation that often accompanies corneal ulceration (particularly when secondarily infected) can reverse or otherwise mask underlying xerosis [92]. Studies on conjunctival transdifferentiation in rabbits support the potential influence that vascular flow can have on epithelial

differentiation, perhaps through alterations in the local availability of vitamin A [140,141]

Despite recognition that inflammation[89,93,142–144] and sudden deterioration of vitamin A status[79,89,131,145,146] can modify the clinical appearance of potentially blinding xerophthalmia, cases have been denied therapy because of the absence of classical conjunctival xerosis [135] Pillat's suggestion[102] that "examination of the conjunctiva is the only guard against a mistaken diagnosis" is unfortunately, and sometimes tragically, incorrect It costs little, and is entirely safe and appropriate to treat every case of suspected xerophthalmia and keratomalacia with high-dose systemic vitamin A (Chapter 10)

Active Bitot's spots (and more extensive xerosis) are almost always bilateral and respond rapidly to systemic vitamin A All lesions will improve within five to seven days, and most will have disappeared entirely by two weeks [144] In his classic report of 1881, Snell[13] noted that most spots began to resolve within four days of taking cod liver oil and disappeared within ten days Similar results have been reported by others [93,101,102,147] As they heal, the lesions shrink and lose their covering debris Before disappearing entirely, they appear as small xerotic patches or tiny epithelial blisters located 1 mm to 2 mm from the limbus Healing usually progresses symmetrically in the two eyes, nasal lesions usually disappearing before temporal ones [44,102] In a closely followed case reminiscent of Sinha's observation, the originally sizeable spots shrank by day 20 to two tiny temporal bubbles, at both one and two months they had reexpanded into small classical Bitot's spots, at four months, both eyes were clear of all abnormalities, but by six months the temporal bubbles had reappeared, despite serum vitamin A levels remaining at their post-treatment high (> 25μg/dl)

Histologic and clinical responses proceed in parallel [82] Keratin and granular cell layers are usually gone within one to two weeks of treatment It takes considerably longer to repopulate the conjunctiva, particularly the inferonasal quadrant, with a normal complement of goblet cells [47,82,137,148]

An important consideration for both assessing the vitamin A status of a population (Chapter 11) and evaluating the impact of an intervention program (Chapters 11, 13–15) is the proportion of cases of Bitot's spots that represent active vitamin A deficiency and are therefore likely to respond to improvement in vitamin A status This will depend upon the prevalence of clinically active vitamin A deficiency in the age group studied and their propensity for developing nonresponsive lesions A therapeutic clinical trial was conducted during the last of seven rounds of observations in rural Indonesian preschool children (200,000 IU versus 700 IU vitamin A), and the children reexamined in double-masked fashion by the same ophthalmologist two to three weeks later Bitot's spots had disappeared in nearly 80% of high-dose recipients (versus a spontaneous "cure" rate of 50%, $p < 05$) [1] An 80% cure rate in only two to three weeks is equivalent to a nearly 100% cure rate over a longer follow-up [1]

Table 4–3 Prevalence of Bitot's Spots (X1B) at Follow-Up—Aceh Mortality Trial

	No Bitot's Spots at Baseline		*Bitot's Spots at Baseline*	
	Number of Children	*X1B, Number (%)*	*Number of Children*	*X1B, Number (%)*
Vitamin A villages	12,277	15 (0 12)	140	20 (14 3)
Non-vitamin A villages	11,342	55 (0 48)	160	20 (12 5)

From E Djunaedi et al [133]

Xerophthalmia rates were also assessed at baseline and follow-up examination (one year after initial dosing and three to four months after the second dose) in the Aceh mortality trial [132] The prevalence of *new* cases of X1B was five times greater in the control group than in the treatment group (0 1% versus 0 5%) But the total prevalence of X1B (new and old cases—despite treatment of all "old cases" at baseline) was only twice as great as the prevalence of *new* cases, representing persistence of refractory or recurrent lesions Among children assigned to the vitamin A prophylaxis group, and who had Bitot's spots at baseline (and received immediate high-dose treatment), the prevalence of Bitot's spots at follow-up was 100 times greater than among those children who didn't have Bitot's spots at baseline (Table 4–3), reminiscent of Sinha s experience [56 129] Hence, over the course of the year in which children with baseline Bitot's spots in the vitamin A group received baseline high-dose treatment for existing lesions and subsequent high-dose prophylaxis, X1B disappeared in 85% of the original cases This is consistent with the "cure' rate of 80%–100% noted above among preschool children followed on Java, six years earlier and 1200 miles away Persistence of Bitot's spots in the other 15%, however, created a point-prevalence of the same order of magnitude created by incident cases among non-vitamin A recipients

In the Sudan, where baseline vitamin A status was probably considerably better to begin with, large dose prophylaxis reduced the incidence of new cases, but to a lesser degree than total vitamin A intake (e g , from dietary sources) [58]

In the MSG-vitamin A fortification trial on Java,[149 150] Bitot's spots prevalence in the fortified villages declined 73% by the five-month follow-up, and 85% by the final examination at eleven months [150] Rates in the unfortified villages remained essentially unchanged (Fig 4–3)

Nonresponsive lesions have reportedly been more prevalent in parts of India, even among young children In the intervention trial conducted by Sinha in West Bengal,[56] the prevalence of Bitot's spots fluctuated between 8% and 20%, over half representing nonresponsive lesions Similarly, almost one-third of Indian children treated for "conjunctival xerosis" at a day care center failed to respond to massive dose vitamin A over a five-week follow-up period [151] In Bangladesh, the prevalence of XN in communities decreased in relation to vitamin A coverage, Bitot's spots prevalence declined much less [43] The relative prevalence of XN and

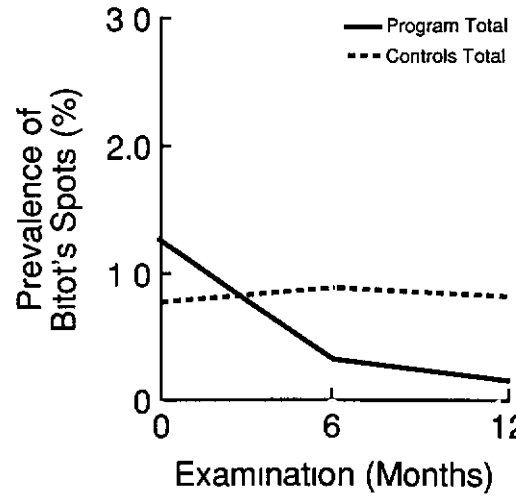


Fig. 4-3. The prevalence of Bitot's spots among children from Indonesian villages purchasing vitamin A-fortified MSG fell precipitously Bitot's spots prevalence in control villages remained essentially unchanged (From Muhilal et al [150])

X1B among those who did not receive a large dose compared with those who did was similar in Indonesia and Bangladesh, 1 9 and 2 7 for XN, and 1 3 for X1B [132]

As already indicated, individuals with responsive lesions are more likely to have other evidence of active vitamin A deficiency (e g , nightblindness, punctate keratopathy) or lesions pathognomonic of the condition (e g , bilateral Bitot's spots present nasally and temporally) However, the prevalence of coexisting evidence or pathogonomic signs is insufficient to distinguish active from inactive vitamin A deficiency on an individual basis For example, a high proportion of children presenting to clinic with Bitot's spots will be nightblind, since it is often the reason help is sought in the first place—80% at the Cicendo Eye Hospital,[1] with comparable rates elsewhere [13 15 48,93 97 110 152 153] But the proportion of X1B accompanied by recognized XN in the field is likely to be lower, representing varying reliability of a history of nightblindness when patients are not sufficiently aware or moved by the condition to seek help it was said to affect only 61% of children with Bitot's spots examined at their homes in neighboring West Java [39] Where an appropriate local expression for XN is not available, the rate can be 10% or less,[58] which may have been the case in the Sudan [58]

Conjunctival pigmentation, much commented on in the past[63 88 142 154] is a chronic change, if in fact it is related to vitamin A deficiency at all [1 44,89 95 115 117 121 155 156] It is not a reliable component or criterion for diagnosing Bitot's spots

Brief interest in the use of "vital stains" (e g , rose Bengal and lissamine green) to enhance the visibility of conjunctival xerosis[117 157] has waned following careful trials indicating the technique is neither sufficiently sensitive nor specific [151 158–161]

Xerophthalmic corneal destruction is classically said to occur in noninflamed "white and quiet" eyes (Plates 17, 22–23) [121 145 162] Nonetheless, conjunctival inflammation (of varying degree) is the rule, not the exception, in corneal ulceration (Plates 13, 15 24–26, 29) [1 93 115 142 143] It may result from secondary infection, concomitant illness (e g , measles), or simply corneal destruction itself It is clear that inflammation often masks or otherwise reverses conjunctival xerosis [92]

Cornea (X2, X3A, X3B)

Corneal changes of vitamin A deficiency, particularly ulceration and keratomalacia, were probably recognized even before those of the conjunctiva Over 250 years ago, Duddel[163] described corneal necrosis in poor children and "sometimes after measles ' There is no mistaking Brown's early 19th century description of a "case of ulcerated cornea from inanition" in a "poor babe "[164] Hubbenet, who first reported the "hemeralopic spot" for which Bitot became famous, may also have been the first to describe early changes in the cornea[8] " as the disease progresses the cornea itself becomes cloudy the vision becomes blurred, and consequently the patient has poor vision in artificial light, objects appearing to him as if they were wrapped in a halo"—exactly what one would expect from early metaplasia (and xerosis) of the corneal epithelium

Within six years of Hubbenet's observations, Blessig[110] described the entire spectrum of ocular manifestations, from nightblindness through corneal melting, in the presence of conjunctival xerosis Excellent descriptions of conjunctival and corneal xerosis, some progressing to ulceration and widespread necrosis, followed [93 94 142 165] Concluding with Bloch,[166] these early investigators had associated diverse ocular abnormalities with the lack of a specific nutrient, often precipitated by severe infections, and responding to foods rich in "fat-soluble A "

A number of classifications for these ocular changes were suggested over the years [1] The one officially recognized today[90 167 168] (Table 4–1) arose from a long-used scheme in Indonesia,[169] modified in 1974[45] and minimally revised in 1980 It retains the practical distinction between reversible corneal changes that heal entirely without sequelae (X2) and those that do not (X3) The latter is further divided into X3A and X3B, for practical purposes, X3A represents localized corneal destruction that, when healed, will leave a scar but preserve central vision, X3B is more extensive, invariably resulting in loss of most or all useful vision

The following discussion refers to a more detailed classification of corneal alterations developed solely for investigational purposes (Table 4–4) [1 170 171]

Punctate Keratopathy

The earliest, mildest corneal change observed in vitamin A-deficient individuals appears to be a fine, fluorescein-positive, superficial punctate keratopathy (SPK)

Table 4–4 Detailed Classification of Corneal Xerophthalmia Developed for Investigational Purposes

X2 ***Categories 1–3*** Corneal xerosis	Minimal inferior haze apparent on handlight examination, through frankly thick, elevated, keratinized plaques on the corneal surface
X3A: ***Categories 4–5*** Corneal ulcers with xerosis	Stromal loss in the form of relatively small, round to oblong, sharply demarcated, shallow to full-thickness ulcers accompanied in most (but not all) instances, by evidence of X2
X3B (I) ***Category 6*** Keratomalacia (localized)	Localized corneal dissolution or necrosis
X3B (II). ***Categories 7–8*** Keratomalacia (generalized)	Generalized corneal dissolution or necrosis of one or both eyes

Cases with punctate keratopathy but otherwise clear corneas (on handlight examination) are classified by their other manifestations of vitamin A deficiency (XN or X1)

From A Sommer [1]

(Plate 12), usually beginning inferiorly, especially inferonasally (Fig 4–4) [128] As the disease progresses, the lesions become more numerous, concentrated, and affect a larger portion of the corneal surface [1] Sixty percent of corneas of patients with confirmed nightblindness and 75% of patients with vitamin A-responsive conjunctival xerosis were affected, even though their corneas appeared crystal clear on handlight examination Among controls and patients with nonresponsive Bitot s spots, punctate keratopathy was far less prevalent and sparse (Fig 4–4) [44] Similar lesions, responsive to systemic vitamin A, have recently been reported in putatively deficient but otherwise asymptomatic adults [38]

Punctate keratopathy responds rapidly to vitamin A,[128] improving within one week of a single oral dose of 200,000 IU vitamin A Healing begins with loss of staining, producing a mixture of staining and nonstaining white specks Within one to two weeks many of the nonstaining specks become lightly pigmented By the fourth week only nonstaining, pigmented epithelial or subepithelial specks remain, most prominently inferiorly, where they may persist for months Eighteen eyes of ten patients with Bitot's spots received low-dose (700 IU) vitamin A as controls [128] By the end of the month none had improved and eleven (61%) had actually deteriorated, four of them alarmingly so These patients were then

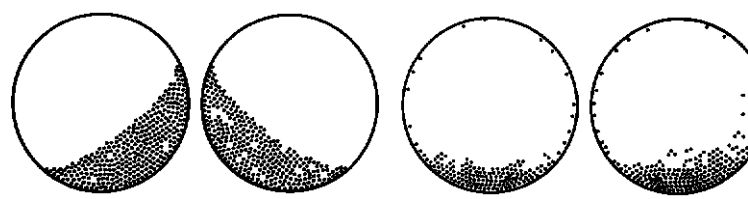


Fig. 4–4. In early xerophthalmia, fluorescein-staining punctate lesions have a strong predilection for the inferior and nasal aspects of the cornea (From A Sommer et al [128])

treated with a single oral dose of 200,000 IU vitamin A, all improved within nine days

By the time punctate keratopathy covers most of the corneal surface, involvement is generally apparent on handlight examination, thus qualifying for the designation "corneal xerosis" (X2)

Corneal Xerosis (X2)

The first visible change on handlight examination is a haziness of the corneal surface, usually most pronounced adjacent to the inferior limbus but occasionally centrally instead (Plates 10–11) The haziness becomes more distinct a few seconds after the lids have been retracted, the tear film having had a chance to run off revealing the dry, "nonwettable," finely irregular surface below Mild involvement may be missed by the casual observer Many such cases were referred to us for nightblindness or Bitot's spots In more advanced disease, haziness and decreased wettability are apparent over a larger portion of the corneal surface, although usually remaining most pronounced inferiorly In the vast majority of cases both eyes are involved, usually symmetrically

The most prominent abnormalities seen on slit-lamp examination are the punctate epithelial changes already described (Plate 12) Some corneas present with a mixture of staining and nonstaining specks, suggesting previous episodes of active disease An occasional case presents with nonstaining, fine epithelial microcysts with or without more typical punctate lesions (SPK) interspersed between them These cysts are often water repellent, appearing even less fluorescent than the tear film itself Within one to two days of systemic therapy the cysts usually disappear, being replaced in many instances by typical staining punctate lesions

The other abnormality apparent on slit-lamp examination is stromal edema As with the surface changes, the inferior aspect of the cornea is usually affected first and most severely

The extent of corneal haze is directly related to the density[1] and extent of the punctate changes and to the degree of stromal edema Dense accumulations of healed, residually pigmented specks may cause inferior haziness to persist indefinitely Central corneal haze, when present, is therefore a better index of active disease Corneas with active disease often have a ground-glass appearance on retroillumination This is most prominent in areas of dense punctate keratopathy, the same sites at which tear film break-up time is usually briefest

In more severe involvement the corneal surface has a dry, granular, peau d'orange appearance (Plates 14, 20), which is usually most prominent inferiorly, either adjacent to the limbus or as a horizontally oval area 2 mm to 3 mm above it Occasionally these changes first become apparent one to two days after initiation of therapy Multiple patches of similar appearance are sometimes distributed about the corneal surface Involvement is generally symmetrical in the two eyes Haziness tends to be more severe

The frankly xerotic nature of the changes is often apparent, even on handlight examination of the fluorescein stained cornea Many eyes have severe, dense punctate staining, while in others the fluorescein stains or pools in cracks on the cornified surface, presenting a "treebark" appearance (Plates 16, 18–19)

"Treebarking" appears to represent a solid sheet of cornified epithelium Although fluorescein pools in the cracks, the surface is usually free of punctate staining (Plate 16) Following therapy, the cornified layer loses its attachment to the surface below and peels off (Plate 16) The free end may fold over, or break off and slough The central, interpalpebral zone is generally the last to clear, presumably because of its protection from abrasive lid action (compare Plates 18, 19)

The severity of edema tends to be greater than in milder disease corneal thickness was at least twice normal in over one-fifth of eyes

Thick, elevated, frankly xerotic plaques are the most severe form of nonulcerating surface change (Plates 18–19, 21) Relative protection from lid abrasion and the greater potential for exposure probably account for their preponderance in the interpalpebral zone Particularly heavy plaques are foamy or amorphous in nature, suggesting an agglomeration of bacteria and fragmented keratin as in thick Bitot's spots Plaques, xerosis, and corneal haziness are present in both eyes Punctate keratopathy is of the densest sort, especially surrounding the plaques The surface of the plaques themselves generally stain as linear or whorl-like crevices With healing, the plaques fragment and slough, sometimes being (transiently) replaced by areas of densely packed, fluorescein-positive punctate lesions

Stromal edema is universally present

At least one-third of our fifty-three cases of X2 presented with a chief complaint compatible with corneal derangement twelve because they were keeping their eyes closed, six because they were avoiding bright lights, and two because they had "itchy eyes " A twenty-four-year-old woman with corneal involvement[47] denied any foreign-body sensation, grittiness, or dryness, but was moderately to severely photophobic

Aside from their ocular appearance, there was little to distinguish cases of mild to moderate X2 Patients with severe X2, however, were sicker and more protein-deficient [1]

- Serum albumen and transferrin were lower in severe X2 (2 5 μg/dl versus 3 4 μg/dl and 151 mg/dl versus 159 mg/dl respectively, $p < 01$), and pedal edema was far more prevalent (60% versus 15%, $p < 001$) Standard anthropometric indices were below normal but similar for all degrees of X2
- A history of recent febrile illness was more common and clinical illness scores more severe in advanced X2, as reflected in their mortality rates (4 2%, 12 5%, and 40 0% for mild, moderate, and severe X2 respectively)

Stromal Loss (X3)

It is more difficult to devise a meaningful clinical subdivision for cases with stromal loss, since different mechanisms, with potentially similar outcomes, might be responsible Categories 4 and 6 contain cases with active, locally destructive corneal lesions of distinctive appearance Category 5 contains cases with large, often perforating ulcers that may represent a transitional stage between the other two Categories 7 and 8 contain cases with complete corneal destruction of one or both eyes

The prevalence of binocular stromal loss increased with the severity of loss in the worst affected eye (24% versus 93% for Categories 4–5 and 7–8 respectively, $p < 01$) [1171]

X3A Corneal Ulceration (Categories 4–5)

Category 4 was characterized by the presence of one or more sharply demarcated ulcers of varying depth The most common form of stromal loss, present in over 70% of these cases, was a punched-out, smooth-walled, cylindrical ulcer, looking as if a cork borer or trephine had been applied to the eye (Plates 22,24) The margins were regular, not undermined, and, in only a minority of instances, swollen In 13% of affected eyes the worst lesion was a smooth, saucer-shaped depression, otherwise similar in appearance to the punched-out form Infiltration, if present, was slight and confined to the base, inner wall, or a thin rim around the ulcer (Plate 26) In rare instances a pinpoint infiltrate was present at a distance from the main ulcer, occasionally at the base of an erosion or second, superficial ulcer In an additional 7% of eyes, such shallow, saucer-shaped, infiltrated ulcers represented the most severe lesion In 9%, the worst lesion was a mild, seemingly transitional stage in which a large, subepithelial bulla (sometimes encountered in corneal xerosis [XS]) ruptured, leaving a densely staining erosion or superficial ulcer

Ninety-one percent of affected eyes contained only a single ulcer, the remainder contained two or three Over three-fourths of all ulcers were located in the inferior and/or nasal quadrants, most commonly the former Over 90% were 0 mm to 2 mm in diameter regardless of location Of those in the inferior quadrant, 45% were horizontally oval, 1 mm to 3 mm in length, and at least 1 mm to 2 mm above the limbus (Plates 17, 25)

The main ulcer was usually between one-fourth and one-half corneal thickness In only one instance (2 4%) was it greater than three-fourths depth

Among the forty-four cases in which both corneas were xerophthalmic, ulcers were present bilaterally in eight In all but one of these eight, the location, shape, size, and depth of the lesions were symmetrical in both eyes

Conjunctival injection, of varying intensity, was present in a majority (79%) of eyes with ulcers A hypopyon, usually less than 20% in size, was present in

one-third of eyes with ulcers (Plates 25–26) It was almost twice as prevalent (34%) among eyes with conjunctival injection as among those without (18%), and often adherent to the posterior surface of the cornea immediately deep to the ulcer

Two patients with corneal xerosis included in Categories 2 and 3 developed ulcers while under observation Both children were ill and severely protein-deficient Their cases illustrate potential mechanisms by which corneal ulceration might develop in xerotic corneas

Case 200/068 A two-year-old, severely malnourished child with rhonchi, pedal edema, stomatitis, and draining right ear was referred for Bitot's spots Both eyes had extensive corneal xerosis with a treebark, cornified layer in the inferonasal quadrant With hospital therapy, his general condition and eyes improved By day 3, the pedal edema had largely subsided, the conjunctival xerosis had disappeared, and the cornified layer had begun to break up and slough By day 7, the cornified layer was completely gone During the second week the child's general condition deteriorated he developed diarrhea, anorexia (requiring a feeding tube), and the pedal edema returned Although the diarrhea was soon controlled, serum albumin had fallen further Both corneas deteriorated by day 15, numerous fluorescein-positive punctate lesions and nonstaining epithelial microcysts appeared inferiorly despite good lid closure On day 17, the surface of the inferior portion of the right cornea developed an irregular appearance due to the presence of five small bubble-like mounds, all sharply demarcated cystic swellings in the superficial stroma The tear film in both eyes was irregular and unstable Within five days, a sharply demarcated, perfectly clear, intensely staining non-infiltrated shallow ulcer replaced the bubblelike mounds inferocentrally The ulcer was surrounded by a moderately hazy zone, the rest of the cornea was minimally hazy, studded with SPK, and 1 5 times normal thickness Three days later, the left cornea assumed an identical appearance By day 28, the erosion/ulcer of the left eye had healed without a trace The child died on day 37

Case 200/069 A severely ill and malnourished three-year-old boy with bloody diarrhea was seen with skin-like changes of his entire conjunctiva, and heavy xerosis with treebark cornification and adherent amorphous white plaques inferiorly in both corneas (Plate 18) His serum albumin was 1 9 and holo-RBP 1 Two days after admission and initiation of therapy, a sharp-margined, punched-out, fluorescein-positive depression formed at the receding, inferior edge of the xerotic plaque (Plate 19) This presented a striking appearance, the crevices of the remainder of the plaque converging on the ulcer, suggesting that underlying epithelium, and perhaps stromas as well, had been lost when the overlying plaque sloughed There was no evidence of infiltration and the margins were not swollen By day 8, the xerotic plaque in the left eye was confined to a small, nasally

remote area inferiorly What remained of the cornified layer was folded upon itself some distance from where the ulcer had been The ulcer itself had reepithelialized, forming a clear, nonstaining, shallow depression The child's general condition also improved, his edema disappeared, and his albumin rose to 2 6 by the end of the first week

A number of cases presented simultaneously with ulcers and potentially precursor lesions In some, ulcers were located amid treebark cornification, in others, they were associated with stromal bullae In one case a classical punched-out ulcer evolved from a cluster of bullae

Case 200/168 The left eye of a two-year-old girl contained a grayish-white, cystic, bulging, necrotic lesion (Category 6) However, the right eye appeared, on handlight examination, to contain a classically small (1 mm) circular, sharply demarcated, hazy lesion with central staining, suggesting a punched-out ulcer Slit lamp examination revealed that the lesion actually consisted of a rosette of clear epithelial or subepithelial microcysts The central cyst had apparently broken, resulting in the staining Of perhaps greater significance was a clear cleft parallel to the surface in the anterior stroma Although centered below the more superficial bullae, and widest at that point, it extended beyond their area of involvement Serum albumin was 3 3 g/dl and vitamin A 1 μg/dl The child received 200 000 IU vitamin A in oil orally and topical retinoic acid By day 4, the bullous lesion had become a classical, one-fourth depth punched-out ulcer By day 10, the ulcer had healed, leaving a small leukoma behind

Another case presented an extraordinary picture containing many of the elements already discussed and suggesting the potential role of localized desiccation and dellen formation [172–175]

Case 200/160 A nineteen-month-old boy had a serum vitamin A of 4 μg/dl, albumin of 3 2 g/dl, and a weight-for-height greater than 90% of standard The right eye was white and quiet, with conjunctival xerosis involving the inferior 180 degrees of bulbar conjunctiva and heavy punctate keratopathy, though the cornea still appeared clear by handlight examination The conjunctiva of the left eye appeared injected but not xerotic Centrally, the corneal surface was irregular (Plate 26), due to alternating mounds (clear epithelial or thin-walled superficial stromal vesicles) and depressions, the latter looking as if there were stromal loss or more likely, desiccation, as in dellen If these were, in fact, dellen, they were atypical areas of superficial desiccation overlying stroma two to three times normal thickness None of these irregularities stained (Plate 27) Breakup time (BUT) in the area was zero seconds Inferior to this irregular patch were two ulcers of peculiar appearance Both were oval with saucer-shaped, gently sloping, nonstaining walls of one-fourth corneal depth (Plate 26) Midway between the outer rim and center of each ulcer, the walls abruptly assumed a more typically cylindrical-shaped, punched-out, intensely staining configuration extending an

additional one-fourth to one-half depth into the stroma (see smaller ulcer, Plate 27) It is tempting to speculate that a clear, central stromal vesicle had broken, resulting in a typically punched-out, staining ulcer surrounded by a nonstaining, epithelial covered, dellen-like depression

As part of a therapeutic trial, the child received 200,000 IU oil-miscible vitamin A intramuscularly, and topical retinoic acid to the left eye three times daily The inferior ulcer proceeded to heal uneventfully at day 2, a sharply demarcated area of whitish (presumably) infiltration appeared at the base, the only area still staining By the next examination, on day 5, the entire area of the ulcer had become a clear, nonstaining corneal depression At day 2, the area of irregularity superior to this ulcer was unchanged (Plate 27) By day 5, however, it had an identical appearance to the inferior ulcer the alternating bumps (clear, intrastromal cystic spaces) and depressions had been replaced by a clear, non-staining, saucer-like depression, and the generalized stromal edema in the area had disappeared

The temporal ulcer did not fare as well as the inferior one By day 2, the complex saucer/cylindrical shape had been converted to a deeper "V"-shaped gutter, now staining edge-to-edge (Plate 27) Although topical antibiotic therapy (garamycin/bacitracin five times daily) was instituted at this point, the ulcer continued to deteriorate By day 5 the area was opaque, its surface a fluorescein-positive bulging mass, like an abscess more typical of cases in Category 6 The patient was removed from the hospital on day 6 and when next seen at three months, had two leukomas a dense one in the area of the initial temporal ulcer, and a much paler one in the area of the initial inferior ulcer. The depression found in the area of original irregularity was barely discernible At least a partial explanation for the poor response in this patient was the use of oil-miscible vitamin A intramuscularly the vitamin A level, initially 4 μg/dl, peaked at 9 μg/dl at 4 h, and was back down to 4 μg/dl at 1 day, where it persisted His serum albumin was a reasonable 3 2 g/dl

As already suggested, one of the earliest responses to therapy is formation of a whitish, presumably infiltrated area, usually at the base or within the wall of the ulcer In some instances, especially where infiltration is prominent or the ulcer large, deep, or adjacent to the limbus, the ulcer "fills in" with marked infiltration/edema in the base and surrounding area, and eventually scars over Shallow, central ulcers tend to reepithelialize uneventfully, usually within two to three days, forming clear depressions easily missed on routine handlight examination

Variations in the appearance of localized stromal loss were distinguished by the presence, in at least one eye, of a large, full-thickness, stromal ulcer (reaching to or beyond Descemet's membrane) (Category 5) These cases were also more likely to have bilateral stromal defects The corneal defects were all localized and sharp-margined They probably represent a modification or late stage of the processes encountered in Categories 4 (surface ulcers) and 6 (necrotic stroma)

Most cases provide interesting transitional changes between these two other groups, often containing lesions typical of one or the other in the same or fellow eye Two such cases are presented below, others elsewhere [1]

Case 200/091 A three-year-old boy with kwashiorkor an albumin of 2 8 g/dl, and transferrin of 30 mg/dl presented with a right cornea that was three times normal thickness and contained a sharp-margined ulcer that had perforated centrally The walls around the perforation were more swollen and opaque than characteristic for Category 4 The anterior chamber was flat The left eye had two distinct areas of treebark xerosis one inferiorly adjacent to the limbus, and another, a sickle-shaped swath 2 mm above the first, which terminated nasally in a 1 mm ulcer of one-half depth and slightly edematous, shaggy appearance Both corneas healed without consequence, the right as an inferior adherent leukoma, the left as a small inferonasal leukoma

Case 200/158 A severely ill, three-year-old female with marasmic-kwashiorkor died on the day of admission Serum vitamin A had been 4 μg/dl, albumin 1 9 g/dl, and transferrin 20 mg/dl The right eye contained a classical punched-out 2 mm shallow ulcer superotemporally of one-quarter corneal depth and two pinpoint ulcers superonasally (Plate 22) Although an anterior synechia had not been noted initially, slit lamp examination following dilation revealed densely pigmented tissue caught up in the posterior aspect of the cornea deep to the shallow ulcer, with clinically normal stroma in between The left cornea displayed a large (5 mm), central, perfectly round, punched-out, full-thickness defect, through which Descemet's membrane and iris behind were bulging (Plate 23) The inferior margin of the defect was moderately hazy

The age distribution of Categories 4 and 5 were identical But cases in the latter group were clearly sicker and more malnourished the prevalence of respiratory tract infection, fever, anorexia, pedal edema, and "life threatening illness" was at least twice as common as among cases in category 4, while their mean serum levels of vitamin A (3 3 versus 7 6 μg/dl), albumin (2 7 versus 3 2 g/dl) and transferrin (75 versus 134 mg/dl) were roughly half as high [1]

X3B Corneal Necrosis (Categories 6–8)

Except for the size of the area involved, the clinical appearance of these lesions is identical and readily distinguished from those of milder damage

Category 6 was characterized by the presence of localized, deep stromal necrosis The lesions were opaque, grey to yellow in color, and ranged in size from 2 mm to involvement of almost the entire cornea (by definition the cutoff between Categories 6 and 7/8) (Fig 4–5, Plates 29, 31)

In general, greyish lesions were extremely edematous and cystic Although their surface often appeared flush with (or even protruding above) surrounding,

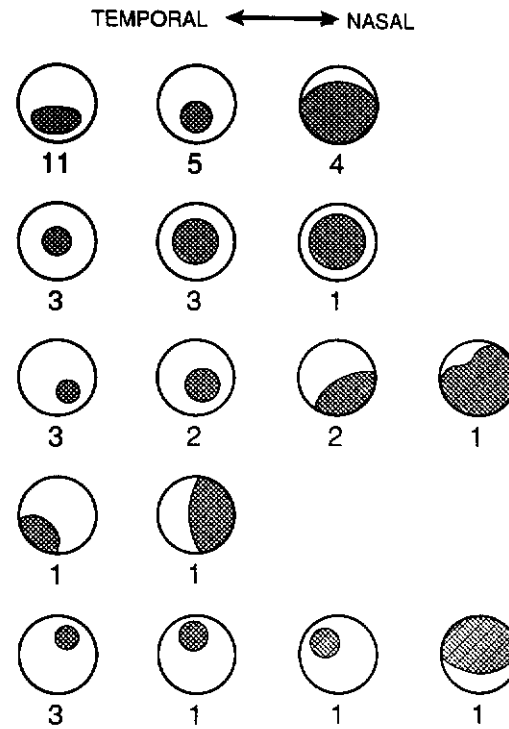


Fig. 4–5. In the vast majority of cases of xerophthalmia with focal corneal necrosis (Category 6), the lesions were located in the inferior and/or nasal quadrants (From A Sommer [1])

generally edematous cornea, they lacked much if not most, of their stroma With treatment, edema disappeared and the lesions collapsed Previously indistinct borders contracted down to sharply demarcated, deep stromal defects, often considerably smaller than was originally anticipated (compare Plates 29 with 30, 31 with 32), suggesting reversible changes surrounding an area of sharply focal, irreversible cylindrical dissolution Occasionally some of the edematous tissue sloughed Deep stromal loss was attended by descemetoceles With inadvertent pressure these could rupture, resulting in perforation and iris prolapse There was rarely any evidence of infiltration about the lesions

Other lesions were yellowish in color, suggesting heavy, focal inflammatory cell infiltration rather than quiet dissolution Some of these cases represented a different stage in the same process In a number of instances these formed protuberant, dome-shaped lesions (Plates 29, 31), sometimes with smooth, glistening surfaces Several failed to stain with fluorescein at the time of admission, suggesting that necrosis might have proceeded below an intact epithelium On the other hand, almost all began to stain within one to three days Whether initial absence of staining was due to some unrecognized factor masking epithelial

loss, or subsequent staining was secondary to changes induced by the reparative process, is uncertain The necrotic tissue sloughed, revealing a descemetocele below Purulent discharge was rarely present

Descemetoceles, especially large (≥ 3/4 corneal surface) perforated ones, scarred extremely slowly Small ones (< 1/4 corneal surface) reepithelialized rapidly (two to five days) and scarred quickly (especially if some stroma remained to serve as scaffolding) The smaller, rapidly healing lesions became adherent leukomas, while the larger, more recalcitrant ones became anterior staphylomas (eventuating in shrunken, phthisical globes)

The inferior quadrant was most commonly affected (Fig 4–5), though the lesions often extended beyond its borders The nasal quadrant was more commonly involved than the temporal

Serum levels of vitamin A and transferrin tended to be lower among patients with larger lesions

In Categories 6–8, the cornea of the ' second" eye was more severely affected than among cases in Category 4 44% suffered stromal loss (versus 18%) Fully 88% had some degree of frank xerophthalmic involvement Of the four corneas seemingly clear on handlight examination, three had extensive SPK and the other had bilateral conjunctival xerosis

Typical necrotic lesions were present bilaterally in nine cases in all, the size and location of the lesions in the two eyes were virtually identical In the six instances in which the stromal lesion in the second eye was milder (Category 4, punched-out type) the location of the lesions in the two eyes was still symmetrical

Generalized stromal edema was extremely common, and often severe Corneal thickness was sometimes three to four times normal with marked shallowing of the anterior chamber, even in the absence of corneal perforation Only rarely was there evidence of corneal exposure

The following case illustrates the reversibility of some areas of apparent necrosis

Case 200/171 A three-year-old girl presented with conjunctival xerosis and two small, classically punched-out staining ulcers in the right eye, and a large, greyish, opaque, nonstaining bulging lesion involving three-fourths of her left cornea Her protein status was reasonably good (serum transferrin 102 mg/dl, albumin 3 5 g/dl), but serum vitamin A was only 2 μg/dl She received 200,000 IU vitamin A orally on admission and again the following day By day 1, the surface of the swollen, opaque lesion in the left eye had begun to stain By day 5, the last follow-up, it had already shrunk to one-third its original size and involved only the inferior area Except for some mild, generalized edema (reduced from three times normal thickness on admission) the rest of the cornea appeared normal This is an extreme example of the fact that areas of apparent "necrosis" are sometimes largely (and rapidly) reversible

In the following case of vitamin A and protein deficiency, classical focal necrosis was precipitated by measles, an all-too-common story (Chapter 7), the rest of the globe was seemingly free of clinical disease, and the extent of irreversible destruction turned out to be less extensive than it initially appeared

Case 200/124 Measles rash and "conjunctivitis" appeared in this nineteen-month-old boy ten days prior to presentation Three days later he closed both eyes and had kept them closed since On admission he was noted to have a "healing" measles rash, pneumonia, and marasmic-kwashiorkor Serum albumin was 2 6 g/dl, transferrin, 115 mg/dl, vitamin A, 4 μg/dl, and holo-RBP 2 μg/ml Both conjunctivae were injected, without evidence of xerosis Neither cornea looked xerotic In the inferior half of the right cornea was a horizontally oval, sharply demarcated, slit-like, one-half thickness ulcer surrounded by an area of moderately heavy haze, which itself was surrounded by less dense haze An opaque, necrotic, "mushy" looking lesion with central descemetocele and probable iris prolapse occupied the inferior one-half to two-thirds of the left cornea The opaque area was greyish and edematous The transition between the lesions and remaining cornea (superiorly) was dramatic, especially in the left eye, where it was crystal clear and free of SPK On vitamin A therapy the two eyes healed uneventfully, the right eye as a faint macula, the left as an adherent leukoma involving only the inferior two-thirds of the extent of the original lesion

In cases with the most severe damage, the necrotizing or melting process involved the entire cornea In some instances the cornea resembled a relatively firm, yellowish-white abscess whose surface was seemingly intact (Plate 28) In others, what cornea was left resembled thick mush or gelatin (Plates 13, 15) The final result was the same the corneal stroma sloughed, usually within one to three days, resulting in an enormous descemetocele (occasionally retaining a thin stromal or fibrinous covering) (Plate 23) or (rarely) complete prolapse The peripheral 1 mm corneal/limbal zone was spared it was usually edematous and opaque on admission but remained vital (Plates 13, 15, 23, 28) Unlike some instances in Category 6 where the surface of the localized "abscess" did not stain initially, all lesions in Categories 7–8 did

Two forms of healing are seen, depending upon the amount of residual stroma Where some stroma remains, a mixture of scarring and (often multiple) descemetoceles develop, all of which eventually scar over Where all stromal tissue has melted away, a peculiarly cross-banded star pattern of iris covered by Descemet's membrane bulges forward in the quadrants (Plate 23) This scars slowly, and the person remains at risk of complete prolapse for weeks to months Both situations commonly result in a large, protuberant staphyloma, which may later shrink as the eye becomes phthisical

In some cases the "necrotic" cornea is completely clear, white, and cystic In one instance, a clear descemetocele was present centrally, as if there were

insufficient stroma left to swell and produce the characteristically opaque appearance In this instance the anterior chamber was formed and iris was in its normal position, it healed as a flat, vascularized scar

The clinical course in several patients suggested that the initial process is one of stromal dissolution, followed by infiltration of the devitalized tissue

Case 200/096 A two year-old girl with kwashiorkor and serum vitamin A of 3 μg/dl, holo-RBP of 2 μg/ml, albumin of 2 9 g/dl, and transferrin of 20 mg/dl presented with an almost pure descemetocele of the right cornea, and white, opaque dissolution of the entire left cornea One to two days later the left cornea was yellow, more opaque, and bulging

In only a single case in Category 7 was the opposite eye entirely normal on handlight examination, while 54% (7/13) of cases suffered bilateral stromal loss By definition, all cases in Category 8 suffered complete, bilateral corneal destruction Otherwise the two groups of cases did not differ, clinically or biochemically

Clinical-Histopathologic Correlations

Despite detailed descriptions of the ocular manifestations observed in vitamin A deficiency and a good deal of animal experimentation, the pathogenesis of corneal destruction remains unclear Clinical-histopathologic correlations, though limited in number, help to illuminate what those precesses might be The following largely represent Indonesian study participants who either died or underwent enucleation [176]

Corneal Xerosis (X2)

Two patients with corneal xerosis, both responding poorly to therapy, died while their corneas were hazy In neither case was there clinical evidence of residual keratinization Histologic appearance was remarkable only for occasional areas of epithelial thinning and facettes in one of the cases The superficial facettes may represent healing of earlier, superficial ulceration

Stromal Loss (Ulceration/Necrosis) (X3)

Histologic material was available from three study participants with active corneal destruction of varying severity and duration [170 176]

Case 200/101, with relatively fresh disease, is among the most informative in the literature One cornea contained small, classically punched-out ulcers The other cornea was almost entirely a descemetocele Light and electron microscopy confirmed the sharply focal nature of both sets of lesions, scarcity of inflammatory cells in many areas of active necrosis, the absence of bacteria, and what appeared to be an intact (if keratinized) epithelium

Case 200/101 A severely marasmic (weight-for-height < 60% of standard) one-year-old boy had a serum albumin of 2 2 g/dl, transferrin of 40 mg/dl, vitamin A of 10 μg/dl, and holo-RBP of 2 μg/ml He had a history of nightblindness The right cornea was largely replaced by a near limbal to limbal descemetocele The left cornea contained two three-quarters depth, sharply punched-out, adjacent ulcers, each 1 mm in diameter, and a deep, intact, anterior chamber These ulcers were surrounded by a moderate (1 mm) rim of infiltrate The child received 200,000 IU vitamin A orally on admission and again the following day, and frequent topical antibiotics and systemic penicillin and streptomycin On day 1, the ulcers were clearly filling in, a process which was more advanced by day 2 On the morning of day 3 the child died and both corneas were biopsied The right cornea, which had received topical retinoic acid in addition to the other agents, had remained unchanged throughout

Histopathologic examination of the right cornea revealed keratinized epithelium overlying a thin layer of lush, young, fibrous tissue lined posteriorly by iris (Fig 4–6a) The iris was infiltrated with mononuclear cells No bacteria were noted

The left cornea contained keratinized epithelium that was markedly edematous but lacking a granular cell layer The basal layer contained large, plump cells with prominent nucleoli There was an abrupt loss of Bowmen's layer and corneal stroma in a wedge-shaped configuration extending three-fourths of the way through the stroma (Fig 4–6b) This area contained strands of necrotic

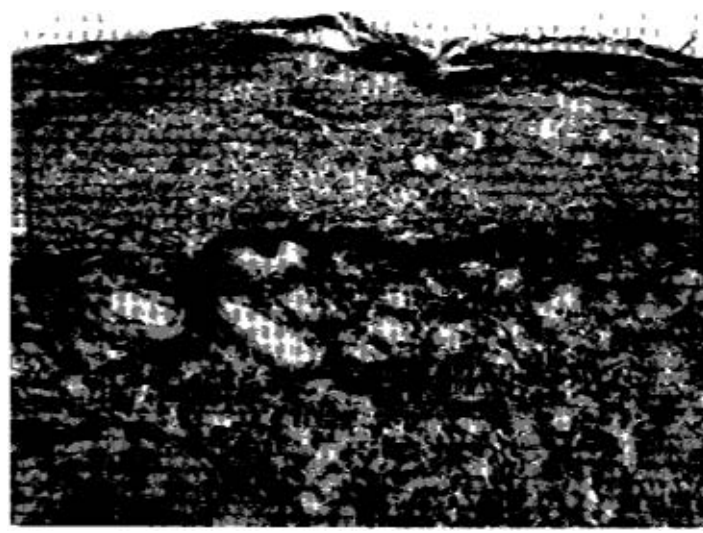


Fig. 4–6a. Right eye of case 200/101 There is total dissolution of corneal stroma Intact keratinized epithelium covers fibrinous material and iris behind No bacteria, and only rare inflammatory cells, were present (periodic-acid Schiff, × 575) (From A Sommer et al [176])

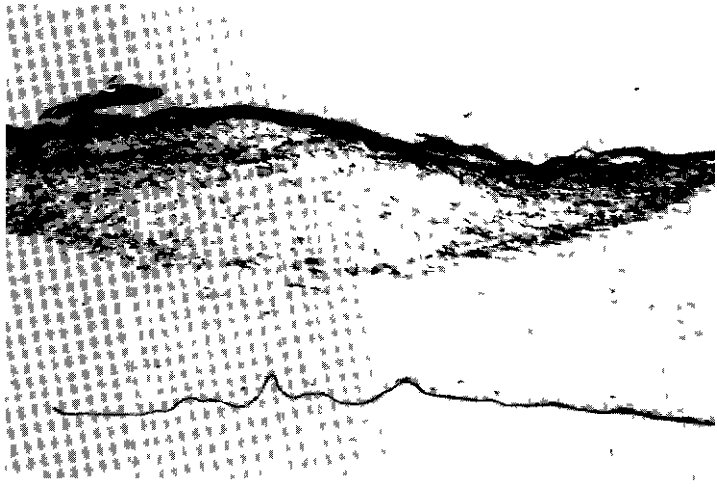


Fig. 4–6b. Left eye of case 200/101 Peripheral area of lesion demonstrates sharp delineation and saucer-shaped configuration of stroma disintegration, which was covered by intact keratinized epithelium (hematoxylin-eosin, × 45) (From A Sommer et al [176])

collagen, a greater number of plump, spindle-stellate-appearing fibroblasts than normal, and marked stromal edema, all below an intact epithelium Most significantly, there was little inflammatory infiltrate and no bacteria in the area of collagen dissolution The keratocytes had been replaced by large fibroblastic-type cells At either side of this sharply demarcated area the stroma was normal except for a paucity of keratocytes In some areas, Descemet's membrane was broached despite the absence of clinical evidence for perforation Such areas were covered anteriorly by edematous, necrotic material, and overlying epithelium (Figs 4–6c, 4–6d)

In the second case, 200/037, corneal material was obtained shortly after widespread necrosis replaced small, sharply demarcated ulcers Although inflammatory infiltration was extensive, it was often most intense in otherwise normal areas surrounding involved stroma

Case 200/037 Ten days prior to admission, an eight-year-old girl developed high fever, five days later, a hemorrhagic rash on her face and extremities, and two days later, "white spots" on both corneas On admission, she was considered moderately ill and only mildly (grade I) malnourished The conjunctiva of both eyes was injected and the corneas hazy, especially inferiorly, 1 mm to 2 mm oval, sharp-margined shallow ulcers were present 2 mm above the inferior limbus, within the area of densest haze Serum transferrin was 58 mg/dl and albumin 2 2 g/dl, which was surprising given the absence of edema or other evidence of kwashiorkor, suggesting acute decompensation of protein metabolism Gentamicin and mycitracin were administered five times a day to the right eye The lids

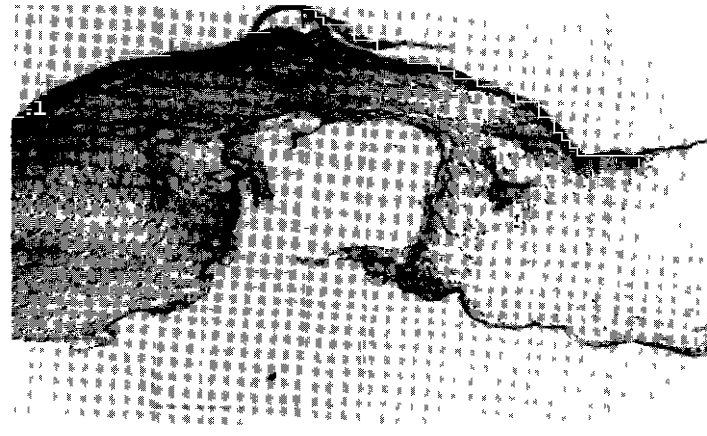


Fig. 4–6c. Same eye as in Fig 4–6b, showing central area of lesion Most of the stroma is destroyed, as is Descemet's membrane behind The sharply delineated saucer-shaped configuration of involvement is readily apparent The entire area is covered by keratinized epithelium (hematoxylin-eosin, × 30) (From A Sommer et al [176])

Fig. 4–6d. Higher-power view of section shown in Fig 4–6c The sharply focal nature of the lesion, and lack of both bacteria and significant inflammatory infiltrate, are readily apparent (hematoxylin-eosin, × 100) (From A Sommer et al [176])

were well opposed during the next two days and the eyes showed minimal improvement On day 3 she became comatose and the lower half of both eyes were noted to be exposed The conjunctivae were white and quiet O U The entire right cornea had a yellow, opaque, necrotic appearance The left cornea was gelatinous centrally, with iris seen through the center She died within a few hours and the nasal quadrants of both corneas were biopsied

Histopathologic changes in the right cornea included necrosis and foci of gram-negative rods Some areas of necrosis were acellular, but surrounded by otherwise normal stroma infiltrated by neutrophils Gram-negative bacilli were present

The specimen from the left eye was lined in places by keratinized epithelium There was an abrupt transition to stromal necrosis with fraying of collagen Polymorphonuclear leukocytes were present throughout the stroma, sometimes surrounding rather than within the area of necrosis Actinomyces-like organisms lined the exposed margin of necrotic stroma in the peripheral area of the descemetocele

The last and most chronic of the three cases, 200/006, documents the risk of secondary bacterial infection and endophthalmitis in the xerophthalmic eye It is also another example of xerophthalmic necrosis precipitated by measles (Chapter 7)

Case 200/006 A four-year-old boy presented with a history of measles, fever, and diarrhea one month prior to admission, and a red, tearing, photophobic eye of two weeks' duration He had received antibiotic eye drops, pills, and injections Serum vitamin A was 6 μg/dl, and albumin 2 4 g/dl The conjunctiva and cornea of the right eye were mildly xerotic The conjunctiva of the left eye appeared mildly injected but not xerotic The left cornea was yellow, soft, necrotic, and bulging, with a gelatin-like central protrusion of vitreous or lens The peripheral corneal/limbal ring was normal A small biopsy was taken of the centrally necrotic and perforated cornea 100,000 IU water-miscible vitamin A was given intramuscularly and preparations made for enucleation of the left eye the following day In the interim, the left eye developed a purulent exudate The biopsy specimen resembled a fibrinous exudate containing a segment of Descemet's membrane and, in some areas, moderate numbers of diplococci Examination of the enucleation specimen revealed an abrupt transition between peripheral cornea, which was relatively intact except for fraying of collagen bundles and areas of acute inflammatory cell infiltrate and vascularization, and the central fibrinopurulent full-thickness exudate The margins of preserved stroma showed a dense neutrophilic infiltrate Epithelium covering the limbal cornea showed minimal keratinization Large gram-positive cocci were noted in the vitreous abscess

Local Pathogenic Factors

A dry (xerotic) ocular surface can result from physical desiccation (a deficiency in the quantity or quality of the tear film) or from primary keratinizing metaplasia Mori[177] thought xerosis accompanying vitamin A deficiency was secondary to disturbed secretions of the lacrimal gland, in part because he noted that tears were, as a rule, scanty or absent in children with xerophthalmia [178] Wolbach[179] correctly identified keratinizing metaplasia as the primary event, at least in rats, the lacrimal glands were unaffected until late in the disease Since then, lacrimation has rarely been mentioned tear production is said to remain unchanged in xerophthalmic rabbits [180]

Despite these negative reports and difficulties in assessing basal tear secretion,[1 181 182] children with xerophthalmia are more likely to suffer markedly reduced tearing than age-sex-neighborhood-matched controls (Table 4–5) [1 183] There is a direct relationship between the severity of xerophthalmia (e g , degree of vitamin A deficiency) and the proportion of children with reduced basal tearing

Corneal cases suffered a particularly severe reduction in tearing over 30% of X2/X3, but only 5% of their controls, had < 5 mm wetting

At least some of this relationship seems to have been a direct result of vitamin A status Among otherwise normally nourished children (serum albumin ≥ 3 5 g/dl and weight-for-height ≥ 80% of standard), the more severe the xerophthalmia, the higher the prevalence of deficient tearing Further, tearing responded to vitamin A therapy In an analysis restricted to outpatients (whose therapy consisted solely of high-dose vitamin A) followed for seven to ten days, the proportion of eyes in which tearing increased was significantly greater than the proportion in which it decreased ($p < 001$ for corneal cases, $p < 01$ for all xerophthalmia) [1 183] In a recent report, half the fifteen adults with hepatic dysfunction studied for subclinical vitamin A deficiency had marked reductions in tearing (though there was allegedly little correlation with individual serum retinol levels) [38]

Other factors besides vitamin A status may well play a role Tear production is also associated with protein status the prevalence of deficient tear production is inversely related to serum transferrin [183] Almost half the cases of X2 with

Table 4–5 Tear Production in Xerophthalmia

		Eyes with < 15mm Wetting	
Clinical Classification	*Eyes (N)*	*(N)*	*(Percent)*
Controls[a]	60	4	6 7
X1	46	18	39 1
X2/X3	54	32	59 3

[a]Controls age sex-neighborhood matched to cases of X2/X3

Test for linear trend $p < 001$

From A Sommer et al [183]

transferrin levels < 50 mg/dl had < 5 mm of wetting, compared with a fifth of cases with higher transferrin levels ($p < 01$) [183] This may, in part, be secondary to functional dehydration, since many of these children appeared "dry" despite pedal edema

The concomitant presence of dehydration, vitamin A deficiency, and protein deficiency should have a marked effect on tear production, and probably explains the severity of corneal xerosis among cases in the severest category of X2

Kogbe et al [184] observed that normal children "produced tears much more readily" than children with measles Whether this is associated in any way with measles-related corneal destruction is uncertain, but they note that all but one child still available for study at three weeks were too ill to provide tear samples, they "had dry eyes as they just cried noisily without shedding any tears " It seems most had secondary systemic infection and malnutrition, presumably they may have been dehydrated as well

Considerably more attention has been given to the loss of mucous-producing goblet cells, which virtually disappear from the bulbar conjunctiva by the time conjunctival xerosis (X1) appears [44 82] Goblet cell density is a sensitive indicator of ocular surface disorders, including vitamin A deficiency [84 185 186] Within two weeks of systemic vitamin A treatment of corneal xerophthalmia, goblet cells begin to repopulate the inferonasal quadrant, they reach normal densities within one month [148]

Mucous stabilizes the tear film[187] by interfacing between its aqueous component and the hydrophobic surface of the cornea [188 189] It seems reasonable to suspect that its loss results in rapid tear-film breakup and epithelial "exposure," with consequent drying and damage to the corneal epithelium, much like primary mucous-deficient states [190] Goblet cells are not, however, the only source of mucin-like glycoproteins in the tear film, and goblet cell counts can be profoundly depressed—out of all proportion to relatively modest reductions in tear film mucin [185] This is consistent with observations that corneal healing in xerophthalmia proceeds more rapidly than does the repopulation of conjunctival goblet cells [148]

While alterations in the quantity and quality of the tear film might exacerbate vitamin A-related xerosis, they do not initiate it, most evidence suggests tear film alterations occur *pari passu* with other changes Keratinizing metaplasia of the corneal epithelium is almost certainly the direct consequence of altered vitamin A metabolism, just as it is in the epithelium of the bladder and renal pelvis[179 191] despite their constant bathing by an aqueous medium Corneal keratinization, in the rat at least, precedes alterations in the lacrimal gland, and in both the rat[179 192] and guinea pig[127] occurs in the presence of abundant conjunctival goblet cells Since goblet cells have already disappeared in mild conjunctival xerosis, it is difficult to imagine how their absence could account for more severe disease, and corneal healing, of all degrees of severity, begins within one to three days and is often completed within four to seven days,[1 193] long before goblet

cells return in any numbers (see Table 10–1) [44 47 137 148] Except for early punctate epithelial erosions,[172] ocular changes in keratoconjunctivitis sicca and other dry-eye states[172 194–198] generally do not resemble vitamin A-related xerophthalmia even though dry eye states may be deficient in both tears and goblet cells [185 199–201] Although it is possible that alterations in the amount of mucus produced by the lacrimal gland[202 203] could account for some of these differences, histopathologic changes in rats whose lacrimal glands are surgically removed are far milder than in those made vitamin A deficient [204] Occasionally patients with Sjogren's syndrome associated with rheumatoid arthritis develop steep-margined, non-infiltrated ulcers similar to those of mild X3 [205]

Composition of skin surface lipids is altered in protein-energy malnutrition [206] It is conceivable that comparable changes in the lipid phase of the tear film could contribute to the xerophthalmic process This, however, has received little attention

Even if changes in tear film dynamics are not responsible for initiating keratinizing metaplasia, they may contribute to the predilection to mild corneal abnormalities (punctate keratopathy and early haze) for the inferior and nasal quadrants, the exaggerated accumulation of keratin debris in severe corneal xerosis, and dellen formation, epithelial degeneration, and the like, especially in functionally dehydrated individuals

Although the vast majority of cases studied in Indonesia appeared to have complete lid closure and a normal Bell's phenomenon, exposure may exaggerate the effects of tear film instability Patients with keratitis sicca who are presumed to have incomplete lid closure during sleep[207] develop punctate keratitis and intraepithelial microcysts in the inferior cornea Some even develop sterile ulcers, most commonly inferonasally, though not of the circular, punched-out variety typical of xerophthalmia

Accumulation of heavy plaques in the interpalpebral zone may be influenced by the relative exposure and lack of lid action at this site

The potential role of tears as a normal and essential mechanism for delivering physiologically active vitamin A to the ocular surface has only recently received attention Topical retinoic acid can speed xerophthalmic corneal healing (Chapter 10) [208–210] Ubels has shown that in rabbits the lacrimal gland stores vitamin A as fatty acyl esters[211] and maintains a 2 μm retinol concentration in tears,[212] similar to the minimal concentration of topically applied retinoic acid needed to reverse corneal epithelial keratinization in vitamin A-deficient rabbits [213] A single large oral dose of vitamin A (110 mg retinyl palmitate) produced a significant increase in tear fluid retinol levels in marginally nourished Thai children [214]

The keratinized surface of xerophthalmic eyes presents a good culture medium for a host of bacteria, particularly the xerosis bacillus, but also *Staphylococcus, Pneumococcus, Hemophilus* and others, which are commonly encountered in large numbers [96 102 154 215] There is less evidence to support suggestions that infection, as opposed to colonization, is more common or responsible for stromal

ulceration and necrosis [121 131 135 216 217] The large numbers of bacteria Pillat discovered in smears from corneal cases disappeared following vitamin A therapy [102]

In advanced experimental xerophthalmia of rats, necrotic corneas frequently contain large numbers of bacteria [215 216,218] But these could be secondary infections and need not reflect the situation in humans Ocular and periocular inflammation, conspicuous in xerophthalmic rats, is often minimal or absent in the human condition [121 145 165 166]

Clinical reports generally describe isolated instances of positive cultures from ulcerated eyes [135 137] But a culture does not distinguish primary from secondary infection, or either from mere colonization Only a tiny proportion of eyes harboring *Pseudomonas*[219] or *Staph aureus*[220] are ulcerated To be of pathogenic significance, positive cultures should be more prevalent in ulcerated than in nonulcerated eyes Valenton et al [217] found that the frequency of recovery of pathogenic organisms (*Pseudomonas, Pneumococci, Moraxella, S pyogenes, Klebsiella, E Coli,* or *Proteus*) increased with the severity of clinical xerophthalmia But in another study[121] that claimed to demonstrate the importance of infection, positive cultures in malnourished children were no more prevalent in those with ulcerated corneas than in those without, even the spectrum of organisms was the same

One bacteriologic study included controls, as well as eyes with xerophthalmia of varying severity [1] Conjunctival/corneal swabs (and scrapings in ulcerated/necrotic corneas) grew a wide variety of organisms The most common and potentially pathogenic were *Pseudomonas, Staph aureus,* and *E coli* Positive cultures were more prevalent among eyes of cases with active xerophthalmia than among controls, though not to a statistically significant degree (Table 4–6)

Table 4–6 Positive Bacterial Cultures in Controls and Cases of Corneal Xerophthalmia (Study II)

		Percent of Eyes with Positive Cultures			
Corneal Status	*Total Eyes (N)*	Pseudomas	*Micro Aur Coag +*	E coli	*Total Positive*
Controls	50	24	24	4	46
X2 (1–3)	117	25	30	9	53
X3A (4–5)	65	31	28	14	60
X3B (6)	44	36	23	11	57
X3B (7–8)	35	31	34	11	54
RELATIVE RATE OF POSITIVE CULTURES (COMPARISON BETWEEN EYES OF DIFFERENT CORNEAL STATUS SHOWN IN FIRST COLUMN)					
X2/control		1 04	1 25	2 25	1 15
X3A/X2		1 24	0 93	1 56	1 13
X3B (6)/X2		1 44	0 77	1 22	1 08
X3B (7–8)/X2		1 24	1 13	1 22	1 02

From A Sommer [1]

The prevalence of positive cultures among ulcerated and nonulcerated eyes of xerophthalmic patients was similar, even by pair-wise comparison of ulcerated and nonulcerated eyes in the same patient (n = 33) Other organisms, which included strains of *Hemophilus, Strep viridans,* diptheroids, and *Aerobacter,* were common in all groups *Proteus* was cultured from a single eye (with corneal xerosis)

Various fungi, which may have been contaminants, were recovered from seventeen eyes (*Penicillium* [twelve eyes], *Aspergillus* [three eyes], and *Candida* [two eyes]) There was no consistent difference in the frequency of positive isolates among the various forms of corneal ulcer/necrosis Positive isolates were more frequent from ulcerated/necrotic corneas (9%) than from purely xerotic ones (3 4%) ($p < 07$)

The lack of etiologic significance of locally infectious agents was confirmed by a formal diagnostic/therapeutic trial one eye of each patient was systematically assigned to receive frequent topical antibiotic ointment (at a minimum, gentamicin plus bacitracin five times a day) Twenty-seven consecutive hospitalized patients with bilateral corneal ulceration were studied before a single nonrecipient eye was found to fare worse than its matched recipient Despite the fact that similar, initial deterioration was occasionally observed in eyes receiving antibiotic, the trial was discontinued at this point and all subsequent cases of ulceration were treated with both topical and systemic antibiotics

Unfortunately, the formal trial was not conclusive most cases required and received systemic antibiotic therapy for nonocular conditions (tuberculosis, respiratory tract infection, gastroenteritis, etc) By including patients outside the formal trial (because they refused hospitalization), observations were available on twenty-two ulcerated (Category 4) or locally necrotic (Category 6) corneas unexposed to either topical or systemic antibiotics All but two (9%) healed rapidly and uneventfully on systemic vitamin A therapy alone One of the two exceptions cultured only a coagulase-negative *Staph albus* , the other contained an ulcer (Category 4) that had enlarged by the next visit, at day 4, but began to resolve spontaneously by day 6

The noninfected clinical appearance of most ulcers, the frequent absence of bacteria and fungi from areas of stromal dissolution studied histopathologically, and the rapid healing induced by vitamin A[102,193] and retinoic acid (see Chapter 10),[210] combined with the previous observations, suggest that local bacterial and fungal "infections" rarely initiate and only occasionally contribute to xerophthalmic ulceration or melting However, there is no reason why ulcerated or perforating lesions should not become secondarily infected[95,221] and even lead, on occasion, to panophthalmitis Sweet and K'ang[95] noted the same sequence observed in our patients corneal edema, then necrosis, eventually followed by "ulceration and secondary bacterial infection " But even *Pseudomonas*—which releases proteases[222] and exotoxin,[223] attracts collagenase and proteolytic enzyme-producing leukocytes,[224] and can cause rapid corneal melting—has a different clinical appear-

ance whereas keratomalacia (Categories 7–8) invariably spares peripheral cornea, *Pseudomonas* recognizes no such limits [225] Even though *Pseudomonas* and other pathogenic organisms are sometimes more frequently encountered in severely xerophthalmic eyes of malnourished children,[217] this may merely represent a potentially transient change in flora, such as observed among burn patients [219]

Animal experiments suggest advanced corneal xerosis increases susceptibility to ulceration from topically applied *Pseudomonas*[226], the relevance of this observation to corneal melting in human xerophthalmia is uncertain

Because of the complexity and significance of the relationship between measles, systemic vitamin A status, and ocular viral invasion (by measles and herpes simplex), these issues are dealt with separately (Chapter 7)

Evolution of Corneal Lesions

Surface changes clearly predominate early in the disease (X2) Minimal punctate keratopathy and inferior haze can occur in the absence of obvious histopathologic alterations on light microscopy, though a presumably similar clinical appearance in the rabbit corresponds to focal areas of squamous metaplasia [155] In many instances, punctate erosions are preceded by, and probably arise from, nonstaining intra-epithelial microcysts These changes are earlier than those generally described Even "prexerosis"[136 154] was more consistent with changes observed in Category 2 xerosis [1] Pillat[102] described a case in which slit-lamp examination revealed "a remarkable number of vacuoles in the epithelial layer," but it is unclear whether this referred to Bitot's spot-like material, attached to or floating on the corneal surface, intra-epithelial microcysts, or the peau d'orange effect

The haziness spreads and patches of cornified epithelium begin to cover the surface, initially inferiorly, but eventually the entire cornea In particularly ill, malnourished children, amorphous, localized plaques may cover the cornified surface, especially in the intrapalpebral zone Their strong resemblance to Bitot's spots suggests aggregation of desquamated keratin and bacteria Histopathologic observations at this stage of the disease, in both humans and animals, indicate frank keratinization of the corneal surface [95 96 179 215 218 227]

The mildest stromal change is generalized edema, which also begins inferiorly Whether this is secondary to changes in the epithelium or endothelium, or a primary effect of vitamin A deficiency on the stroma itself, is unclear In general, the severity of edema parallels the severity of surface changes But it also parallels the severity of vitamin A deficiency quite independently of surface changes, as evidenced by the fact that edema was more prominent in nonulcerated xerotic "other" eyes of cases in Category 6 (focal necrosis) than in Category 4 (punched-out ulcers) (There is no convincing histopathologic evidence of endothelial alterations early in the course of corneal xerophthalmia, although vacuolated endothelial cells[180] and a reduction in mean endothelial cell counts[228] have been observed in the vitamin A-deficient rabbit)

Stromal loss appears to occur through a multiplicity of mechanisms, some primarily anterior (Categories 4–5), others posterior or at least simultaneously full thickness in nature (Categories 6–8)

Traumatic baring, if not loss of anterior stroma, probably initiates the process in some heavily xerotic corneas As pieces of the cornified layer are sloughed, they may take a full-thickness plug of epithelium (with or without deeper tissue) with them In one case, the ulcer was located adjacent to a large plaque, exactly at the point where a large, inferior segment had recently broken off and sloughed These ulcers might also represent a breakdown of dellen caused by their proximity to a steep-margined plaque [173 174]

The other mechanism of anterior stromal baring or loss does not require a heavily keratinized surface, and, in fact, commonly occurs in its absence Deep epithelial or superficial stromal bullae, observed in our cases as well as the vitamin A-deficient rabbit,[180] may rupture The mechanism of cyst formation is unclear In several of our cases it appeared to result from dellen formation in a central, depressed, nonstaining area of apparently superficial stromal desiccation In at least one animal model, drying is most intense centrally, occasionally resulting in epithelial loss followed by stromal dissolution [174]

Posterior and rapid full-thickness stromal necrosis, in some instances apparently deep to an intact epithelial layer, is not easily explained by currently accepted mechanisms of stromal dissolution It is appealing to think[229 230] that collagenases and other proteases known to be released by regenerating epithelium,[231–234] infiltrating leukocytes,[235–237] and even keratocytes,[96,238] and present in the stroma of ulcerating eyes,[239] may be responsible for corneal melting in xerophthalmia Apparently epithelial changes may even control polymorphonuclear cell infiltration [236] Whether this accounts for the cylindrical shape of anterior-posterior ulcers and sharply demarcated appearance of (localized) full-thickness necrosis remains to be seen But it fails to explain deep stromal necrosis beneath a seemingly intact epithelium, as suggested by several of our cases, those reported previously,[95 102 166] and occasionally seen in the vitamin A-deficient rat [227] An intriguing report suggests the existence of a collagenase gene promoter that is specifically repressed by retinoic acid, and that the retinoic acid-receptor (RAR) may exert its effect through protein-protein interaction that inactivates a protein that normally enhances collagenase gene expression [240 241]

Especially disturbing is the paucity of inflammatory cells in the recently melted cornea Corneal "infiltration" has been described as an early, prominent component of the xerophthalmic process, before epithelial integrity is lost [142 166 242] But it is unclear whether these observations refer to inflammatory cell infiltration, which in our experience and those of others[68 93 94 102 121 165 169] rarely occurs until late in the course of human disease, or merely stromal edema Although inflammatory cell infiltration is prominent histopathologically in corneas of vitamin A-deficient animals,[179 215 216 218 227] it has not been convincingly demonstrated in pre-ulcerated or newly ulcerated xerophthalmic human corneas Most important, several cases,

especially those in which histopathologic material was obtained soon after necrosis commenced, contain large areas devoid of keratocytes and suffering collagen fragmentation and dissolution in the absence of significant numbers of inflammatory cells. Smith also commented on the paucity of inflammatory cells in his relatively recent case of keratomalacia [103] This suggests the possibility that sufficiently impaired vitamin A metabolism results in devitalization of corneal stroma, with secondary infiltration by inflammatory cells The intact keratinized rabbit cornea displays autolysis of superficial epithelial cells and stromal keratocytes in the absence of inflammatory cells and myeloperoxidase activity [243] The ulcerating cornea is invaded by inflammatory cells and protease levels rise only after the epithelial layer is scraped off As in the few human specimens, dense infiltrations are observed at the edge of the ulcer, with fewer cells in the central ulcerating region [243] The cornea of the vitamin A-deficient rat is more susceptible to melting following trauma than is that of pair-fed controls,[175,243,244] while epithelial wounds heal more slowly [245,246]

Specific vitamin A receptors have been found in corneal epithelium, stroma, and endothelium,[247] among other tissues, while vitamin A appears to be required for normal glycoprotein biosynthesis in the rat cornea [248] The manner in which protein deficiency may contribute to these changes, and the role of systemic infection, will be dealt with later The beneficial effects of ascorbate therapy in the burned rabbit cornea[249,250] suggest an additional mechanism that may bear on the problem, though corneal melting is not observed in classical scurvy Nor were our cases clinically scorbutic

Punched-out ulcers (X3A, Category 4) can proceed to typical necrosis (keratomalacia) Whether this is the common course of events or merely incidental is unknown The fact that all xerophthalmic alterations can be seen in the same case and occasionally in the same eye suggests that if one does not contribute to the other, they at least represent staging posts along the course of a common disease

Corneal dissolution is sharply focal, both clinically and histopathologically The area of ultimate stromal loss may be considerably smaller than initial clinical observation suggests, particularly in focal necrotic cases (X3B, Category 6) Perhaps the area of (seemingly) normal collagen devoid of keratocytes that surrounds a focus of infiltration and frank dissolution represents viable and reversible corneal damage Alternatively, we may simply be witnessing resolution of optically disruptive edema, or in some instances, inflammatory infiltration

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5

Anemia and Iron Metabolism

Anemia has long been recognized as a potential consequence of vitamin A deficiency Soon after the discovery of vitamin A, Bloch noted that Danish orphans with xerophthalmia were "weak and thin, markedly anemic "[1] Wolbach and Howe noticed loss of stem cells in the marrow of vitamin A-deficient rats, with advanced deficiency they found an even more striking void of hematopoietic and lymphoid cells, accompanied by heavy hemosiderin deposits in the spleen [2] Hematopoietic disturbances characterized by a fall in hemoglobin (Hb) and pallor of red blood cells preceded eye signs and inanition in vitamin A-deficient rats [3] Koessler was convinced that "blood regeneration" could not take place without vitamin A, and he prescribed a vitamin A and carotenoid-rich diet for anemia [4] Berglund described a form of anemia in China that he thought was secondary to vitamin A deficiency and that responded to cod liver oil [5] Blackfan and Wolbach observed sequestered iron deposits (as hemosiderin) in the liver or spleen of almost all malnourished infants who had died with histopathologic evidence of vitamin A deficiency [6]

Early investigators also recognized that the hematologic response to vitamin A deficiency was biphasic, characterized by an initial fall in both Hb level and erythrocyte count followed by their rise late in deficiency This pattern was attributed to initial interference with hemoglobin production, succeeded by hemoconcentration secondary to dehydration from reduced water intake (and possibly diarrhea) as vitamin A deficiency became more severe [3,4] These early observations have withstood the test of time [7-16]

Although as early as the 1930s anemia was viewed as one of several consequences of vitamin A deficiency, it was not considered an important public health factor until forty years later, following the classic study by Hodges and colleagues in the mid-1970s Five of eight healthy, adult, male volunteers maintained on a vitamin A-deficient/iron-sufficient diet for up to approximately two years became

both vitamin A-deficient (evidenced by serum retinol <10 μg/dl or abnormal dark adaptation) and moderately anemic (mean Hb declined from 161 g/liter to 114 g/liter) The anemia was refractive to medicinal iron, but responded to minimal amounts of vitamin A or beta-carotene (up to 150 μg retinol equivalents [RE] per day, or ~25% of the FAO-recommended intake[17]) introduced into the diet [18]

Effect of Vitamin A on Anemia

Controlled trials that followed the work of Hodges and associates confirmed the hemoglobin response to improved vitamin A nutriture—whether achieved through food fortification, daily supplementation, or single, large periodic dosing (Table 5-1) In Indonesia,[19] the mean Hb level of preschool-aged children rose ~10 g/liter over baseline and exceeded the levels in control children after vitamin

Table 5-1 Randomized, Controlled Trials of the Impact of Vitamin A Supplementation on Hemoglobin Level

				Hemoglobin (g/liter)					
				Baseline			*Follow-up*		*Follow-up Interval*
Country/ Reference	*Age (years)*	*Treatment*	*Dosage*	*n,*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	*(months)*
Indonesia[a19]	< 6	Control	0 RE	240	114	(16)	112	(15)	5
		MSG-A	~0 24 mg RE/d	205	113	(16)	123	(16)***	
Guatemala[21]	1–8	Placebo	0 RE/0 Fe	20	104	(7)	107	(6)	2
		VA	≤ 3 mg RE/d	25	103	(8)	112	(8)*	
		Fe	3 mg/kg/d	30	105	(6)	119	(9)***	
		VA & FE	Both	24	106	(6)	120	(7)***	
Indonesia[22]	17–35	Placebo	0 RE/0 Fe	62	103	(6)	105	(6)	2
	(pregnant)	VA	2 4 mg RE/d	63	103	(4)	109	(5)**	
		Fe	60 mg/d	63	103	(5)	113	(6)***	
		VA & FE	Both	63	103	(5)	118	(6)***	
Thailand[24]	3–9	Control	0 RE	58	115	(—)[b]	115	(—)	0 5
		VA	60 mg RE	54	115	(—)	118	(—)*	
Thailand[25]	1–8	Control	0	71	112	(—)	116	(—)	2
		VA	60 mg RE	61	112	(—)	116	(—)	

[a]Fortification trial was not randomized

[b](—), SD not reported

*$p \leq 0\,05$

**$p < 0\,01$

***$p < 0\,001$

A-fortified monosodium glutamate (MSG-A) was consumed for five months (providing ~810 μg RE/d, or about twice the FAO-recommended safe level[17]) (Chapter 15) Supplemented children still maintained this Hb advantage (not shown) six months later, although it did not increase further, raising the possibility that dietary iron may have been required to normalize hematologic status [19] Hodge's work suggests that the amount of vitamin A actually required to evoke this further improvement may have been less than was administered [18] In another field trial in Indonesia, brick salt was fortified with vitamin A at a level of intake that provided preschool-age children with only ~135 μg RE/d (< 50% the FAO level) After six months, the prevalence of anemia (hematocrit < 35%) among recipients was reduced by one-third (26%, versus 38% among controls, $p < 0.02$) [20]

Randomized clinical trials have clearly demonstrated the value of vitamin A supplementation in reducing anemia and improving iron status in children[21] and pregnant women[22] (Table 5–1) Two months after approximately 100 anemic children in Guatemala were assigned to receive 1500–3000 μg RE/d, Hb concentration rose by 9 g/liter on average, compared with a 3 g/liter increment among placebo recipients (Δ = +6 g/liter) This response was consistent with improvements in indices of iron status Serum iron in vitamin A recipients increased by 16 μg/dl ($p \leq 0.01$) and percent transferrin saturation (% TS) rose by 4 5% ($p < 0.07$) over the mean changes observed in placebo controls Children receiving iron without vitamin A showed an even greater increase in Hb over controls (Δ = +11 g/liter) Adding vitamin A to iron did not increase Hb further, though serum iron and % TS improved further in children given both nutrients [21] This suggests vitamin A deficiency impairs iron utilization, hence, vitamin A alone can improve such utilization, usually by increasing Hb to the point that available iron becomes the limiting factor Adequate iron supplementation by itself, however, seems capable of compensating for the reduced efficiency of iron utilization imposed by vitamin A deficiency [21,22]

An additive effect of vitamin A over iron supplementation alone was reported among Indonesian women who were enrolled in a randomized trial between their fourth and sixth months of pregnancy After two months of daily vitamin A supplementation (2400 μg RE/d), Hb levels increased by 4 g/liter over that seen in placebo controls[22] (Table 5–1) Although daily iron supplementation elevated Hb by 8 g/liter over controls, the greatest response was observed in women taking both vitamin A and iron tablets, in whom Hb increased by 13 g/liter over controls The response to vitamin A alone reduced the prevalence of anemia (Hb < 110 g/liter) by 23% compared with controls, whereas iron alone reduced anemia by 62% Double supplementation virtually eliminated nutritional anemia (98% reduction) [22] The hematologic response to a vitamin A-plus-iron supplement was also superior to iron alone in a study among pregnant women in India [23]

There appears to be a hematologic response to a high-potency vitamin A supplement (e g , 200,000 IU), but the effect on Hb or hematocrit (Hct) may be

less sustained than when vitamin A intake is improved by increased daily intake (Table 5–1) In one of two randomized trials in Thailand, vitamin A supplementation increased mean Hb concentration by 3 g/liter two weeks after a single large supplement,[24] but children were not followed further In a second trial, a single high-potency dose of vitamin A failed to elicit a positive Hb (or Hct) response as long as two months to four months following supplementation [25] A third trial, in Indonesia, observed no change in mean Hb five weeks after a large-dose supplement, although Hb levels had improved in children who were initially anemic (Hb < 110 g/liter) at baseline [26] These findings suggest that Hb may be elevated for only a short duration (possibly for a month or less) following a single, large dose of vitamin A, paralleling the rise and fall of serum vitamin A [27–29] However, in each of these trials one or more indicators of iron status (serum ferritin, serum iron, or % TS) responded favorably,[24–26] suggesting an early and perhaps more sustained effect on iron mobilization than on Hb

Since the most sustained Hb response is associated with daily ingestion of vitamin A in amounts that are close to recommended levels of intake,[17,30] reduction in anemia following vitamin A supplementaion appears to represent a normal, physiologic response to the reduction in vitamin A deficiency rather than a pharmocologic effect

Sugar Fortification with Vitamin A in Guatemala

As a case study, Guatemala's experience with vitamin A-fortified sugar (Chapter 15) offers unique insights into the impact produced by increasing dietary vitamin A intake over a prolonged period of time [31–33] A cohort of some seventy-five preschool-age children routinely consuming vitamin A-fortified sugar (~15 μg RE/g) that increased their dietary intake threefold (~350 μg RE) were followed for two years Changes in serum vitamin A and in multiple indices of iron status (serum ferritin, serum iron, total iron-binding capacity [TIBC], and % TS) were evaluated, although Hb measurements were not taken [34–36] At the end of six months, serum retinol had increased, consistent with a positive effect on vitamin A status (Table 5–2) Serum iron rose while serum ferritin declined, reflecting changes in iron status consistent with mobilization of existing iron from body stores TIBC also increased initially, suggesting that the iron-carrying capacity of transferrin may have improved as part of a broader response of protein synthesis to this initial improvement in vitamin A nutriture [37]

At the next evaluation, at twelve months, serum iron and TIBC had both declined slightly Serum ferritin had begun to rise, while % TS was unchanged relative to baseline [34] None of these increments was statistically significant, but each appeared to foretell a shift in the longer-term response to improved vitamin A nutriture that became evident by eighteen and twenty-four months At these visits serum iron, % TS, and serum ferritin were all significantly elevated over

Table 5–2 Change in Serum Retinol and Iron Status in Preschool Children Following Sugar Fortification with Vitamin A in Guatemala

	Interval (months)			
	0–6 (N = 77)	*0–12 (N = 75)*	*0–18 (N = 46)*	*0–24 (N = 51)*
Retinol (μg/dl)	+ 5 1*	+5 2*	+ 3 6*	+ 2 5
Serum iron (μg/dl)	+ 4 5*	−3 6	+13 1*	+ 9 1*
TIBC (μg/dl)	+18 3*	−8 8	− 7 8	−13 6
% TS	+ 0 6	−1 0	+ 3 8*	+ 2 2*
Serum ferritin (ng/ml)	− 3 3*	+2 1	+ 5 5*	+ 4 9*

*p ≤ 0 05

From Mejia 1982 [14]

baseline while TIBC was lower, although not significantly so Dietary iron intake remained stable throughout the intervention period [34]

Mejia et al concluded that the increase in vitamin A intake achieved via fortified sugar initially enhanced iron mobilization (and presumably its utilization in erythropoiesis), leading to an initial decline in iron stores They hypothesized that lower stores triggered an increase in the efficiency of iron absorption in the presence of a constant dietary iron intake The improved absorption gradually restored and maintained iron reserves and stabilized the delivery of essential iron for hematopoiesis at levels that were signficantly higher than before sugar fortification with vitamin A had begun [35 36] Although not controlled, the longitudinal changes observed in this study are consistent with iron kinetics of animals and humans, and suggest the likelihood and magnitude of changes in hemoglobin and iron status that may be expected from programs that control vitamin A deficiency (in communities of similar nutritional status, dietary intakes of vitamin A and iron, and rates of disease)

Extent of Vitamin A Deficiency-Associated Anemia

Preadolescent children and women of reproductive age appear to be the principal groups at risk of anemia due to vitamin A deficiency Although the extent of this form of anemia remains to be elucidated, the problem appears widespread where vitamin A deficiency exists, regardless of dietary iron intake (which is generally suboptimal)

Children

Cross-sectional studies generally show that chronically mild-to-moderately vitamin A-deficient children are anemic relative to their non-deficient peers Lower

Hb or Hct levels have been observed among school-aged children with xerophthalmia or serum retinol < 0 70 μmol/liter [38] In Central America,[38] Africa,[39] and South Asia,[40 41] significant correlations of r = ~0 20 to 0 30 have regularly been reported between serum retinol and Hb, suggesting that serum retinol explains 4% to 10% of the variation in Hb level among preadolescent children A stronger correlation among anemic[38] or vitamin A-deficient[40] children supports findings that Hb is more responsive to vitamin A in the presense of anemia [21 26 40]

In preschool children the association between circulating retinol and Hb levels is similar to that in older children, although usually not significant [38 42] The relationship is clearer with respect to clinical (more severe) vitamin A deficiency Preschool-aged children with mild xerophthalmia exhibit Hb levels that are lower than non-xerophthalmic children [20 40] In the western Pacific, where xerophthalmia and subclinical vitamin A deficiency are widespread in the relative absence of wasting malnutrition,[43-45] distributions of Hb[43] and Hct[44] were shifted to the left among preschoolers with abnormal conjunctival impression cytology (CIC) This suggests that children with physiologically impairing vitamin A deficiency (but without severe clinically detectable vitamin A deficiency or wasting malnutrition) are more prone to anemia than children with apparently normal vitamin A-related function and ' status" (Fig 5–1)

That anemia of vitamin A deficiency is responsive to vitamin A repletion is apparent from the findings of Mejia et al , which show that ~65% of the Hb response in anemic children can be obtained by increasing the intake of vitamin A alone [21]

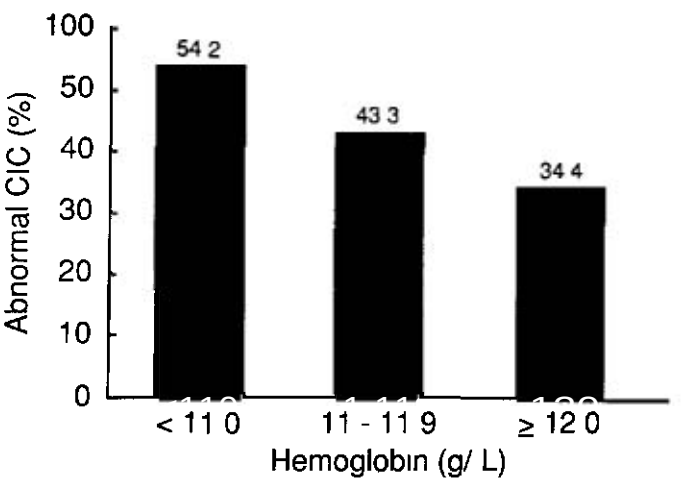

Fig. 5–1. Relationship between hemoglobin level and prevalence of vitamin A deficiency assessed by conjunctival impression cytology (CIC) in children 3–6 years of age in Truk, Micronesia (From M A Lloyd-Puryear et al [44])

Women of Reproductive Age

Following their initial study in the 1970s,[18] Hodges and his associates carried out an ecologic reanalysis of data on nonpregnant, nonlactating women who were examined during the nutrition surveys conducted by the Interdepartmental Committee on Nutrition for the National Defense (ICNND) in the 1960s In eight developing countries, selected for low estimated dietary vitamin A intakes, there was a linear relationship between mean plasma vitamin A and mean Hb levels ($r = 0.77$, $p < 0.05$) [18,35] In Indonesia, hemoglobin and hematocrit, in addition to serum iron levels, were significantly and positively associated with serum retinol in pregnant women after adjustment for multiple confounders [46] An initial estimate of the extent of vitamin A deficiency-induced anemia can be derived from the findings of Suharno and coworkers in Indonesia [22] They observed that approximately one-third of the Hb decline in anemic, pregnant women (in a population where 85% of all pregnant women were anemic [Hb < 110 g/liter]) could be attributed to vitamin A deficiency, and that improving vitamin A intake through daily, low-level supplementation could eliminate anemia in a quarter of these women [22]

Potential Mechanisms

Anemia observed in vitamin A-deficient children and women may be due to impaired release or transport of iron from body stores, sequestration of iron in tissue depots in response to infection, defective hematopoiesis within bone marrow, or any combination of these and other factors

Effects on Iron Storage

Vitamin A deficiency in experimental animals restricts the release of depot iron, as evidenced by a consistent rise in hepatic and splenic iron reserves [2,10–12,38,47,48] The rise in iron storage in the vitamin A-deficient rat occurs regardless of the presence of infection,[2,10–12,38,47,48] suggesting that these dynamics are not mediated solely by concomitant, infection-induced sequestration of iron

The relationship between iron stores and vitamin A deficiency is not as clear in humans as in animals, in part because human studies generally employ serum ferritin as a proxy for iron reserves Although serum ferritin is widely considered a sensitive indicator of iron tissue storage,[49–52] it is also a positive acute phase reactant[53–55] affected by infection[56,57] and acute malnutrition [58] Where wasting malnutrition, vitamin A deficiency, and chronic infection coexist, serum ferritin is increased [57,59] This may reflect higher iron stores (as seen in vitamin A-deficient animals), the acute phase response of ferritin to infection,[57,60,61] or the chronic

response seen in patients with long-term inflammatory, malignant or hepatic disorders [50,51,62]

Serum ferritin is often found to be unassociated,[26,37,46] or only weakly so,[25] with hyporetinemia[37,46,63] and mild xerophthalmia, even where protein-energy malnutrition (PEM) is less severe and infection is not obvious [26] Serum ferritin can be unresponsive to vitamin A for as long as four months after supplementation,[21,22,24,25] during which time the risk of serious infection should have been reduced However, other studies have produced different results

In Indonesia, serum ferritin increased (+11 4 μg/liter versus placebo, $p < 0.001$) within five weeks after vitamin A-deficient children (half of whom had xerophthalmia) were dosed with 209 μmol (200,000 IU) of vitamin A [26] Mechanisms underlying this response are unknown, but may involve a rapid increase in iron absorption in response to the rapid correction of vitamin A deficiency in severely deficient children who had only marginally depleted iron stores (serum ferritin = 27 μg/liter) [26] In Guatemala, serum ferritin initially decreased but then slowly increased in children over the two years that followed sugar fortification with vitamin A [34–36] Change in serum ferritin was inversely correlated with change in serum retinol ($r = -0.29$, $p < 0.05$) The gradual, positive response in serum ferritin in these children was consistent with improved iron uptake and release following prolonged increases in vitamin A intake and improvement in vitamin A status

Effects on Iron Transport

Vitamin A deficiency may impair the mobilization and transport of iron Cross-sectional studies in children and women indicate serum iron varies directly with serum retinol, with correlations generally ranging from $r = 0.17$ to 0 42 [37,38,46,59,63] Thai preschoolers with nightblindness had a mean serum iron concentration that was ~70% the level among their non-xerophthalmic peers ($p < 0.01$) [63] A similar but nonsignificant difference was observed in India among hyporetinemic children, some of whom had mild xerophthalmia [40] In the vitamin A-compromised state the decline in plasma iron is often accompanied by a fall in % TS,[25,37,38,59] reflecting impaired capacity to deliver iron to the bone marrow

Vitamin A supplementation appears to mobilize the delivery of iron to the erythron in poorly nourished populations Increases in both circulating (plasma) iron and % TS have been observed in randomized, clinical trials two weeks[24] to two months[21,22,25] following known vitamin A administration to children[21,24,25] and pregnant women,[22] whether the vitamin A was delivered as a daily supplement[21,22] or a high-potency (209 μmol) capsule [24,25] Increases in both serum iron and % TS among vitamin A-supplemented children were 25% greater than the increase in controls [21,24,25] Relative increases of these two indices in pregnant women have been more modest than in children ($\leq +5\%$) [22]

Effects on Hematopoiesis

Vitamin A is required for normal hematopoiesis in the presence of apparently adequate iron bioavailability Most of the advances in our understanding of the mechanisms responsible, however, have emerged from in vitro studies of the regulatory effects of retinoids on maturation of hematopoietic cell lines (e g , the HL-60 human promyelocytic cell line) [64-66] Retinoids, but particularly all-trans retinoic acid induce differentiation and proliferation of pluripotent hematopoietic cells derived from bone marrow [64 65] The nature of the effects appears to depend on the type of cell, stage of maturation, nature of stimulus, type of retinoid being tested, and many other factors [66]

In animals, experimentally controlled vitamin A depletion produces an unequivocal decrease in Hb and Hct [3 4 7 10–12 47 48 67] This is accompanied by decreased uptake of isotopically labeled iron by erythrocytes compared with controls,[11] indicating a defect in heme formation Rats on a low-vitamin A diet develop mild anemia concurrent with their decline in vitamin A status weeks before showing other signs of impaired iron metabolism, including changes in iron storage and absorption [12] This suggests that deranged erythropoiesis may initiate anemia in vitamin A deficiency, which could, in turn, lead to impaired iron metabolism rather than vice versa [12 64]

Available data suggest that vitamin A deficiency is an important contributing factor in nutritional anemia The responsiveness of Hb and indices of iron metabolism following vitamin A supplementation, alone or in combination with supplemental iron, in diverse populations suggests that vitamin A-responsive anemia is widespread A number of mechanisms might be involved, including disruptions in the transport, absorption, and storage of iron, as well as a direct influence on hematopoiesis The anemia of vitamin A deficiency may be expected to initiate or contribute to the same cascade of defects in oxygen transport and metabolism,[68] reduced resistance to infection,[68 69] and impaired cognition and performance[70-72] attributed to primary iron deficiency anemia of similar severity The prevention of vitamin A deficiency should be considered, along with iron supplementation, as a potentially important approach to the control of nutritional anemia

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6

Growth

> There is no low plane of intake of [vitamin A] which can be said to maintain an animal without loss of vitality When the minimal amount necessary for the prevention of loss of weight is approached the life of the animal is jeopardized if the diet is persisted in
>
> —McCollum and Simmonds, 1917[1]

Mammalian growth requires vitamin A A mixed diet apparently satisfactory in energy and all other nutrients will not sustain growth, vigor, or life in a young animal without a minimum amount of vitamin A This discovery, made eighty years ago by McCollum and Davis[2,3] and almost concurrently by Osborne and Mendell[4] provoked an enduring interest in the nature, timing and sequence, mechanisms, conditioning factors, and consequences of vitamin A deficiency As a result, the growth response to changes in vitamin A nutriture has been reliably established in several animal models Inevitably, this has proven difficult to study in children due to the numerous influences on their growth that are difficult to control

The basic growth response to vitamin A in well-controlled animal experiments provides insight into this relationship in children More important, it also establishes approximate thresholds at which vitamin A deficiency may restrict child growth, other factors remaining stable

Animals

The effects of vitamin A deficiency on growth have clearly and consistently been observed in animals The sensitive changes in weight gain of rats fed varied amounts of an ether-soluble fraction of egg yolk[2,3] and butter[4] originally led to

the discovery of "fat-soluble factor A", its deficiency led to growth cessation (Fig 6–1) [1–3] In the phase of vitamin A depletion leading up to growth failure, however, animals are not mildly deficient in vitamin A Rather, they are systemically depleted in vitamin A with attendant health complications, including a rapid rise in mortality [1]

Only after several weeks on a vitamin A-deficient diet do small animals begin to grow poorly, characterized by deceleration in weight gain This is followed by a stable weight "plateau" and, eventually, precipitous weight loss [2–9] Liver stores of vitamin A are generally depleted several weeks before an animal reaches the weight plateau [10 11] During the pre-plateau phase, slowing weight gain coincides with declining levels of circulating retinol [9 12] Presumably, recycling of extrahepatic retinol helps to sustain peripheral availability of vitamin A[13] and extends the duration of near-normal tissue function, including those mechanisms involved in growth However, numerous other functional abnormalities appear before hepatic vitamin A is depleted, or weight gain ceases These include an increase in cerebral spinal fluid pressure,[14 15] defects in cellular and humoral immune response (Chapter 9), epithelial changes in the intestinal mucosa,[16 17] and epidermoid metaplasia of the respiratory,[9 18–20] glandular and urinary[21] tracts Reductions in weight gain velocity also correspond to losses in efficiency of protein and energy metabolism [22–25] Risk of infection and death increase prior to weight plateau, often long before xerophthalmic eye signs appear [11,26 27] Cessation of weight gain heralds clinically apparent, severe vitamin A deficiency, and signals a collapse of normal metabolic adaptation

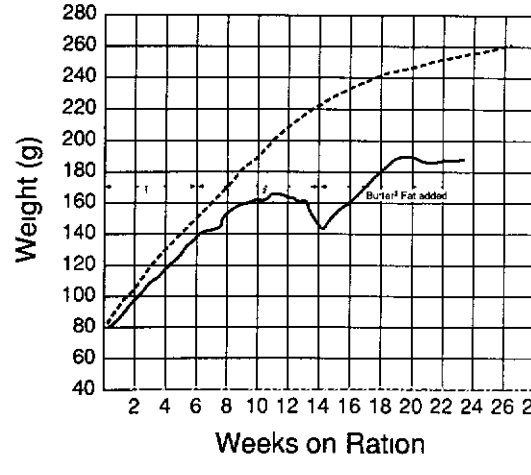

Fig. 6–1. Growth curve of rat number 141, reported by E V McCollum in 1913, showing (1) initial, slightly subnormal weight gain while consuming a purified ration lacking a small amount of butterfat with "fat-soluble A", (2) growth faltering (slowed rate followed by a "plateau" and weight loss) during advanced deficiency, and (3) a distinct and rapid ponderal growth response following reintroduction of butterfat [2]

Repletion of an experimentally deficient diet with small amounts of vitamin A (inadequate to return hepatic or circulating retinol levels to normal) can enhance survival,[28–31] halt weight loss and reestablish normal or even catch-up ponderal growth [2 7 9–11,13 28,30,32] Thus, weight gain in the young animal is not easily disturbed by vitamin A deficiency,[33] it lags behind depletion and responds rapidly to a diet containing a minimum amount of vitamin A

In contrast to the well-defined vitamin A-related change in weight, the effects of dietary vitamin A manipulation on linear growth, bone formation and body composition in animals are less clear Early studies reported continued growth in tail and total body length during chronic vitamin A deficiency despite deceleration and loss in body weight,[5] raising questions about the utility of weight change as a valid, single index of permanent growth of animals Subsequent studies in calves[15] and ponies[34] have shown little[34] to no[15] effect of vitamin A supplementation on (witheral) height This apparent absence of effect is surprising given data demonstrating retarded endochondral growth in the epiphyses, and a consequent shortening of long bones of vitamin A-deficient dogs,[35] chicks,[36] ducks,[37] and rats [38] Some have attributed these endochondral changes in vitamin A deficiency to inanition that accompanies advanced vitamin A deficiency[15,39] and not to a specific effect of vitamin A deficiency itself Others have demonstrated abnormal sulfation of cartilaginous bone in the vitamin A-deficient rat,[40] as well as deficient somatomedin production in vitamin A-deficient children [41] These observations support a regulatory role for vitamin A in osteogenesis that could, in part, underlie linear growth stunting in vitamin A deficiency

The need for body vitamin A stores to be exhausted, or nearly so, before ponderal growth is affected in animals may be relevant to understanding ponderal growth of vitamin A-deficient and supplemented children

Children

Few would doubt the importance of vitamin A in child growth However, the ability to conclusively demonstrate its precise impact is difficult given the different environments in which children live, with variations in their status of other nutrients, nutritional demands (i e , as posed by repeated infection), and study designs Children are often deficient, to varying degrees, in other nutrients essential for growth Thus, vitamin A deficiency may truly limit growth when it is the most deficient of the essential nutrients, or may be associated with poor growth when it is correlated with another, growth-limiting factor The association would be evident by a lower attained growth or a deceleration in growth rate in children who are vitamin A-deficient, especially those with severe deficiency (e g , xerophthalmia) Children in whom vitamin A deficiency is truly "growth-limiting" would be expected to respond to vitamin A replenishment, at least to the point at which other nutrients become limiting Where vitamin A deficiency is not the most

limiting factor, replenishment may not result in a measurable growth response, although it might still induce other health benefits (such as a reduction in mortality)

Attained Growth during Vitamin A Deficiency

Cross-sectional studies reveal instances in which vitamin A status and attained growth are associated Children with vitamin A deficiency are often stunted and occasionally wasted compared with apparently normal children of similar age Differences inevitably represent a mix of direct and indirect effects, the latter often secondary to generalized malnutrition and infection that can accompany vitamin A deficiency Indeed, vitamin A deficiency could increase the frequency, severity, and duration of infections (Chapter 3), which in turn affect vitamin A and general nutritional status (the "infection-malnutrition complex") While static assessment cannot establish temporality or cause-and-effect, the consistency with which poor achieved growth and vitamin A deficiency coincide suggests a causal role for the nutrient

Height-for-Age

Children with corneal xerophthalmia are almost always stunted in linear growth,[42–44] an association that is more strongly correlated with age (as a surrogate for duration of deficiency) than degree of corneal involvement [44] Older preschool children with active corneal disease tend to be more severely stunted than younger children, reflecting, in part, the cumulative impact of underlying malnutrition[45,46] and repeated infection[47–49] that occur with age and may precede blinding xerophthalmia [43,44,50] The first Indonesian countrywide survey in 1978–1979 identified a sufficient number of cases with which to examine this relationship (n = 20, 1–4 years, n = 1 at age 5) [44] Stunting was severe among all children with active corneal disease (X2/X3), but was relatively more severe in children three to four years of age (Fig 6–2), suggesting a longer duration of preexisting deficiency

Growth stunting is also observed at less severe stages of vitamin A deficiency Frequently,[44,51–56] though not always,[57–60] children with mild xerophthalmia (XN or X1B) are stunted compared with non-xerophthalmic children Indonesian children with X1B were shorter at every age, by an average of 2 6 cm, from 1 to 5 years compared with matched controls and the national random sample of children[44] (Fig 6–2), suggesting that vitamin A deficiency and linear growth retardation coexist very early in life The constant difference in stature with age might indicate that children with xerophthalmia, possibly once recovered, tend to reestablish a growth rate comparable to that of the non-xerophthalmic (but still vitamin A-deficient) population norm, but do not exhibit catch-up linear growth. The absence of catch-up growth was documented in a longitudinal study of vitamin A supplemented xerophthalmic children [61] Where a xerophthalmia-

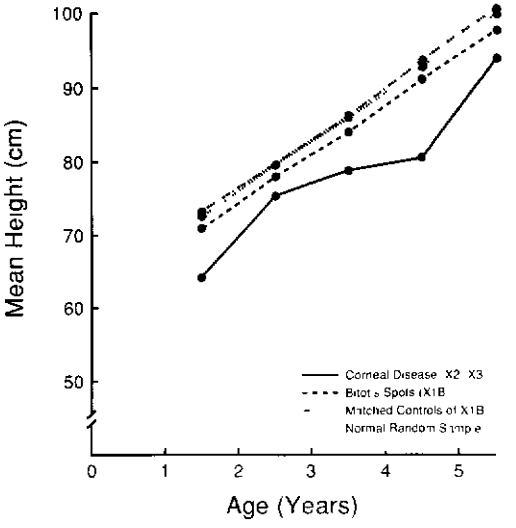

Fig. 6–2. Height-for-age and ocular status of Indonesian children assessed during the countrywide survey of 1978–1979 Children with corneal disease (X2/X3) were shorter than children who had Bitot's spots (X1B) ($p < 0.01$) children with X1B were shorter than their matched controls ($p < 0.01$) There were no significant differences in height between randomly sampled children without xerophthalmia (normal random sample) and matched controls [44]

stunting relationship has not been observed,[57–60 62] it may be that other, more widespread and severe nutritional deficiencies are limiting growth in the population as a whole (with which the xerophthalmic children's growth is compared) [63]

Stunting appears less consistently at subclinical stages of deficiency, at which point competing determinants of poor linear growth might dominate Excess stunting has been observed among children with abnormal conjunctival impression cytology (CIC), an index of functional vitamin A deficiency (Chapter 11) [55 64 65] In Micronesia, the prevalence of vitamin A deficiency (abnormal CIC) rose in a dose-responsive fashion with the severity of low height-for-age (Fig 6–3) [64] Senegalese preschool children with abnormal CIC were 1 7 times (95% CI 1 4–2 0) more likely to be stunted (< −2 height-for-age Z-scores [HAZ][66]) than their normal peers [65] An inability to obtain CIC specimens from a large proportion of subjects may have biased findings of a recent study that failed to show this relationship [67]

Stunting is often associated with hyporetinemia among both preschoolers[54 67–69] and school-aged children,[70] although not, as expected, in all populations studied [71 72] Age- and neighborhood-matched controls of xerophthalmic children in Indonesia—who had a lower serum mean retinol and, therefore, were at higher risk of subclinical vitamin A deficiency than children in other neighborhoods due to its tendency to cluster[51 73–75] (Table 12–2)—also did not differ appreciably in height from the rest of the population (Fig 6–2) Few studies have attempted

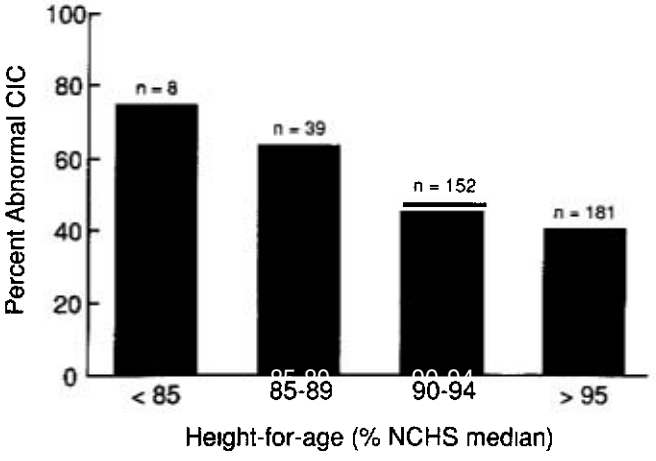

Fig 6–3 Prevalence of vitamin A deficiency (abnormal CIC) by height-for-age of preschool-age children in Truk, Micronesia The percentage who were vitamin A-deficient rose with increased stunting relative to the National Center for Health Statistics (NCHS) median [139] (From M A Lloyd-Puryear et al [64])

to relate attained growth to dietary intake of vitamin A One such study in Peru that incorporated longitudinal dietary assessment for a number of years prior to status assessment found that long-term beta-carotene intake was strongly associated with attained stature, independent of dietary protein, energy, and other nutrient intakes, but only in male children [76] While not fully understood, the gender effect is consistent with a propensity for boys to be more vitamin A-deficient than girls [77] Sex-specific differences in growth responses to vitamin A deficiency and repletion have been repeatedly observed in animals [78]

Seasonality may confound the relationship between vitamin A status and attained height-for-age, given the sharp seasonal variation of the former[53,79–83] and modulated or lagged seasonality that can attend the latter [84,85]

In aggregate, the above findings suggest that vitamin A deficiency needs to be severe enough to impact on linear growth Prospective studies and intervention trials tend to bear this out

Weight-for-Height

Children with corneal xerophthalmia are nearly always wasted,[42,44,50,86,87] reflecting their general state of frailty [44,88,89] This fact has been recognized for over half a century [42] Less obvious is that children with non-corneal xerophthalmia (XN or X1B) may also be thinner than normal, or more so than hospitalized, malnourished peers matched on similar anthropometric categories [90] Occasionally, in food-sufficient populations, mildly xerophthalmic or subclinically vitamin A-deficient children are observed to be slightly more wasted than their normal peers

below two years of age [51 91] Otherwise the association cannot be seen,[44 51 59 64 72 91 92] perhaps because of higher rates of mortality among wasted children with xerophthalmia [91] On the other hand, mild degrees of wasting, reflected by a lower weight-for-height[94] or arm circumference,[93 94] often accompany non-corneal xerophthalmia in populations that face chronic food shortage This relationship is especially apparent during drought, when both wasting malnutrition and high rates of mild xerophthalmia coexist [95–98]

Weight-for-Age

Weight-for-age represents the combined effects of stunting and wasting malnutrition, corrected for age Because some studies have only reported anthropometric status by weight-for-age,[99 100] they shed little light on the wasting-stunting continuum in relation to vitamin A status

Growth Velocity during Vitamin A Deficiency

The extent to which vitamin A deficiency may alter growth velocity of children can best be gleaned from prospective, natural history studies such as that conducted in West Java from 1978–79 [44] Growth measurements were repeated at regular intervals as children waxed and waned in their clinical vitamin A status [61] Height and weight were measured and an ocular examination was conducted on ~4000 preschool children every three months over an eighteen-month period (Fig 2–2) Growth increments were determined in temporal relation to xerophthalmia status (XN or X1B), adjusted for age, sex, history of diarrhea, and clinically evident respiratory illness at the outset of an interval The clinical examination of children at the beginning and end of 15,816 intervals permitted interval status to be classified as (1) normal (N–N), (2) xerophthalmic (X–X), (3) deteriorating in vitamin A status as children went from normal status at one exam to having xerophthalmia three months later (N–X), or (4) improving in vitamin A status as children recovered from xerophthalmia during an interval (X–N)

Compared with children who remained normal (N–N) those who developed xerophthalmia during a 3-month interval (N–X) showed a decline in ponderal and linear growth velocities by 199 g and 0 28 cm respectively (Table 6–1) Children who were mildly xerophthalmic on two consecutive occasions (X–X), reflecting a state of chronic vitamin A deficiency, gained 120 g less in weight and 0 21 cm less in height than children who were clinically normal but subclinically deficient (with nearly half having a serum vitamin A < 0 70 μmol/liter) [44 73] This is equivalent to an annual deficit of ~560 g and ~0 9 cm for children who develop xerophthalmia and do not improve during the year

In contrast, children who recovered from xerophthalmia during an interval (X–N) gained more weight (+124 g) than normal children, accounting for more than half of the 200 g decrement in weight gain that occurred during periods of

Table 6–1 Differences in Three-Month Weight Gain and Linear Growth between Xerophthalmic and Non-Xerophthalmic Preschool Indonesian Children

		Change in Weight (g)			*Change in Height (cm)*		
Interval Clinical Status[a]	*Intervals (N)*	*3-Month Interval*	*Difference from N–N*		*3-Month Interval*	*Difference from N–N*	
			Crude	*Adjusted*[b]		*Crude*	*Adjusted*
N–N	14,743	348	—	—	1 77	—	—
		(6)[c]			(0 02)		
N–X	353	139	−209	−199	1 41	−0 36	−0 28
		(43)		(57)	(0 08)		(0 08)
X–X	252	211	−137	−120	1 38	−0 39	−0 21
		(43)		(36)	(0 09)		(0 08)
X–N	368	459	+111	+124	1 68	−0 09	0 09
		(41)		(41)	(0 09)		(0 09)

[a]N–N Clinically normal at the outset and end

N–X Clinically normal at the outset and xerophthalmic at the end

X–X Xerophthalmic at the outset and end

X–N Xerophthalmic at the outset and clinically normal at the end

[b]Adjusted by least squares linear regression for age, sex presence of respiratory infection and history of diarrhea at the start of each interval

[c]± 1 SEM

From Tarwotjo et al., 1992 [61]

declining vitamin A status Thereafter, clinically recovered children continued to gain weight (at least through the next interval) at a rate that was comparable to normals as long as they remained free from xerophthalmia However, recovering children only slowly caught up in linear growth relative to normals (who themselves had mean vitamin A levels below 20 μg/dl) 0 09 and 0 06 cm more, respectively, during concurrent and subsequent three-month intervals

Although cause and effect cannot be conclusively inferred, these findings suggest that vitamin A deficiency, at a level that is consistent with mild xerophthalmia, can indeed modulate the tempo of weight gain and linear growth in children The estimates may also be used to define the range of relative weight gain (up to ~500 g/yr) and linear growth (up to ~1 cm/yr) velocity that could be singly affected by vitamin A deficiency and, therefore, responsive to vitamin A intervention, given that some of the total growth response during periods of recovery might well be due to an overall improvement in health and diet

In this study, recovery from xerophthalmia favored catch-up weight gain in the short term but a slower response in linear growth, similar to that seen in early animal studies[5] Presumably growth improved up to a nutritional plane where other deficiencies became restrictive These growth dynamics could underlie the frequently seen pattern of minimal wasting with more apparent

stunting[44,52–54 64] of mildly xerophthalmic children, particularly where episodes of xerophthalmia repeatedly occur throughout the preschool years.[44 61 80]

Given a tendency for stunting among subclinically vitamin A-deficient children,[54,64 65,67,68] it may be expected that such children grow less than their peers However, this was not the case in rural Nepal, where a cohort of non-xerophthalmic preschool-aged children participated in a control group of a randomized community trial [101] They were classified as CIC-normal (n = 222) or CIC-abnormal (n = 64) at baseline, and their growth was followed every four months CIC status was not reclassified at each visit, as was done in the study in West Java Nonetheless, age- and baseline status-adjusted changes in linear growth, weight, arm circumference and skinfold thickness of CIC-abnormal children were comparable to children with normal CIC status throughout sixteen months of follow-up (K P West et al, unpublished data, 1995) It appears that mild vitamin A deficiency (abnormal CIC), consistent with early epithelial metaplasia, may not be growth-limiting in a markedly stunted (mean ≈ −2 5 HAZ[66 102]) and wasted (mean ≈ −1 00 weight-for-height Z-score [WHZ][66 102]) population potentially suffering other factors more influential to growth

Growth Response to Vitamin A

Vitamin A supplementation trials provide an opportunity to investigate the specific contribution of vitamin A status, in and by itself, to growth Well-designed, randomized, controlled trials conducted in different populations and with varying constructs have yielded differing results These range from improvement in ponderal[102–104] and linear[102 105] growth and apparent body composition[102 103] in certain groups of children, to little[102 106] or no[107 108] effect in others This is not surprising given the complexity and apparent nonspecificity of growth to a host of factors (diet, infection) under free-living conditions Variation in response to vitamin A supplementation depends on the nutritional ecology of the population, including the coincident severity and seasonality of protein-energy malnutrition (PEM), vitamin A and other micronutrient deficiencies, the age, health status, breast-feeding, and other coexisting dietary habits of children, and perhaps the frequency duration and dosage of vitamin A supplementation

Large-Dose Vitamin A Supplementation

Data from a randomized, double-masked community trial in Nepal[102] (NNIPS) permit a detailed inspection of the growth response to periodic vitamin A supplementation in a moderately stunted and chronically wasted population [101 102 109 110] A cohort of approximately 3500 children aged twelve months to sixty months were dosed with 200,000 IU or 1000 IU (control group) of vitamin A at baseline and every four months for sixteen months A smaller group of infants was also followed after receiving 50,000 IU (less than one 1 month old) or 100,000 IU

(one month to eleven months old) versus placebo Linear and ponderal growth, including changes in height or length, weight, and mid-upper arm circumference (MUAC), were evaluated at each visit Subscapular and tricipital skinfolds, permitting derivation of upper arm muscle (including humoral bone) and fat areas,[111] were also measured at the first and fifth visits

Non-Xerophthalmic Children The basic question posed by this trial, as with other intervention studies,[103,105–108] was whether vitamin A supplementation alone could improve overall child growth in an area of endemic vitamin A deficiency The response was modest in clinically normal children (without xerophthalmia at baseline) who were twelve months of age or older at the outset (Table 6–2) All growth comparisons were adjusted by sex, age and indicator status at baseline Vitamin A supplementation appeared to exert small, relative effects on weight gain that were seasonally dependent During the first perıharvest four-month interval, vitamin A-supplemented children gained 121 g less weight than controls This represented less gain in soft tissue, reflected by a −0 11 relative change in WHZ ($p < 0\ 001$), with similar rates of linear growth in both groups During the next eight "leaner" months, throughout which rates of weight gain fell in both groups and arm circumference remained relatively constant (K P West et al , unpublished data, 1995), vitamin A-supplemented children gained slightly more weight than controls, leaving essentially no difference between groups after the first year of intervention (differences in ΔWHZ = 0 02 and ΔHAZ = 0 01) During the last (perıharvest) four-month interval observed in the trial, weight gain was again smaller in the vitamin A group, causing the relative deficit in weight gain (−72 g) and WHZ (−0 07) ($p < 0\ 01$) to reappear in the continued absence of any effect on linear growth

Changes in upper arm muscle and fat and truncal fat suggested that the perıharvest deficit in weight gain relative to controls may have been due to reduced accumulation of fat Arm circumference initially increased by 0 16 cm in the vitamin A group over unsupplemented controls, a modest advantage that was generally maintained throughout the trial After sixteen months, the difference between groups was due entirely to a 25 mm^2 increase in muscle (plus bone) area ($p < 0\ 001$) (Table 6–2), similar to what was seen among boys receiving vitamin A in the Indonesian Aceh Study [111] While this difference accounted for only 2% of the children's initial muscle area (≈ 1100 mm^2), it represented a 30% relative increase over the muscle growth of controls [102] However, since long-term change in weight-for-height (WHZ) was more strongly correlated with apparent change in fatness ($r \approx 0\ 65$ for skinfolds) than with change in musculature ($r = 0\ 32$ for MA), seasonal fluctuations that were observed in WHZ were more likely to have been reflecting variation in body fat than in lean body mass

Growth effects observed in (non-xerophthalmic) infants were far more perplexing and require considerable investigation In a baseline cohort of infants under six months of age who were dosed and followed for sixteen months, vita-

Table 6–2 Differences (Δ)[a] in Growth Increments between Non-Xerophthalmic Children in the Vitamin A (VA) and Control (Ct) Supplement Groups, 12–60 Months of Age at Baseline, Sarlahi, Nepal

	Number		*WT(g)*[b]		*HT(cm)*		*AC(cm)*		*MA(mm²)*		*FA(mm²)*		*SS(mm)*	
	Ct	*VA*	Δ	*SE*	Δ	*SE*	Δ	*SE*	Δ	*SE*	Δ	*SE*	Δ	*SE*
4 mo	1450	1592	−121	19**	0	0 1	0 16	0 02**	—	—	—	—	—	—
12 mo	1405	1578	38	16	0 1	0 1	0 23	0 02**	—	—	—	—	—	—
16 mo	1361	1481	−72	27*	0 1	0 1	0 13	0 02**	25	4**	0	4	0	0 1

[a]Differences represent VA group minus CT group increments estimated by a regression coefficient (b) adjusted for sex, age and measurement status at baseline

[b]WT = weight, HT = length (12–23 mo) or height (≥ 24 mo), AC = left mid-upper arm circumference, MA = upper arm muscle area, FA = upper arm fat area, SS = left subscapular skinfold, Δ = VA increment minus CT increment, SE = standard error of b

*$p \leq 0\,01$

**$p \leq 0\,0001$

From West et al , 1995 [102]

min A supplementation was associated with decrements of 0 16 cm in MUAC ($p < 0\ 05$) and 23 mm^2 in arm fat area ($p < 0\ 05$) relative to controls, that is by the time these infants reached sixteen months to twenty months of age All other indicators also showed slower growth but were not significant On the other hand, when initial four-month increments were examined for infants enrolled across all visits and seasons of the trial, there were practically identical rates of linear growth, weight gain, and MUAC for infants one month to 5 months of age But neonates less than one month of age, who received 50,000 IU, showed improved weight (290 g, $p < 0\ 03$) and MUAC increases (+0 3 cm, $p < 0\ 08$) over controls (K P West et al , unpublished data, 1995)

These data suggest seasonal, age, and cohort effects of vitamin A supplementation on the growth of children However, they also indicate the possibility that 50,000 IU or less may be preferred to 100,000 IU when supplementing very young infants

Xerophthalmic Children Unlike the apparently modest impact of vitamin A supplementation on the growth of non-xerophthalmic children, consistent and positive differences in growth were evident among xerophthalmic children (with XN and/or X1B) supplemented with vitamin A (Table 6–3) At baseline, they weighed more and were taller than non-xerophthalmic children because they tended to be older[102] (after age-adjustment, the status of xeropthalmia cases was poorer than normals[55]) Four months after being treated with 400,000 IU and referred at baseline because of their xerophthalmia, cases in the *unsupplemented control group* (who received no additional vitamin A from field teams after their initial treatment) gained more weight (256 g) than non-xerophthalmic children, yielding a ΔWHZ advantage of +0 16 ($p \leq 0\ 05$) in the absence of a discernable, initial effect on height (+0 1 cm), their relative gain in arm circumference was 0 21 cm ($p \leq 0\ 01$) Sixteen months later, the treated xerophthalmic cases (in the control group) exhibited greater gains in both ponderal (+280 g) and linear (+0 7 cm) growth This was reflected by a relative increase by cases in height-for-age (+0 12 HAZ, $p < 0\ 05$), but no difference in weight-for-height (ΔWHZ = 0 04) Although the early advantage in MUAC had largely disappeared, upper arm muscle area of cases increased by 24 mm^2 over unsupplemented controls but this was not statistically significant ($p < 0\ 1$) Thus, a single, large dose of vitamin A given to xerophthalmic children may have increased the efficiency of dietary protein and energy utilization in the short run, reflected by greater tissue deposition This appeared to translate into a modest increase in linear growth (a cumulative, nonreversible effect) and, perhaps muscle mass discernable more than a year following treatment

Xerophthalmic children in the *vitamin A-supplemented group* (Table 6–3) received the same 400,000 IU vitamin A treatment and referral (plus an additional 200,000 IU dose at home) and continued to be dosed with vitamin A every four months along with their peers Their initial (four-month) increment in soft tissue

Table 6–3 Differences (Δ)[a] in Growth Increments between Xerophthalmic Children Treated with Vitamin A and Non-Xerophthalmic Children within Supplement Groups, 12–60 Months of Age at Baseline, Sarlahi, Nepal

	Number		*WT (g)*		*HT (cm)*		*AC (cm)*		*MA (mm^2)*		*FA (mm^2)*		*SS (mm)*	
	Nml[b]	*Xero*	Δ	*SE*	Δ	*SE*	Δ	*SE*	Δ	*SE*	Δ	*SE*	Δ	*SE*
Control Group														
4 mo Δ	1450	57	256	74***	0 1	0 1	0 21	0 08**	—	—	—	—	—	—
12 mo Δ	1405	55	284	86***	0 4	0 2*	0 16	0 09	—	—	—	—	—	—
16 mo Δ	1361	52	282	106**	0 7	0 2**	0 08	0 10	24	12	−8	12	0 2	0 2**
Vitamin A														
4 mo Δ	1592	47	160	79*	0 4	0 1**	0 17	0 08*	—	—	—	—	—	—
12 mo Δ	1578	45	279	93**	0 7	0 2**	0 27	0 10***	—	—	—	—	—	—
16 mo Δ	1481	44	413	109**	0 9	0 3***	0 38	0 10***	52	15***	26	15	0 6	0 2

[a]Differences represent xerophthalmic child minus non-xerophthalmic child increments, estimated by a regression coefficient b adjusted for sex, age, and measurement status at baseline

[b]Nml = non-xerophthalmic, clinically sound children, xero = xerophthalmia cases See Table 6-2 for other abbreviations

*p ≤ 0 05

**p ≤ 0 01

***p ≤ 0 001

From West et al , 1995 [102]

mass was similar to that of xerophthalmic children in the control group an additional gain of 160 g in weight, 0 4 cm in height and 0 17 cm in MUAC (all $p < 0\ 05$) over non-xerophthalmic children However, unlike cases in the control group who were only treated at baseline, periodically supplemented cases continued to experience some relative gain thereafter by sixteen months those who were initially xerophthalmic had gained over 400 g more in weight, 0 9 cm more in height (reflected by gains in both ΔHAZ [+0 19, $p < 0\ 01$] and ΔWAZ [+0 18, $p < 0\ 05$]), 0 38 cm more in arm circumference and 0 6 mm more in subscapular skinfold over the growth rates of their non-xerophthalmic, supplemented peers Upper arm muscle and fat areas increased by 52 mm^2 ($p < 0\ 001$) and 26 mm^2 ($p < 0\ 1$) respectively in initially xerophthalmic cases over that of clinically normal children

The longer-term growth advantage from periodic supplementation remained when initially xerophthalmic children in the two supplemented groups were compared Subjects who were treated and continued to be periodically dosed showed slightly more gain in weight (+103 g) and height (+0 2 cm), although these differences were not statistically significant More evident was the continued relative increase in arm circumference (+0 34 cm, $p < 0\ 1$), arm muscle area (+50 mm, $p < 0\ 1$) and subscapular skinfolds (+0 5 mm, $p < 0\ 05$), compared with children with xerophthalmia in the assigned control group, who were treated only at baseline [102]

The poorer initial status, sustained growth, and apparent change in body composition of children who initially had moderate-to-severe vitamin A deficiency (i e , xerophthalmia), after adjusting for baseline differences, suggest that vitamin A deficiency was more likely to have been growth-limiting in these children than in less deficient children Xerophthalmic subjects, however, were also likely to have returned to a deficient state (given an inadequate diet that continues long after treatment[112] and no change in their environment) during the intervals between dosing, explaining why subsequent dosing had some cumulative effect

The effects observed in Nepal can be reconciled, at least partly, with growth responses to a large dose of vitamin A in other settings One randomized, controlled trial in a cohort of 312 preschool-aged children in West Bengal, India,[80] showed that four-monthly vitamin A supplementation (200,000 IU) for a year reduced incident xerophthalmia[113] but had no effect on the incidence of diarrhea, respiratory infection, or on growth by weight-for-age [85] However, growth effects were not analyzed longitudinally, partitioned into ponderal and linear components, or stratified by initial xerophthalmia status Secondary analysis by others suggested that general nutritional status may have declined among female vitamin A recipients late in the dry season, a period of declining food availability and growth that may have affected the efficacy of the supplement [114] A second, larger randomized trial among children under three years of age (n = 592) in Vellore, India, also found no significant effects of vitamin A on annual weight gain (+30

g), increase in arm circumference (+0 04 cm), or linear growth (+0 19 cm) [107] Skinfolds were not measured to allow arm muscle and fat area changes to be derived Children with xerophthalmia were also treated with vitamin A and, unfortunately, excluded from further study Findings from Nepal suggest a growth and body composition response might well have occurred among such children

A community-based, clinical trial in China among 168 children six months to thirty months of age observed no significant change in weight gain (−300 g, p = 0 17) or linear growth (+0 6 cm, p = 0 22) following two doses (one, and another six months later) of vitamin A (200,000 IU) [108] Absence of xerophthalmia among all subjects suggests that vitamin A deficiency may not have been severe enough to limit growth

In the nonwasted population of Aceh, Indonesian children twelve months to seventy-one months of age (n = 2012) were randomized by village to receive two six-monthly rounds of vitamin A (200,000 IU) over a twelve-month period Vitamin A enhanced ponderal growth over the year, reflected by age-specific relative increases of 110 g to 263 g in weight gain ($p < 0\ 05$) and 0 2 cm in arm circumference ($p < 0\ 05$), the latter mostly explained by a 36 mm^2 increase in arm muscle area ($p < 0\ 05$) (Fig 6–4) While these changes were similar to those seen in Nepal,[102] the effects were observed only beyond the breast-fed years (two years to five years of age), only in males, and not restricted to xerophthalmic

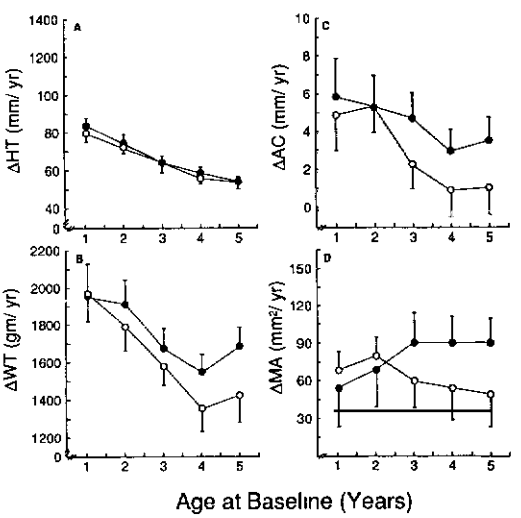

Fig. 6–4. Age-specific, annualized growth increments (Δ) (± 2 standard error of the mean) of boys in vitamin A distribution (●) and control (○) villages during the Aceh (Indonesia) study A, length (< 2 years) or height (2–5 years) (HT), B, weight (WT), C, left mid-upper-arm circumference (AC), D, arm muscle area (MA) (From K P West et al [103])

children There were no consistent effects observed in females and no linear growth response in either sex [103]

Gender differences in the ponderal growth and body composition following vitamin A dosing in Aceh may reflect a combination of phenotypic variation, based on animal evidence showing that male animals respond more rapidly and completely to both vitamin A depletion[11,22,24,115] and repletion [32,116–119] However, these influences may also be dominated by environmental factors such as sex-related differences in dietary intake of vitamin A [120,121] While the sex- and growth-specific findings from Aceh are difficult to reconcile with other studies, the growth response in older males was internally consistent with their higher prevalence of (and more complete recovery from) xerophthalmia[122] and the greater impact of vitamin A on male mortality [123]

Significantly faster weight gain, in association with more rapid recovery, has also been observed in measles cases following large-dose vitamin A treatment [104] Children with moderate to severe measles who were randomized to receive 400,000 IU on two consecutive days following hospital admission had gained 1290 g versus 900 g among placebo recipients (+390 g, $p < 0.05$) by six weeks after hospital discharge, at which time an additional 200,000 IU or placebo supplement was administered Four and a half months later (six-month post-admission follow-up), vitamin A-recipients had gained an additional 1600 g versus 1470 g among controls (no length or body composition measures were reported) Although not statistically significant, the latter difference suggests that the early ponderal response associated with vitamin A treatment was sustained for at least six months [104] These growth changes occurred in the presence of a significant reduction in the incidence and severity of secondary infections In a population-based survey in Malawi, recent fever was associated with a sixfold increase in risk of wasting (< −2 WHZ) among children under thirty months of age However, this risk was halved (and no longer statistically significant) among children who had been dosed with vitamin A during the preceding six months,[124] suggesting less severe febrile episodes and a nutritional "sparing" effect among vitamin A-replete children It is, therefore, difficult to distinguish a primary effect of vitamin A on growth from a secondary effect mediated through its impact on infection and morbidity

Frequent Vitamin A Supplementation

Providing a physiologic supplement of vitamin A on a frequent, routine basis may elicit a growth response different from that to large, periodic doses Small, frequent supplements reverse depletion more slowly, but produce a more incremental, sustained impact on survival In West Java, Indonesia, the routine consumption over eleven months of commercially marketed monosodium glutamate fortified with ~810 IU of vitamin A (MSG-A), at usual levels of MSG intake for age, reduced preschool-child mortality[105] (Chapter 2), increased hemoglobin

levels by ~10 g/liter (Chapter 5), and produced a ~1 cm increase in linear growth at each year of age ($p \sim\leq 0.05$) compared with controls. However, there was no apparent effect on weight gain.[105] The initial vitamin A status of growth responders was not known, nor was it known whether short-term changes in weight had occurred during the study.

During the Guatemalan national vitamin A-sugar fortification program evaluation, the seasonally adjusted prevalence of severe stunting (< 85% of the median height-for-age) decreased from ~22% to ~16%,[68] consistent with the impact on linear growth observed in Indonesia.

In drought-prone Tamil Nadu, southern India, delivery of a small, weekly dose of vitamin A (8333 IU or ~9 μmol) for a year to preschool children older than six months (n = 15,419) had a much less obvious effect, increasing annual linear growth by ~0.27 cm only in nonwasted (≥ −2 WHZ) children ($p < 0.05$). Supplementation had no effect on linear growth in wasted children nor, as in the Indonesian MSG trial, any impact on weight gain in any subgroup.[106] The population was clearly suffering from vitamin A deficiency, evidenced by a 38% prevalence of hyporetinemia (< 0.70 μmol/liter) and an 11% xerophthalmia rate at the outset of the trial, and by a 54% reduction in mortality attributed to the vitamin A intervention.[95] The population was also clearly wasted at the start of the trial (42% < −2 WHZ), presumably due to drought. This situation dramatically improved, along with diet, during the study,[106] which may have masked a potential vitamin A effect on growth. Unfortunately the growth responses of xerophthalmic children were not analyzed separately.

These three studies suggest that slight improvement in linear growth may occur when adequate preformed vitamin A is consumed on a regular basis by (presumably) subclinically vitamin A-deficient children (free of xerophthalmia). The role of protein-energy status in modifying vitamin A effects on growth remains unclear.

Conclusions

> It is thus quite logical to assume that adequacies of dietary protein, energy, vitamins and minerals can individually or in combination determine how well an individual approaches his or her genetic potential.
>
> —George G. Graham, 1981[76]

Our understanding of the mechanisms and conditioning factors that determine the role of vitamin A in child growth remains incomplete. Animal experiments identify specific and, at times, sequentially reliable tissue responses to changes in vitamin A nutriture.[7] However, differences attributable to species and experimental design abound, leaving most questions relevant to humans—other than the basic essentiality of vitamin A for mammalian growth—unanswered. There

may be a general nutritional threshold, or series of tissue-specific thresholds,[125] that must be transgressed relative to other growth-determining nutrients in order for vitamin A deficiency to retard linear and ponderal growth, or affect body composition The effect among children with ocular involvement (xerophthalmia), reflecting severe vitamin A deficiency, indicates that a linear, ponderal, or body compositional vitamin A-responsive deficit may exist Xerophthalmia also indicates the need for sustained dietary intervention beyond immediate treatment [112 126] Unless the child's diet or environment is changed, deficiency will likely recur[44 127–129] with its attendant consequences for growth, health, and survival

Given the wide variations in the prevalence and severity of other environmentally critical factors, the effect of vitamin A supplementation on less deficient children is variable In communities characterized by food insufficiency and wasting malnutrition, there is evidence that routine supplementation can protect or increase lean body mass despite seasonal effects on weight gain,[102] and may produce a small increase in linear growth among nonwasted children over the course of a year [106] In more food sufficient cultures, where less severe (nonxerophthalmic) vitamin A deficiency limits growth, supplementation may stimulate modest increases in weight gain[103] or linear growth [68 105] Animal studies suggest that the type and extent of the growth response probably depends on the quality[117 130–138] and quantity of diet available to the child Indeed, there is also some evidence in animals that vitamin A repletion increases appetite,[7] suggesting yet another intermediary mechanism for a vitamin A influence on growth Similar explanations may also apply to the effects of other micronutrients on growth [63]

The variability and nonspecificity of the observed growth response to vitamin A supplementation in children suggests that improved growth should not be a criterion for assessing the efficacy or effectiveness of a vitamin A intervention program Studies that seek to measure growth effects of vitamin A supplementation need to enroll adequate numbers of potential responders (i e , children with significant vitamin A deficiency), include a battery of measurements to observe changes in body composition in addition to linear growth and weight gain, consider the child's environment and other factors that may influence growth, and reexamine children frequently and for a sufficient duration to characterize the complex growth response

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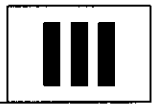

Mechanisms

7

Contributory and Precipitating Events

> Children with respiratory and/or diarrheal disease are more likely to become vitamin A deficient This in turn reduces their resistance to infection, increasing their susceptibility to (severe) respiratory disease and diarrhea, with further consequences for their vitamin A status sight and lives
>
> —Sommer et al 1987[1]

> It is doubtful whether a deficiency disease ever occurs uncomplicated in man or animals
>
> —E V McCollum et al 1925[2]

Vitamin A deficiency exists in a complex milieu of food availability and dietary preference, child rearing practices and educational background, hygiene standards and health services As a rule, severe, blinding deficiency thrives under conditions of social deprivation and poverty But materially poorer populations sometimes suffer less severe deficiency than richer ones by virtue of dietary habits, hygiene practices or immunization coverage For example, income, clothing, housing and other indices of economic well-being were better in West Java than in East Java, but the xerophthalmia rates were far higher as well [3] Furthermore, milder, nonblinding deficiency, widespread throughout the Third World (and pockets of wealthier Western societies), may account for half of all vitamin A deficiency-related deaths [4–7]

Recent recognition of the public health significance of milder deficiency makes it more important, but also more difficult, to document the many factors that contribute to it Xerophthalmia is the only readily recognized, clinically distinct manifestation of vitamin A deficiency, it is therefore the one for which we have the most cause-related data The situation will certainly change as studies

increasingly employ more sensitive tests capable of identifying non-xerophthalmic children who are nonetheless vitamin A deficient (Chapter 11) [8–10] In almost all instances, however, vitamin A deficiency will prove to be the product of multiple, intersecting influences

Vitamin A deficiency, particularly its severe, blinding form, is largely (but not wholly) a disease of preschool-age children (Fig 12–2) This age group is most vulnerable to dietary deprivation (dependent as it is on fetal stores, breast feeding and weaning practices), diarrhea and other common infections related to poor sanitation, and to childhood exanthems It overlaps with the peak ages of protein-energy malnutrition (PEM) and the "malnutrition/infection complex "[11 12]

A number of diseases have traditionally been associated with xerophthalmia For some, like cystic fibrosis[13–19] and chronic small bowel disease,[20–24] vitamin A malabsorption is clearly responsible For others, like liver disease, the relationship is more complex [25–31] It may be due to poor diet,[32 33] impaired absorption,[21 23 32 34 35] decreased capacity for storing or transporting the vitamin,[21 36–38] urinary loss,[39–41] or the influence of concomitant disease

Seventy years ago, Bloch recognized that vitamin A deficiency arose from three basic sources an inadequate diet, malabsorption, and infections,[42 43] to which he and others added a fourth, the "spring growth spurt" and other factors that increase metabolic demand (Chapter 6)

The association with infectious diseases is the most prevalent and complex of all, hardly surprising given the ubiquity of these diseases in developing cultures (turn-of-the-century Europe as much as today's Third World) and their intimate interaction with nutritional status, feeding practices, and one another Despite the consistent, repeated associations between indices of vitamin A deficiency and evidence of infectious episodes (Table 3–1), it is not always clear from these data "which was the chicken and which the egg " In some instances, vitamin A deficiency clearly increases the risk of infectious morbidity, in other instances, it seems that the association is better explained (in part or in whole) by the impact infection has on vitamin A status These differences depend on factors such as how a study was conducted, the particular infection, and the manifestation of vitamin A deficiency with which it was "associated " For example, chronic diarrhea probably leads to progressive vitamin A deficiency, which may in part explain the frequent, strong correlation between a *past* history of diarrhea and the presence of Bitot's spots Measles, on the other hand, is more consistently associated with acute corneal dissolution (X3) and systemic infectious complications in the absence of preexisting clinical xerosis, suggesting acute decompensation of previously marginal vitamin A status The evidence that vitamin A deficiency increases the risk of severe infectious episodes was described in Chapters 2 and 3 Here we examine the other side of this potentially vicious cycle infections and other systemic conditions, notably PEM, that contribute to the development of vitamin A deficiency and its clinical expression

Systemic Infections

As already noted, vitamin A-deprived weanling rats raised in conventional surroundings grow for only a short period of time, with cessation of growth soon followed by death When equally deprived rats are maintained in a germ-free environment, growth continues, albeit at a reduced rate, and the animals survive longer [44–46] It seems likely that the germ-free environment reduces vitamin A demand and otherwise prolongs vitamin A stores

Serum retinol levels decline in a variety of infections,[22 23 47–49] sometimes by as much as 20 μg/dl to 30 μg/dl [48] In some instances, the degree of decline is related to the severity and duration of the infection [23] In part this represents a nonspecific response to fever, which may persist for days after the temperature has returned to normal [23 50] In children with rheumatic fever, serum levels dropped by 50% when their temperature exceeded 100°F [51] Elevation in acute phase proteins, evidence of significant (if subclinical) infection, is associated with reduced serum retinol Following nonspecific insults like myocardial infarction and surgery, C-reactive protein levels rise and the concentration of serum retinol binding protein (RBP) declines, the latter returning to baseline value after three to seven days [52] Rosales et al suggest RBP production may be reduced under these circumstances, acting like albumin as a "negative acute-phase reactant "[53] In some regions malaria probably constitutes a significant proportion of such morbidity [54–56] The magnitude and rapidity of the fall and subsequent recovery of serum retinol in children with some acute infections and/or fever suggests direct interference with vitamin A release and transport, in addition to the potential depleting effects of impaired absorption and increased utilization and excretion The degree to which an infection diverts vitamin A to other sites, reduces its release from the liver, or actually increases metabolism and excretion and thereby reduces stores probably varies with the specific pathogen and the state of the child As we shall see, measles seems to do some or all of the above, causing a marked but transient decrease in serum retinol that may or may not return to normal following the acute insult

An unforeseen outbreak of chickenpox vividly demonstrates the devastating impact that common childhood exanthems can have on vitamin A reserves [8] Serum vitamin A and RDR values were obtained on "deprived" children attending day care centers in Recife, Brazil, at baseline (before administering a dose of 200,000 IU vitamin A) and again at one, four, and six months An outbreak of chickenpox, at about three months, affected thirty-six of the ninety-three original subjects The vitamin A status of the infected children began to diverge from the others at the next evaluation, one month later By the six-month exam (three months after the epidemic), 74% of infected children, but only 10% of noninfected children, had an abnormal RDR (Table 7–1)

These results are particularly dramatic because the children seem to have begun with serum vitamin A levels traditionally considered well within the normal

Table 7–1 Effect of Varicella Infection on Vitamin A Status

Days after Supplementation	Number of Infected Children[a]		Retinol (μg/dl) Infection		RDR Positive Infection	
	+	−	+	−	+	−
0	36	57	37 ± 14	38 ± 14	42%	38%
42	30	42	49 ± 9	47 ± 9	0%	0%
120	31	31	38 ± 7	41 ± 6	10%	0%
180	31	29	26 ± 9*	41 ± 14	74%	10%

[a]Varicella infection at approximately three months

*$p < .001$ for difference between infected and control groups

From F A C S Campos et al [8]

range (mean = 38 μg/dl) Further, the relative dose-response (RDR), as a surrogate for liver stores, was abnormal in a greater proportion of children after the large dose of vitamin A and subsequent infection than it had been at baseline, before the large dose was given As expected, the opposite was true for the cohort of uninfected children

Baseline status nonetheless affected the outcome Among the infected children, over 90% (10/11) who had been RDR positive at baseline were again RDR positive at six months, compared with only 63% (12/19) who had been RDR negative at baseline. The former group, with poorer vitamin A status to begin with continued to exist in a more vitamin A deprived environment and/or the large dose did not adequately compensate for their original degree of vitamin A depletion, particularly given the stress of the intervening exanthematous infection

Infection-related reduction in vitamin A reserves may be sufficient to result in xerophthalmia (moderate to severe vitamin A deficiency) In Bangladesh, a history of recent chickenpox was associated with subsequent xerophthalmia (Odds Ratio [OR] = 1 48) [57]

As already described (Chapter 2), over 3000 rural preschool Indonesian children were reexamined every three months for eighteen months [1,3] The risk of a child developing xerophthalmia (XN/X1B) by the end of each interval was calculated for children with and without respiratory disease and with and without diarrhea at the start of each interval

Children with respiratory disease were more than twice as likely to develop mild xerophthalmia (XN, X1B) than were their peers ($p < .05$) (Table 7–2) The excess cases occurred among children three years and older, in whom the rate was increased threefold to sixfold The magnitude of the increased risk of xerophthalmia associated with prior respiratory disease was relatively constant across strata of different degrees of wasting malnutrition

The relationship between a history of diarrhea during the month preceding the start of an interval and the presence of xerophthalmia three months later

Table 7–2 Age-Specific Incidence of Xerophthalmia for Children with and without Respiratory Disease[a]

Age (years)	*Child Intervals* Respiratory Disease		*Developed Xerophthalmia*[b] Respiratory Disease		*Xerophthalmia Rate (per 1000)* Respiratory Disease		*Relative Risk of Xerophthalmia* Respiratory Disease	
	−	+	−	+	−	+	−	+
≤ 1	5533	595	6	0	1 1	0	1	—
2	3001	417	8	1	2 7	2 4	1	0 9
3	3061	257	10	3	3 3	11 7	1	3 6
4	3042	170	11	2	3 6	11 8	1	3 3
≥ 5	3657	137	13	3	3 6	21 9	1	6 2
Total	18,294	1576	48	9	2 6	5 7	1	2 2*

[a]Presence or absence of respiratory disease at examination initiating the three month interval

[b]Xerophthalmia absent at baseline but present three months later

*$p < .05$ 2-tailed

From A Sommer et al[1]

was even more pronounced (Table 7–3) A positive association was present at every age, with an average relative risk (RR) of 2 5 ($p < .05$) Once again, the relative risk associated with diarrhea was comparatively constant across strata of wasting malnutrition 2 0 and 2 8 for wasted (< 90% weight-for-height) and better nourished children respectively[1]

Results are particularly striking because the children at greatest risk of xerophthalmia (e g those with the most severe respiratory disease and diarrhea)

Table 7–3 Age-Specific Incidence of Xerophthalmia for Children with and without Diarrhea[a]

Age (years)	*Child Intervals* Diarrhea		*Developed Xerophthalmia*[b] Diarrhea		*Xerophthalmia Rate (per 1000)* Diarrhea		*Relative Risk of Xerophthalmia* Diarrhea	
	−	+	−	+	−	+	−	+
≤ 1	4990	465	4	2	0 8	4 3	1	5 4
2	3038	289	8	1	2 6	3 5	1	1 3
3	3045	172	11	1	3 6	5 8	1	1 6
4	2979	151	11	2	3 7	13 2	1	3 6
≥ 5	3644	93	14	2	3 8	21 5	1	5 7
Total	17,696	1170	48	8	2 7	6 8	1	2 5*

[a]Presence or absence of a history of four or more loose stools one or more days within the month preceding the examination initiating the three-month interval

[b]Xerophthalmia absent at baseline but present three months later

*$p < .05$ 2-tailed

From A Sommer et al[1]

were provided symptomatic treatment and referred to local health centers in the interim

It is evident that infectious episodes can materially affect vitamin A status If, as seems likely, vitamin A deficiency increases the risk of diarrhea and respiratory disease, while respiratory disease and diarrhea impair vitamin A status, children get trapped in a particularly lethal cycle that induces and perpetuates ocular and systemic morbidity [1]

Diarrhea and Gastroenteritis

Aside from the incidence data noted above,[1] most evidence linking diarrhea to vitamin A status stems from cross-sectional prevalence surveys (Table 3–1), with their inherent difficulties in distinguishing cause from effect

Data relating the severity of vitamin A deficiency with diarrheal illness also comes from hospital and clinic-based studies [3] Differences in vitamin A status were more pronounced for children presenting to the Cicendo Eye Hospital with a history of diarrhea ($\geq$ 4 loose stools in a single day during the preceding month) than for children with clinically detectable gastroenteritis, presumably because of the cumulative impact of past diarrhea on vitamin A status Sixty-six percent of cases of X1B and 75% of X2/X3 had a positive history, versus 23% of the controls of corneal cases (RR > 3 0, p < 001) By case-control analysis, children with a history of diarrhea during the previous month were at thirteen times the risk of active corneal disease than were children with a negative history Interestingly, a majority of the diarrheal episodes were said to have occurred within the previous week—it's unclear whether this was an acute, precipitating event or merely a surrogate for frequent diarrhea in the past, confounded by the historical nature of the data

The Indonesian countrywide survey yielded comparable results, supporting the ability to generalize findings to the population of Indonesian children at large A history of diarrhea in the past two months was present in 61% of cases of X1B (close to the 66% in the clinic study), and a recent history (during the past week) was three times as common as among controls (10 1% versus 3 5%, p < 001) Among children presenting to the Dhaka Hospital of ICDDR,B in Bangladesh, Indonesia, those with xerophthalmia (XN) had lower serum retinol levels, were more undernourished, and were significantly more likely to have suffered a prolonged illness with dysentery attributed to *Shigella* and *E Histolytica* [58] A community-based survey in the same city demonstrated that protracted diarrhea was significantly associated with the presence of XN (OR = 4 1) [57] In a population-based study in southern Nepal, the risk of xerophthalmia increased with the reported duration of dysentery during the previous week (OR = 2 1 if 1–6 days, 5 8 if $\geq$ 7 days) [59]

In contrast with the high frequency of diarrhea among patients admitted to the Cicendo Hospital, gastroenteritis was present in only 2% (1/50) of children

presenting with responsive Bitot's spots (X1B), and in 7% (11/156) with active corneal disease (X2/X3) The prevalence of gastroenteritis was twice as common in cases of corneal involvement than it was in their matched controls Whether the gastroenteritis was a result of severe vitamin A deficiency, the significant PEM that frequently accompanies corneal involvement,[60] and/or a precipitating cause of acute, severe vitamin A deficiency is uncertain It's likely that one or all three factors were at work, to different degrees in different children

Feachem[61] has pointed to the inevitable difficulties in sorting out cause and effect, and of isolating direct relationships from the confounding issues of nutritional and socio-educational status Two approaches help The strongest epidemiologic evidence is the consistency and strength of the relationship across multiple studies The other is the existence of biological mechanisms to explain the relationship These include anorexia, reduced absorption of ingested vitamin A that accompanies diarrhea, and cultural practices such as the proscription of food during diarrhea Absorption is seriously impaired in steatorrhea,[62] in gastroenteritis not accompanied by fat malabsorption,[63] in mild to moderate undifferentiated diarrhea,[64] in chronic salmonellosis,[65] and even in asymptomatic infection with parvovirus and strains of *E coli* [66]

A recent study of Peruvian children suggests acute diarrhea, particularly from rotavirus, increases urinary excretion of vitamin A tenfold Much of the increase appears to have been related to accompanying fever [41] Diarrhea may be associated with *Giardia* and *Ascaris,* both of which reduce vitamin A absorption, in some instances by as much as 70% [67–70] Parasitic infestation may therefore exaggerate the effects of borderline dietary intake, though the mechanisms are not entirely clear a recent report failed to identify increased absorption of vitamin A by *Ascaris* or increased excretion of orally administered vitamin A in the stools of infected children [71]

Vitamin A deficiency and parasitic infections are commonly linked in the popular mind Indonesian villagers refer to Bitot's spots as "worm feces"[72] and blame roundworm infestation for their presence Although roundworms are found in a large proportion of xerophthalmic patients,[73–76] worm infestation is so prevalent in depressed communities that it is difficult to assess the significance of this observation in the absence of suitable controls In a cross-sectional survey in the Philippines, roundworms were no more common in cases of (mild) xerophthalmia than in the rest of the population,[77] although in Jordan they were[78]—at least 43% of normals and 60% of abnormals harbored the parasites

In the representative Indonesian countrywide survey, a history of shedding worms during the preceding month was present in 22% of children with Bitot's spots, 14% of their matched controls, and 9% of other children ($p < .01$ for linear trend) [3] Although the beneficial effect of deworming on vitamin A absorption lasts at least two months,[68] reinfestation is extremely common [67] Deworming in combination with a large oral dose of vitamin A (200,000 IU) every six months was not superior to vitamin A alone in boosting and maintaining serum retinol

among urban slum-dwelling Indian children [79] But in Brazil, where *T trichuris, A lumbricoides* and *G lamblia* are common, treatment with mebendazole and metronidazole prior to relatively small daily doses of vitamin A (500 mg retinyl acetate) was essential for increasing serum retinol [80]

Since Third World children generally obtain a significant proportion of their vitamin A from beta carotene, and the absorption and conversion of dietary sources of carotene to vitamin A is even more susceptible to intestinal alterations,[81–83] the true health impact of intestinal infections on vitamin A status is probably greater than isolated studies on preformed vitamin A (or even carotene) might suggest

Milder degrees of vitamin A deficiency are only beginning to be studied, primarily through tests of physiologic function like conjunctival impression cytology (CIC) and measures of body stores such as RDR Indian children with mild, chronic diarrhea and entirely normal eyes were much more likely to have abnormal CIC and RDR than their age-sex-neighborhood matched controls [84 85]

Diarrhea affects a variety of intestinal functions, which lead not only to vitamin A deficiency but also to PEM,[86] another vicious cycle with which disadvantaged children must contend The impact of PEM on multiple facets of vitamin A metabolism is discussed below

Respiratory Disease

Respiratory disease, like diarrhea, is strongly associated with mild xerophthalmia (Chapter 3) The evidence is about equal that respiratory disease influences vitamin A status and that vitamin A status influences the risk of respiratory disease

Active respiratory disease (fever plus cough/rales) was the most common condition accompanying xerophthalmia in children presenting to the Cicendo Eye Hospital in Bandung, Indonesia [3] The prevalence of respiratory disease increased dramatically with the severity of vitamin A deficiency (xerophthalmia) (Fig 3–5) This is made all the more striking by the fact that the children presented for their eye problems, not for their systemic disease As with diarrhea, a recent history of respiratory disease suggests it is contributory to, rather than merely a consequence of, vitamin A deficiency A history of significant cough was more common in severe deficiency (X3B) than in milder xerophthalmia (X2, X3A), 27% versus 16% respectively ($p < 01$) It was also more common among corneal cases (X2/X3) than among their matched controls (30% versus 9%, $p < 05$) [3]

A number of factors may explain the particularly high frequency of respiratory disease among cases of corneal necrosis (X3B) These children were generally more malnourished and debilitated than children with milder disease, conditions generally associated with a high prevalence of respiratory infection [87] As already discussed (Chapter 3), vitamin A deficiency may increase the risk (or severity)

of subsequent respiratory tract infection, while severe or repeated respiratory disease can have a profound influence on vitamin A status (which in turn contributes to the severity of vitamin A deficiency and xerophthalmia)

Serum vitamin A levels are routinely depressed in respiratory disease [22,23,48,49,88] Ordinarily, vitamin A does not appear in the urine, but during respiratory infections, enormous amounts (in excess of 3000 IU/d) may be lost by this route [39,89,90] Recent studies in patients with severe pneumonia and sepsis suggest retinol excretion results from impaired retention of retinol binding protein (RBP) [40] Respiratory infections also interfere with absorption of beta carotene and vitamin A Beta carotene absorption may fall by as much as 50%–80%, remaining at this level for as long as two weeks after the last day of fever [24] Dietary intake ordinarily capable of raising serum vitamin A may fail to maintain preexisting levels [88] Absorption of vitamin A administered in moderate amounts, usually 90% or higher, may fall to as low as 30% (mean of 74%) [63] The average rise in serum vitamin A levels following a massive oral dose in persons with tuberculosis is only half that in normals [91]

A history of recent respiratory disease, like a history of recent diarrhea, is also more common among children with mild ("pre-xerophthalmia") vitamin A deficiency detected by CIC Micronesian children with abnormal CIC were 2 6, 2 3, and 1 7 times more likely, respectively, to have reported diarrhea ($\geq$ 4 loose stools), respiratory illness (frequent cough with rapid breathing, $p < 05$), and high fever, during the prior two weeks than were their CIC normal controls [10] Results were comparable for an earlier, clinic-based study using similar techniques [92] In the United States, serum vitamin A levels dropped over the first months of life in very-low-birth-weight (VLBW) infants with repeated episodes of airway infection when compared with controls [93]

Measles

One of the most intriguing aspects of the vitamin A "story" to unfold in the past two decades has been our increased understanding of the interrelationship between measles infection and vitamin A status, and its implications for sight and life

As already discussed (Chapters 3, 4), vitamin A status plays a major role in determining the incidence and severity of measles complications and associated blindness and mortality By the same token, measles impacts on vitamin A status

During the acute phase of measles, vitamin A levels are markedly depressed in comparison to the predisease state[94] or a noninfected reference population [95–100] As many as a quarter or more of children studied have had deficient levels ($< 10\mu g/dl$), even in New York Serum retinol often rises spontaneously and dramatically once the acute phase of measles has passed [94,97,98,100]

The genesis of the decline in vitamin A levels accompanying active measles is uncertain but probably multifactorial Given its acute and often transient

nature, it is unlikely to reflect reduced intake and absorption More likely, there is a disturbance in the release and transport of vitamin A stores coupled with a dramatic increase in metabolic demand secondary to an intensely catabolic state The latter results from measles invasion, epithelial desquamation, fever, and secondary infection Measles induces a protein-loosing enteropathy[101] that exacerbates underlying PEM,[36 102 103] providing an additional mechanism to explain reduced RBP syntheses and release[104] in the face of potentially adequate hepatic reserves [105] A number of issues remain puzzling the near-universal depression in circulating retinol and spontaneous recovery despite a persistent ability to mount a holo-RBP response to exogenous sources of vitamin A[106], and the low incidence of vitamin A deficiency-related clinical complications in children who are otherwise well nourished and have good vitamin A reserves—as if serum retinol does not necessarily reflect the physiologic adequacy of vitamin A at the level of target tissues Nonetheless, sudden, dramatic, and severe depression in circulating retinol can and does occur during the acute phase of measles Levels are most depressed in children with severe disease, complications, pneumonia, high and persistent fever, and PEM [94 96 98]

In addition to its acute impact on vitamin A status, measles can initiate events leading to increasingly severe, chronically progressive vitamin A deficiency and PEM, the latter exacerbating the former Voorhoeve[107] described the typical course in a Nigerian child initial acute measles-associated ocular discomfort resolved spontaneously, but measles-induced diarrhea persisted for four weeks when the child presented as a "marasmic skeleton" with perforations of both eyes By itself, fever accompanying measles increases protein breakdown in excess of synthesis, resulting in negative protein balance [108] This is quite apart from measles-induced protein-loosing enteropathy[101], dietary intake that is reduced by anorexia, stomatitis, and common cultural practices that dictate withholding food and fluid from children with measles[109–113], and recurrent diarrhea and pneumonia,[100 110 112 113] which occur as long as six months or more after the original measles attack [114–116]

It is hardly surprising that in some instances a quarter of affected children lose 10% of their body weight,[112] or that measles is frequently cited as an important precipitating event in severe PEM [87 94 112 113] They are dragged downward into the spiral of the "malnutrition/infection complex,"[11] which may have a significant, negative impact on growth that persists for years [117] Vitamin A supplementation during the acute attack can reduce the risk of growth faltering and malnutrition, at least in part, by reducing post-measles infections [100 118]

Measles has long been associated with corneal ulceration and melting resembling severe xerophthalmia Given the impact of measles on vitamin A status, it is not surprising Duddell makes reference to it in his treatise on the cornea of 1729 [119] Measles was included by Spicer, in 1892, as one of several systemic diseases precipitating "large perforating ulcers "[120] Bloch described a Danish case of keratomalacia that "appeared simultaneously with measles "[42] Since then,

a number of authors have called attention to the potential relationship Corneal ulceration resembling xerophthalmia has been observed to follow measles in Europe,[25 27 42,119 121 122] the Middle East,[109 123] Asia[74 124–127] and Africa [107 111 128–134] Fifty-seven percent of El Salvadorian[135] and 16% of Haitian[136] children with corneal scarring typical of xerophthalmia claimed that their active disease had been accompanied by measles

Malnourished, hospitalized children in El Salvador with typical corneal ulceration and necrosis were more likely to have had a recent history of measles than were malnourished children with normal eyes [137] In the Indonesian nationwide survey, 36% of cases of X2/X3 claimed to have had measles during the preceding month, a rate ten times that of the rest of the population, 79% of X3 and 51% of XS claimed the onset of ocular disease had been preceded, by one to four weeks, by measles [3] This is in sharp contrast with cases of X1B, which were as likely to have suffered measles during the past month as their non-xerophthalmic peers (4 8%),[3 138] a finding consistent with that of the Philippines and Nepal [77 139]

Among xerophthalmia patients with X2/X3 presenting to the Cicendo Eye Hospital in Bandung, almost 10% had evidence of active or recent measles, usually within the preceding month [3] They were likely to have the severest forms of corneal necrosis, and all were markedly vitamin A-deficient One case is particularly illustrative He was first seen twelve days after onset of measles The cornea contained three inferocentral, saucer-shaped, infiltrated ulcers in a perfectly straight horizontal line in the left eye, and a linear central ulcer resembling a traumatic lesion in the right eye He had been successfully treated for nightblindness with low-dose vitamin A pills at a local clinic three months previously A week prior to admission he developed fever, cough, diarrhea, and measles rash coincident with the return of nightblindness Initial serum retinol was extremely low (5 μg/dl) but protein status was reasonable (serum albumen 3 5 g/dl and transferrin 244 mg/dl) He was given high-dose vitamin A, and the ulcerations disappeared within three days

As in the nationwide survey, none of the patients presenting to Cicendo Eye Hospital with Bitot's spots (and normal corneas) had a recent history of measles [3]

Classical xerophthalmia, with conjunctival and corneal involvement, has been long recognized following post-measles deterioration of vitamin A status [111 129–131 140–142] Sudden corneal ulceration and necrosis accompanying the acute phase of measles, often without coexisting conjunctival xerophthalmia and occasionally in populations in which xerophthalmia is not commonly recognized, have obscured the central role vitamin A plays in a significant number of cases This has been especially true in Africa, where measles-associated corneal destruction is commonly cited as the major cause of acquired pediatric blindness [110 128 143–153] The situation has been further clouded by the common African practice of placing herbal medicines in the eyes of children with active measles [112 154 155] An area of Zambia in which the problem was once particularly common, the Luapula Valley, achieved notoriety as "the valley of the blind "[140] "Luapula blindness" came to

be attributed to the "three Ms" muti (local medicines), malnutrition, and measles Most children with corneal complications suffered particularly virulent measles[113 128 156] and significant malnutrition [107 111 113 129 130 146 156–158] The virulence of their measles may have been related to concomitant malnutrition,[112 129 157 159] overcrowding,[160] vitamin A deficiency,[96 99 100 161] and other factors [114 162 163] Indeed, blindness was noted to be much less prevalent in Luapula Valley communities in which red palm oil (an excellent source of pro-vitamin A carotenoids) was consumed than in those in which it was not [132]

In 1978, Whittle and colleagues called attention to the potential role of herpes simplex in measles complications, particularly those affecting the eye [113 166] They identified herpes virus by culture or immunofluorescence in a significant proportion of ulcerating eyes Most of the subjects were malnourished, those with the worst corneal necrosis, severely so The researchers concluded that the combined immunosuppressant effects of measles and PEM predisposed to secondary (presumably recrudescent) herpes [164] Vitamin A deficiency was considered less important, because a significant proportion of cases had serum RBP levels considered by the authors to be in the "normal range " The latter conclusion is arguable half the cases of clinical corneal xerophthalmia *un*associated with measles reported from India[165] and Indonesia[3] had similar levels, above 15 μg/dl

Typical herpetic corneal lesions have been observed in children with concomitant measles and malnutrition in Africa[129] and elsewhere [166] Children with measles[167–172] and malnutrition[173–176] are particularly immuno-compromised and susceptible to secondary herpes [177] Indeed, fatal herpes in neonatal African children is frequently preceded by measles [178] This does not necessarily mean vitamin A deficiency plays no role concurrent measles and malnutrition cause greater depression of vitamin A than either condition alone[179], and vitamin A deficiency reduces immune-competence (Chapter 9)—combined with measles and PEM, the suppression should be even greater Vitamin A-deficient rats infected with herpes simplex virus (HSV-1) (placed on mildly abraded epithelium) in the early stages of vitamin A deficiency (prior to or at the start of the weight plateau) develop more rapid and severe epithelial ulceration and corneal necrosis than do their pair-fed controls [180] They are also more likely to develop disseminated disease Interestingly, topical application of herpes virus to nonabraded, keratinized corneas of rabbits with more severe, prolonged vitamin A deficiency produced less extensive epithelial infection,[181] a difference ascribed to an increased barrier to infection provided by the heavily keratinized corneal surface

McLaren, long a pioneer in this area,[182 183] drew attention to Foster's contribution[184] to sorting out the pathogenesis of measles-associated corneal ulceration Foster studied 130 Tanzanian children with active corneal ulceration from all causes by clinical examination, response to therapy, and biochemical parameters Herpes simplex was responsible for one-third of the ulcers, vitamin A deficiency, for one-quarter, and traditional medicines, for one-seventh (Fig 7–1) Vitamin A deficiency was associated with more severe disease and a less favorable out-

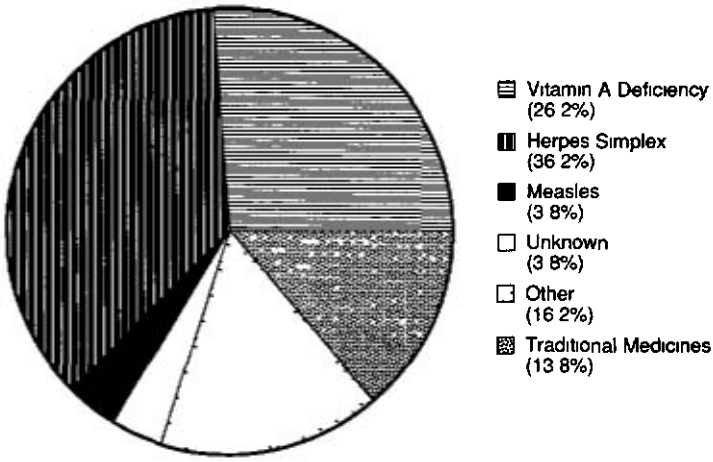

Fig. 7–1. The presumptive etiology of corneal ulcers among 130 children presenting to the Mvumi Hospital in Central Tanzania Measles was responsible for less than 4%, vitamin A deficiency, for more than 25% (From A Foster et al [184])

come In over one-third of the children, both eyes were ulcerated Vitamin A deficiency was responsible for over half such cases, herpes, for less than a quarter

A *history* of measles within the preceding month was present in one-third of all cases of corneal ulceration and in 57% of those with bilateral ulcers Vitamin A deficiency was responsible for half the measles-associated corneal ulcers, herpes, for one-fifth, and traditional medicines, for nearly 17% (Fig 7–2)

In this same study, three-quarters of all corneal ulcers due to vitamin A deficiency, but only one-fifth of those caused by herpes simplex, had a recent history of measles The longer the duration between measles and onset of ulceration, the more likely that the ulcer was secondary to vitamin A deficiency, suggesting late sequelae of chronic, post-measles deterioration of vitamin A status

The clinical diagnosis was almost always supported by the biochemical data (Fig 7–3) Occasional discrepancies between clinical diagnoses and serum retinol values point up the complexity and multifactorial nature of corneal ulceration in rural African children In retrospect, two cases diagnosed as xerophthalmia that appeared to have normal vitamin A levels may have been caused by traditional medicines One case of measles-associated ulceration with extremely low vitamin A and RBP levels had initially been diagnosed as xerophthalmia, but the diagnosis was changed to "traditional medicines" when the child failed to respond to vitamin A therapy There is no reason the child could not have suffered from both

Eleven percent of measles-associated ulcers were round, epithelial, and central, occurring within ten days of onset of rash These healed quickly on antibiotics and patching and probably represented confluent measles keratitis accentuated

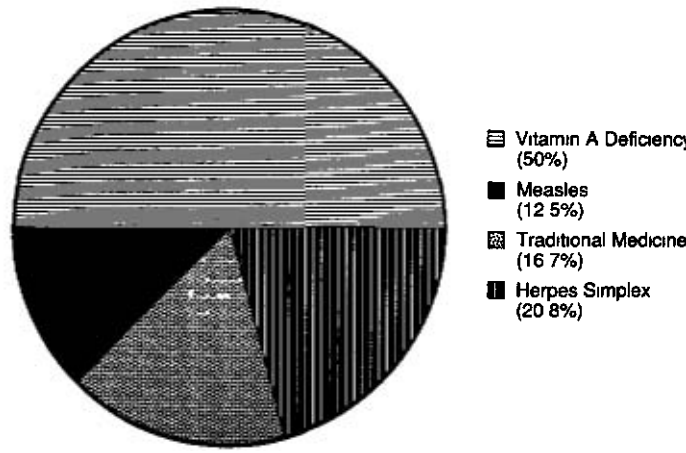

Fig. 7-2. Over one-third of the children presenting to the Mvumi Hospital with corneal ulcers had a history of measles within one month of onset of their corneal lesions In half these measles-associated cases, the underlying etiology was vitamin A deficiency (n = 48 children) (From A Foster et al [184])

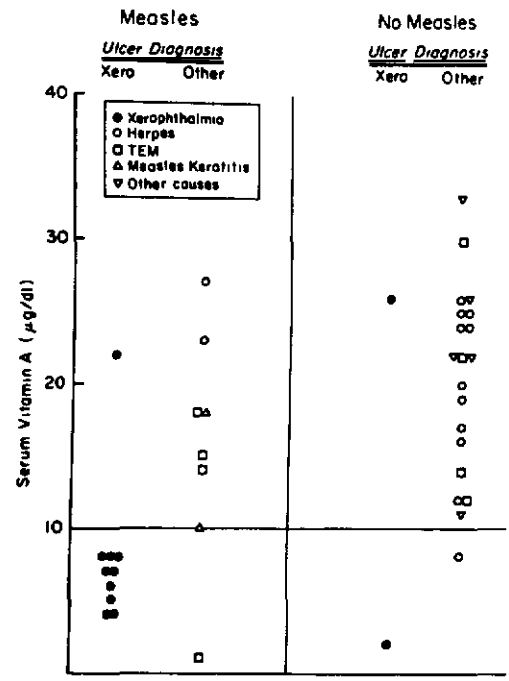

Fig. 7-3. Serum vitamin A levels from forty Tanzanian children with corneal ulcers studied consecutively at the Mvumi Hospital The etiologic diagnosis was established on clinical grounds, before serum vitamin A levels were known (From A Foster et al [184])

by desiccation of the cornea from exposure Herpetic ulcers were usually unilateral, mild, and limited to the epithelium

Foster and Yorston[185] compared childhood corneal ulcers at one hospital for the periods 1982–1984 and 1986–1988, during an interval when measles immunization rates rose from 30% to 80% Measles admissions fell 78%, ulcers "associated with measles" (through whatever mechanism) fell 87%, and ulcers due to vitamin A deficiency (frequently precipitated by measles) fell more than 75%

In conclusion, measles can precipitate severe vitamin A deficiency, resulting not only in increased systemic morbidity and mortality, but corneal ulceration and blindness The exact mixture of contributory causes of measles-associated blindness are likely to vary from one population to another, but the constituents seem clear Measles directly invades the conjunctiva and cornea,[129 186] giving rise to punctate keratitis of varying severity The lesions, however, are superficial and generally resolve spontaneously In a number of children, herpes simplex ulceration occurs Exactly how or why is uncertain Given the common recurrence of herpes lesions elsewhere, principally the skin and mouth, it is likely these reflect reactivation secondary to reduced immune competence from measles, PEM, vitamin A deficiency, and quite possibly other factors, such as malaria,[187 188] alone or in combination Herpes lesions are generally unilateral and superficial, rarely leaving the child blind

At the same time, vitamin A deficiency appears to account for the majority of cases of measles-associated bilateral, severe, blinding corneal ulceration

Two factors that seem to have most confused investigations in the past are the frequent absence of other evidence of xerophthalmia (X1B/X2) in measles cases, and the seeming rarity of mild xerophthalmia in the population at large The latter may simply reflect failure to adequately assess the status of poor, often rural, remote populations (personal communication, Erika Sutter, 1979) [189] On a first consultation to Tanzania (by A S) in 1981, the existing "lore" concluded xerophthalmia was a rarity, despite claims by Sauter[128] that vitamin A prophylaxis would prevent serious measles-associated corneal ulceration in that country Active surveillance subsequently identified large numbers of cases,[190] later confirmed by formal surveys [191]

Typical xerophthalmia, in the absence of precipitating measles, has been observed in most areas of Africa in which measles-associated blindness occurs and in which it has been sought out [107 110 128–130 132 140–142,144 149 154 192–196] Estimates that one-fourth to one-half the cases of corneal involvement are associated with measles[128,132 141 155 184 195 197 198] are consistent with observations in Indonesia[3 138] and reports from El Salvador,[135] Vietnam,[126] Japan,[127] and Jordan [123]

It is also likely that most children in Africa, as elsewhere, have mild vitamin A deficiency (e g , are "pre-xerophthalmic") Where measles leads to chronic, repeated reinfections, diarrhea, and PEM, vitamin A status may deteriorate slowly, providing ample opportunity for nightblindness and keratinizing metapla-

sia (X1/X2) to become apparent But where measles is severe and vitamin A status precarious, precipitous deterioration of "borderline" deficiency ("marginal status") results in rapid stromal melting (ulceration and keratomalacia, X3) before clinically detectable xerosis develops In some instances, corneal ulceration and its attendant ocular inflammation may reverse or otherwise mask preexisting conjunctival xerosis (Chapter 4) [199]

Measles may also work through local ocular factors to potentiate the xerophthalmic process Measles keratitis no doubt increases corneal requirements for vitamin A and may contribute to those still-obscure processes responsible for xerophthalmic melting of the corneal stroma Measles is also associated with reduced tearing[200 201] and alterations in tear composition While the importance of tears for the delivery of vitamin A to ocular tissues is uncertain, tears do contain vitamin A in a concentration that varies with vitamin A status [202]

The mutual interaction between measles and vitamin A nutriture, and their effect, in turn, on systemic morbidity, mortality, and blindness, provide a powerful and urgent incentive for further enhancing measles immunization The facts are that immunization coverage falls well below 100%, immunization does not protect a substantial proportion of those reached, and children too young to be successfully immunized (less than six months to nine months) are at risk of blinding and deadly measles These are cogent reasons for ensuring that all children with measles receive large-dose vitamin A treatment[203–205]—as should all children who have less than adequate vitamin A stores [206]

Interestingly, five cases of post-measles corneal melting were observed in Leipzig, Germany, in a three-month period in 1955 [207] The authors could not explain its genesis Although the children were generally very ill, the authors ruled out vitamin A deficiency by the absence of any significant clinical response to vitamin A therapy But the vitamin A was administered as an oily injection (which is relatively ineffective) late in the course of corneal disease (when little viable corneal tissue may still have been present) One of the earliest reports of classical vitamin A deficiency (by dietary history) and xerophthalmia had been published from this same clinic forty years earlier [208]

Protein Energy Malnutrition

The association between PEM and the presence or risk of vitamin A deficiency, particularly severe, blinding xerophthalmia[3 42 74 209–217] and measles-related corneal destruction,[107 111 113 130 146 156–158 218] has been repeatedly noted In Indonesia, the severity of xerophthalmia, especially corneal xerophthalmia, was linked with the severity of PEM as measured by a variety of indices, including anthropometric status, pedal edema, and serum levels of albumen and transferrin [3 219 220] Seventy percent of cases of corneal xerophthalmia, but only 30% of vitamin A-responsive Bitot's spots (and none of the matched controls), had serum albumin levels below 3 5

gm/dl Children with nightblindness (XN) admitted to ICDDR,B's Dhaka facility were more likely to be malnourished than were their non-xerophthalmic peers [57]

The pervasive relationship between malnutrition, particularly protein deficiency, and vitamin A status can arise from at least two sources The first is common origins dietary habits, hygiene standards, and their interplay (the "infection/malnutrition complex") [211] In general, children deficient in protein are likely to be deficient in other nutrients as well [2,221] This noncausal relationship helps explain individual variations in the association between protein and vitamin A status [220]

The presence of PEM increases the subsequent risk of xerophthalmia (by roughly the same order of magnitude as does the presence of respiratory disease or diarrhea) [1] This suggests a second source of association, arising from a direct, causal interaction between protein status and vitamin A metabolism As discussed in Chapter 8, protein plays an important role in transporting vitamin A from liver stores to target cells RBP synthesis, hence retinol transport from the liver (as holo-RBP), depends on an adequate supply of both vitamin A and protein Rats deprived of vitamin A suffer a fall in serum and liver vitamin A concentration, but a fourfold rise in liver apo-RBP [222] Administration of a large dose of vitamin A results in a rapid outpouring of holo-RBP Rats deprived of both vitamin A and protein suffer a much more muted holo-RBP response [223]

Children react in much the same way Serum retinol levels of protein deficient children are depressed, even in the presence of adequate vitamin A liver stores, and rise in response to a high protein diet[102,105,224–227] (serum retinol and RBP doubling within eight days[226]) By the same token, the rapidity and extent of the holo-RBP response following a large (oral or parenteral) dose of vitamin A is directly related to a child's baseline protein status (Table 10–3),[3,228,229] just as in the RDR assay of vitamin A reserves (Chapter 11) [37]

These biochemical differences have their clinical counterparts While most cases of xerophthalmia will respond to a massive dose of vitamin A (even in the presence of severe protein deficiency) (Chapter 10), protein status affects the speed and duration of that response Corneal xerophthalmia healed uneventfully in 74% of Indonesian children with PEM given standard, large-dose vitamin A, the response, however, was slower than that of better-nourished children [230] Fully 90% of cases that suffered clinical relapse had significant PEM (serum albumin < 3 0 g/dl or transferrin < 50 mg/dl, weight for height < 70%, or pedal edema) [3,228] The transient nature of the clinical and holo-RBP response to large-dose vitamin A therapy suggests impaired vitamin A transport and storage In rats, at least, protein deficiency can interfere with liver storage of vitamin A [231–233]

While the complexity and nuances of vitamin A and protein interaction are only beginning to be understood, even crude attempts to quantify their relationship are revealing [3] Despite the multiple factors that can transiently alter serum retinol levels (rendering it a relatively coarse index of vitamin A status), the cross correlation between serum retinol and albumin (a surrogate for protein

"status") provides a reasonably sensitive and specific biochemical index for differentiating xerophthalmia patients of varying clinical severity—an index superior to either of its components alone [3] These relationships are internally consistent For example, in non-xerophthalmic children, one expects and finds a positive correlation between serum albumin and retinol This contrasts with xerophthalmic corneal dissolution, where vitamin A metabolism has presumably collapsed In this extreme circumstance, patients whose protein status is relatively normal would need to be *severely* retinol depleted, those only modestly vitamin A deficient would need to be *severely* protein deficient As a result, the slope of serum vitamin A on albumin would be negative, which indeed can be the case [3 220] This would explain why serum carotene levels are sometimes higher in xerophthalmic children with kwashiorkor than in those without[225] with their better protein status, children without kwashiorkor must be more severely deprived of vitamin A to develop xerophthalmia Further, this observation is consistent with the frequent finding that the severest forms of xerophthalmia are commonly accompanied by protein deficiency, and that in the absence of severe (primary) vitamin A deficiency serious xerophthalmia is largely limited to severely malnourished individuals [234]

It remains uncertain whether defects in vitamin A transport secondary to protein deficiency or other factors can result in clinical xerophthalmia in the absence of at least some degree of primary vitamin A deficiency Hussey has raised this possibility to explain measles-related, vitamin A-responsive complications among children thought to have been otherwise vitamin A sufficient [99] As important as protein status may be to vitamin A metabolism, it clearly does not dominate the clinical manifestation of vitamin A status How else to describe the wide variation in the frequency and severity of ocular involvement reported in kwashiorkor,[235] or the ability of large-dose vitamin A to produce a modulated but effective therapeutic response in the majority of severely malnourished patients?

It is likely that protein deficiency acts at multiple loci of vitamin A-dependent function In addition to affecting apo-RBP production, cellular RBP may be depressed in protein deficiency [236] Protein status would also be expected to influence vitamin A regulation of gene expression—whose primary outcome, after all, is the production of highly specific proteins

Chronic protein deficiency can have a paradoxical effect on vitamin A status by inhibiting growth and reducing metabolic demands,[237] it may conserve existing vitamin A stores [224 238] Protein deficient rats utilize RBP at only half to two-thirds the normal rate [223 239] Hence, anything that increases vitamin A demands, including a protein-induced growth spurt,[240] can precipitate deficiency and its consequences among individuals with marginal vitamin A stores Gopalan[225] observed malnourished children fed protein-rich diets develop xerophthalmia Bloch[43] attributed the seasonality of xerophthalmia in Denmark to demands placed on limited vitamin A reserves by the spring growth spurt

Our understanding of the molecular mechanisms by which protein status affects vitamin A-dependent functions remains extremely limited, particularly as it relates to the myriad of subtle, non-ocular consequences of "vitamin A deficiency " In the meantime, it is clear that successful prevention and treatment of vitamin A deficiency and its consequences require careful attention to the population's protein status

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8

Biochemistry of Vitamin A and Carotenoids

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Our understanding of the biochemistry of vision developed much more rapidly than that of cellular differentiation Since the identification in 1987 of nuclear receptors for retinoic acid that induce gene expression, however, we have been rapidly gaining insight into the nature of the latter process Our past and present knowledge about vitamin A and carotenoids has been summarized elsewhere [1-3]

Nature, insofar as possible, protects us from the ill effects of both inadequate and excessive intakes of the vitamin The physiological and biochemical processes underlying this protection are described in this chapter

Chemistry and Nomenclature

Chemical Aspects

Vitamin A and more than 600 carotenoids have been crystallized and fully characterized by a variety of chemical and physical methods Furthermore, vitamin A and many of its analogs, as well as selected carotenoids, have been synthesized chemically from simple, readily available precursors Mainly because of the structure of conjugated double bonds that are characteristic of both vitamin A and carotenoids, these substances are sensitive to oxidation [23]

Vitamin A is now considered chemically as a subgroup of the retinoids, which are defined as a class of compounds consisting of four isoprenoid units joined

in a head-to-tail manner and customarily containing five conjugated double bonds [4,5] The term "vitamin A" is used as a generic descriptor for retinoids exhibiting qualitatively the biologic activity of retinol The numbering system for all-*trans* retinol is depicted in Figure 8–1 (point A) Other naturally occurring retinoids of biologic interest, shown in this figure, are B through J Retinoids K and L are synthetic compounds with high biological activity [3]

The nomenclature of carotenoids primarily is based on β-carotene or, more formally, on β,β-carotene [5,6] The formulas and numbering system for β-carotene and α-carotene are given in Figure 8–1 (points M and N) The term provitamin A carotenoids is used as a generic descriptor for all carotenoids exhibiting qualitatively the biologic activity of vitamin A [3–6]

A large number of geometric isomers both of retinol (n = 16) and of β-carotene (n = 272) can exist All sixteen of the vitamin A isomers and several of the β-carotene isomers have been synthesized Interestingly, the all-*trans* and three of the four mono-*cis* isomers of vitamin A—the 9-*cis,* 11-*cis* and 13-*cis* forms—are known to play specific physiologic roles in nature

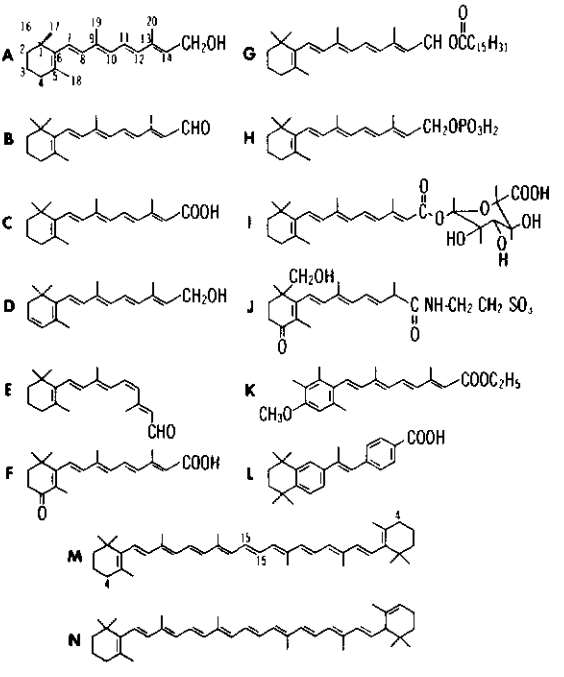

Fig. 8–1. Formulas and numbering systems for retinoids and carotenoids A, all-*trans* retinol, B, all-*trans* retinal, C, all-*trans* retinoic acid, D, 3-dehydroretinol (vitamin A_2), E, 11-*cis* retinal, F, 4-oxoretinoic acid, G, retinyl palmitate, H, retinyl phosphate, I, retinoyl β-glucuronide, J, retinotaurine, K, trimethylmethoxyphenyl analogue of ethyl retinoate, L, tetrahydro, tetramethylnaphthylisopropenylbenzoic acid, M, all-*trans* β-carotene, N, α-carotene (From J A Olson [3])

Vitamin A and carotenoids are soluble in most organic solvents, but not in water In their extraction from plasma and tissues, therefore, the cell structure must be disrupted, the proteins denatured, and the lipid fraction dissolved in some solvent, such as hexane or dichloromethane, which is immiscible with water In crystalline form or when dissolved in oil containing an antioxidant, vitamin A is stable for long periods, providing it is kept in the dark in a sealed container under a dry nitrogen or argon atmosphere Carotenoids, although less stable than retinol, are also preserved well under similar conditions [2,3]

Vitamin A and the carotenoids are sensitive to oxidation, isomerization, and polymerization when dissolved in dilute solution under light in the presence of oxygen, particularly at elevated temperatures The destruction of these compounds is particularly rapid when they are adsorbed as a thin surface film in the presence of light and oxygen [3]

Vitamin A is stable when stored in frozen liver tissue in the dark at a temperature below −20°C, and in frozen serum stored at −70°C in sealed vials under ideal conditions Carotenoids present in stored frozen serum or tissues tend to be more sensitive than vitamin A to destruction, but are stable at −70°C in the dark under argon [3]

Vitamin A and carotenoids are usually separated by high-pressure liquid chromatography and detected by UV-visible absorption spectrophotometry,[7,8] although other physical and chemical procedures for detection exist Vitamin A shows a characteristic ultraviolet (UV) absorption spectrum with an absorption maximum (λmax) of 325 nm and a molecular extinction coefficient of 53,000 cm^2M^{-1} ($E^{1\%}_{1cm}$ of 1850) in hexane

Carotenoids also show characteristic absorption spectra, β-carotene, for example, has a λmax of 450 = rm in hexane with a molecular extinction coefficient of 136,900 cm^2M^{-1} ($E^{1\%}_{1cm}$ of 2550)

Biologic Aspects

Whenever appropriate, vitamin A and individual retinoids and carotenoids are preferentially expressed in molar terms in accord with the Systeme International (SI) Thus, serum concentrations of retinol are given in micromolar terms (μmol/liter) rather than in micrograms per deciliter (μg/dl), and liver concentrations are given as micromoles per gram, not as micrograms per gram In this expression, 1 μg retinol is equal to 0 003491 μmol or, conversely, 1 μmol of retinol equals 286 46 μg of retinol [3] SI units are less applicable to food sources

In nutrition, the primary unit of biologic activity for vitamin A is 1 μg of all-*trans* retinol, whether present as the free alcohol or as one of several natural or synthetic fatty acyl esters

To express both preformed vitamin A and provitamin A carotenoids in foods as a single nutritive value, the retinol equivalent (RE) was created One μg RE is equal to 1 μg of all-*trans* retinol, to 6 μg of all-*trans* β-carotene, or to 12 μg

of other provitamin A carotenoids in foods The bioavailability of carotenoids varies greatly, however, depending on their physical state in foods Thus, carotenoids in oil are well utilized, but those in uncooked whole vegetables are poorly absorbed

A unit of historical value, which is still extensively used in food composition tables and in labeling of vitamin A supplements, is the International Unit, or IU One IU equals 0 300 μg of all-*trans* retinol, or a corresponding amount of retinol in ester linkage Thus, whether the vitamin A in a given solution is present as free retinol, retinyl acetate, or retinyl palmitate, the number of IUs will be the same [3] One IU of all-trans β-carotene was defined at 0 6 μg The nutritional equivalency of β-carotene relative to vitamin A is three times higher when this nomenclature is used than when RE is employed Thus, it is useful to distinguish IU as IU_a for vitamin A, and as IU_c for β-carotene The RE system, which is better based physiologically, is less confusing and, consequently, is preferred

The all-*trans* isomers of both vitamin A and provitamin A carotenoids are the most nutritionally active forms, *cis*-isomers usually show 50% or lower activities relative to the all-*trans* forms

Physiologic Processes

Digestion and Absorption

Preformed vitamin A and carotenoids in the diet are largely released from protein during proteolysis in the stomach Vitamin A and carotenoids tend to aggregate with lipids into globules, which then pass into the small intestine The upper intestine is the major site of lipid hydrolysis Dietary fat, protein, and their hydrolytic products stimulate, through cholecystokinin, the secretion of bile, which first emulsifies lipids and then forms micelles Bile salts also stimulate pancreative lipase, which hydrolyzes triglycerides and other esterases that hydrolyze retinyl esters and cholesteryl esters Retinyl esters are hydrolyzed primarily by an enzyme located in the brush border of intestinal mucosal cells Hydrolysis of retinyl esters greatly enhances the bioavailability of vitamin A The product, retinol, in a bile salt-containing micelle is well absorbed (70% to 90%) by mucosal cells of the small intestine Vitamin A seems to be absorbed by a carrier-mediated process at low concentrations, but mainly by diffusion from the micellar phase at high doses [3,9–11]

Hydrocarbon carotenoids are not as well absorbed as retinol, possibly because of their awkward length and their specific requirement for bile salts In the presence of fat, 30%–50% of moderate amounts (< 15 mg) of carotenoids are absorbed by humans As the amount increases however, the absorption efficiency declines Highly polar carotenoids are poorly absorbed

Plasma Transport

Chylomicra

Within intestinal cells, newly formed chylomicra contain retinyl ester, cholesteryl ester, some retinol, phospholipids, much triglyceride, and apolipoproteins A-1, A-4, B, and several others In the complex conversion of the secreted chylomicra into chylomicron remnants in the plasma, the triglyceride content is markedly reduced by the hydrolytic action of lipoprotein lipase, the predominant apolipoproteins on the chylomicron remnant become B and E, and the relative concentrations of retinyl ester increase per remnant particle [3 9–11]

Retinol-Binding Protein (RBP)

Human RBP, a single polypeptide chain with 182 amino acids in a known sequence, has a molecular weight of 21,230 The protein contains an eight-stranded anti-parallel β-barrel at its core, within which all-*trans* retinol is bound [12] Thus, little if any bound retinol is exposed at the surface of the protein The 1 1 molar complex of all-*trans* retinol and RBP is called "holo-RBP " Within the plasma, holo-RBP is found in large part as a 1 1 complex with transthyretin (prealbumin), which specifically binds one thyroxine molecule per tetramer [13]

In well-nourished adults, the total RBP concentration in plasma is 1 9 μmol/liter to 2 4 μmol/liter (40 μg/ml to 50 μg/ml), 80% to 90% of which exists as holo-RBP In children up to the age of puberty, the total RBP concentration is approximately 60% of the adult level [3 13] Protein-energy malnutrition, infections, and parasitic infestations all lower steady state concentrations of holo-RBP Thus, the vitamin A status of an individual often is not predictable on the basis of holo-RBP concentrations alone (Chapter 11)

Serum Retinoids and Carotenoids

Mean values and ranges of retinoids in serum as a function of age and sex are presented in Table 8–1 [14] These values, drawn from the first U S National Health and Nutrition Examination Survey in 1971–1974 (NHANES I), clearly show that mean plasma retinol concentrations in young children are approximately 60% of adult values until puberty, when they increase Male and female children show the same serum retinol values, whereas adult males have values 20% higher than adult females

The concentrations of specific and total carotenoids in the plasma are highly dependent on diet Of the six major carotenoids in the plasma of American residents, lycopene is the most common, followed by lutein plus zeaxanthin and then by β-carotene Of the total plasma carotenoids, the percentage of provitamin

Table 8–1 Serum Retinol Concentrations (μmol/liter) as a Function of Age and Sex in American Residents, 1971–1974[a]

Age	*Total*	*Males*	*Females*
3–5 yr	1 28 (0 73–2 0)	1 29 (0 70–2 1)	1 26 (0 77–2 0)
n	1414	725	689
6–11 yr	1 31 (0 84–1 9)	1 30 (0 84–1 9)	1 32 (0 84–1 9)
n	1857	930	927
12–17 yr	1 58 (1 0–2 3)	1 62 (1 1–2 3)	1 53 (1 0–2 2)
n	2035	1026	1009
18–44 yr	1 94 (1 2–2 9)	2 08 (1 4–3 0)	1 80 (1 1–2 8)
n	7035	2164	4871
45–74 yr	2 20 (1 3–3 3)	2 29 (1 4–3 5)	2 11 (1 3–3 2)
n	6111	2911	3200

[a]The 5th and 95th percentile values are given in parentheses

Derived from S M Pilch [4]

A carotenoids in adults is usually 40%–50% The plasma carotenoid patterns in adults have been fairly well studied,[15,16] but as yet few studies have been conducted in children [17,18] Serum carotenoid patterns from these studies are summarized in Table 8–2 In malnourished children in Senegal, serum concentrations of both retinol and total carotenoids were approximately half those in well-nourished American children,[17,18] although provitamin A carotenoid concentrations were similar (Table 8–2)

In addition to retinol and the cited carotenoids, lower steady-state concentrations (< 0 1 μmol/liter) of retinyl esters, retinoic acid, retinyl β-glucuronide, retinoyl β-glucuronide, and at least twelve other carotenoids have been identified in fasting plasma [19–21] Besides retinoic acid, some of these minor components may also play significant roles in nutrition and function

Tissue Uptake and Storage

Uptake of Chylomicron Remnants

By interaction with cell surface receptors on liver parenchymal cells for apolipoprotein E, and possibly for apolipoprotein B, chylomicron remnants are internalized by receptor-mediated endocytosis Retinyl esters are hydrolyzed, combined with cellular retinol-binding protein (CRBP) in the cytosol of the hepatocyte, and then subjected to several possible metabolic routes [3,9,11] Lipid-rich chylomicra are cleared more slowly from plasma than are lipid-poor chylomicra [11] Chylomicron remnants are also taken up well, presumably by a similar mechanism, by bone marrow, and to a lesser degree by other peripheral tissues

Table 8-2 Mean Serum Carotenoid Values (μmol/liter) as a Function of Nutritional Status and Age[a]

				Nutritional			*Carotenoids*[b]					
Years	*Place*	*Sex*	*Number*	*State*	*ROL*[b]	*Total*[c]	*β-Car*	*α-Car*	*β-Cryptox*	*Lut*[d]	*Lyc*	*Ref*
2–4	Senegal	MF	271	Poor	0 61 ±0 22	0 74 ±0 61	0 16 ±0 14	0 03 ±0 06	0 02 ±0 02	0 46 ±0 28	0 07 ±0 11	17
2–14	Michigan	MF	10	Good	1 42 ±0 45	1 41	0 20 ±0 12	0 03 ±0 02	—	0 38 ±0 20	0 80 ±0 39	18
18–45+	Boston, MA	M	137	Good	2 2 (1 3–3 3)	1 05	0 34 (0 13–1 1)	0 08 (0 01–0 20)	—	—	0 63 (0 23–1 2)	15
18–45+	Boston, MA	F	193	Good	1 85 (1 1–2 8)	1 39	0 59 (0 11–1 4)	0 14 (0 02–0 40)	—	—	0 66 (0 23–1 2)	15
59±10	Washington, DC	M	55	Good	2 6 ±0 64	1 22	0 31 ±0 20	0 05 ±0 04	0 15 ±0 10	0 32 ±0 15	0 39 ±0 23	16
59±7	Washington, DC	F	55	Good	2 3 ±0 66	1 39	0 44 ±0 27	0 07 ±0 04	0 18 ±0 10	0 35 ±0 16	0 35 ±0 04	16

[a]Under the mean values, values in parentheses are 5th and 95th percentile values whereas ± values are standard deviations

[b]Abbreviations ROL retinol, β Car, β-carotene α-Car, α-carotene, β-Cryptox, β-cryptoxanthin Lut Lutein, Lyc Lycopene

[c]Total carotenoids are estimated as the sum of measured components

[d]Lutein values include a minor portion (ca 20%) of zeaxanthin

Uptake from Holo-RBP

Holo-RBP, derived largely from the liver but also from other tissues, is taken up from plasma by all tissues of the body Two mechanisms have been postulated (1) interaction of holo-RBP with a specific cell-surface receptor, followed by internalization of the complex[22], and (2) dissociation of holo-RBP in the plasma into apo-RBP and retinol, followed by incorporation of retinol into the plasma membrane [11 23] Thereafter, CRBP may draw retinol out of the membrane into the cytosol [11 23]

Although the retinal pigment epithelial cells apparently possess cell-surface receptors for holo-RBP on their external surfaces,[22] their presence on other cells is much less clear [11 23] Whatever the mechanism of uptake, however, retinol and its esters are found in significant amounts in adipose tissue, kidney, testis, lung, bone marrow, and the eye, in addition to the liver, and in smaller amounts in other tissues

Storage

Retinyl esters in chylomicron remnants are hydrolyzed within hepatocytes to retinol, which then can be esterified with long-chain fatty acid esters and stored in specialized lipid globules

Alternatively, retinol may be transferred to stellate cells, also termed lipocytes, Ito cells, and fat-storing cells, where retinol is also esterified and stored in vitamin A-containing globules Under normal physiologic conditions, stellate cells contain 80% to 90% of the stored vitamin A, hepatocytes 10% to 20%, and other liver cells only a few percent The retinyl ester stored in stellate cells and hepatocytes can be readily and completely mobilized and used by the organism [3 9 11]

The major ester of stored retinyl esters is the palmitate, with smaller amounts of the stearate, linoleate, oleate, and others The major synthetic route is by transacylation from the α-position of phospholipids, although direct acylation via coenzyme A derivatives can also occur [11]

Although the liver clearly is the major storage site, most other tissues also possess stellate cells and store retinyl esters Retinol may be transferred from parenchymal cells to stellate cells as intact holo-RBP,[24] as free retinol,[11] or by yet unidentified carriers or processes [11]

Release from Tissues

Within the hepatocyte, a precursor of RBP—preapo-RBP—is first formed It is then proteolytically cleaved, with the loss of a peptide, to apo-RBP All-*trans* retinol combines with apo-RBP in a specific 1 1 molecular complex to form holo-

RBP The latter is transported through the Golgi apparatus and then is secreted into the plasma, probably as a complex with transthyretin [3,11]

Retinol might be released into plasma from liver stellate cells by three routes (1) by its transfer back to parenchymal cells, followed by holo-RBP release, (2) by its direct release into the plasma as an endogenously formed RBP complex, or (3) by its transfer to extracellular apo-RBP at the cell membrane Whether all of these routes are active and, if so, which route predominates under given physiological conditions is uncertain [3,11,24]

Although vitamin A is primarily stored in the liver, all tissues contain some vitamin A Because messenger ribonucleic acid (MRNA) for RBP has been identified in the kidney, lacrimal gland, adipose tissue, and bone marrow as well as in the liver, nonhepatic tissues may well synthesize and secrete RBP RBP in plasma from hepatic and extrahepatic tissues, however, seems to be identical Thus, it has not yet been possible to ascertain the relative amount derived from each tissue

Recycling and Excretion

Retinol released as holo-RBP from the liver is taken up by peripheral tissues Much of this retinol is later released from peripheral tissues as holo-RBP, as lipoprotein-bound retinyl esters, or as water-soluble retinyl β-glucuronides All of these forms are then transported back to the liver This recycling is extensive and efficient, as shown by in vivo kinetic analysis [24,25] When vitamin A intakes are very low, the efficiency of recycling increases [24,25]

The glucuronides of vitamin A, which are secreted in the bile, are also reabsorbed from the intestinal lumen and transported back to the liver [3,9,11] This enterohepatic circulation also helps to maintain the vitamin A status of an individual during periods of low intakes

Approximately 5% to 20% of ingested vitamin A and a larger percentage of carotenoids, depending on their nature, bioavailability, and amount, are not absorbed from the intestinal tract and consequently are excreted in the feces A significant portion (10% to 40%) of absorbed vitamin A is oxidized or conjugated in the liver and then is secreted into the bile Although, as already mentioned, some of these biliary metabolites, such as retinoyl β-glucuronide, are reabsorbed in the intestine and transported back to the liver, most of the biliary metabolites are excreted in the feces

Vitamin A that is oxidized and chain-shortened in various tissues ultimately is excreted in the urine Finally, carbon dioxide that is released by the oxidation and cleavage of the side chain of vitamin A is excreted in the expired air In quantitative terms, an average of 10% of dietary vitamin A is not absorbed, 20% appears in the feces through the bile, 17% is excreted in the urine, 3% is released as CO_2, and 50% is stored, primarily in the liver [3,11]

Metabolic Processes

Binding Proteins

In addition to plasma RBP, a set of specific binding proteins for retinol, retinal, and retinoic acid has been identified within cells, as well as in the intercellular matrix between the retinal pigment epithelium and the rod outer segments [26] Major specific binding proteins of mammals are listed in Table 8–3

Other retinoid-binding proteins that are at least partly characterized have been identified in the epididymis, uterus, fetal liver, and Sertoli cells Some other proteins, such as several fatty acid-binding proteins, serum albumin, and β-lactoglobulin also bind retinoids and, in particular, retinoic acid Several of these proteins are closely related structurally [27]

Thus, the retinoids clearly are chaperoned by a set of highly specific proteins in vivo and can bind as well to other structurally related proteins Specific binding proteins seem to function as transport agents, as sequestering entities, as coligands in enzymatic transformations, and as coligands in genetic expression [3 13] The physiologic role played by nonspecific binding proteins is still unclear

Vitamin A

The major reactions of vitamin A metabolism are esterification, oxidation at C-15, oxidation at C-4, conjugation, isomerization, other miscellaneous oxidative reactions, and chain cleavage Retinol and retinal, as well as other metabolites reversibly converted to them, all possess significant biologic activity Retinoic acid and its glucuronide are active in growth but not in vision or, in most species, in reproduction Except for 14-hydroxy-retro retinol, more oxidized products—such as 4-hydroxyretinoic acid, 5,6-epoxyretinoic acid, and C-19 metabolites—are largely devoid of biologic activity

Retinoyl β-glucuronide, retinyl β-glucuronide, and retinoic acid, as already mentioned, are normally present in small amounts (3 nmol/liter to 11 nmol/liter, or 1 μg/liter to 5 μg/liter) in human plasma Retinoyl β-glucuronide is not hydrolyzed in some cells and only slowly in vivo Retinoic acid, besides being physically bound by serum albumin and by cytosolic and nuclear binding proteins, can also be covalently bound to proteins, possibly by means of a coenzyme A intermediate The cellular retinoid-binding proteins play a major role in the oxidation/reduction and transesterification of retinol Intestinal CRBP-II, CRBP, and CRABP have been particularly well studied in this regard [3 11 28,29]

Stored retinyl esters are hydrolyzed to free retinol by a group of intracellular retinyl ester hydrolases [11] Some of these hydrolases depend on the presence of bile salts or other detergents, and some do not Apo-CRBP stimulates hydrolase activity, presumably by serving as an acceptor of released retinol [11]

Table 8–3 Major Retinoid-Binding Proteins

Name	*Abbreviation*	*Location*	*Molecular Weight (KDa)*	*Major Ligands*
Retinol-binding protein	RBP	Plasma	21 2	all-*trans* retinol
Interphotoreceptor retinol-binding protein	IRBP	Extracellular matrix of the eye	135	11-*cis* & all-*trans* retinol
Cellular retinol-binding protein, type I	CRBP-I	Cytosol	15 7	all-*trans* retinol all-*trans* retinal
Cellular retinol-binding protein, type II	CRBP-II	Cytosol small intestine	15 6	all-*trans* retinol all-*trans* retinal
Cellular retinoic acid binding-protein, type I	CRABP-I	Cytosol	15 5	all-*trans* retinoic acid
Cellular retinoic acid binding-protein, type II	CRABP-II	Cytosol fetal tissues	15 0	all-*trans* retinoic acid
Cellular retinaldehyde-binding protein	CRALBP	Cytosol eye	36 0	11-*cis* retinol, 11-*cis* retinal
Retinoic acid receptor-α	RAR_α	Nucleus	50	all-*trans* retinoic acid
Retinoic acid receptor-β	RAR_β	Nucleus	50	all-*trans* retinoic acid
Retinoic acid receptor-γ	RAR_γ	Nucleus mainly skin	50	all-*trans* retinoic acid
Retinoid X receptor-α	RXR_α	Nucleus	51	9-*cis* retinoic acid
Retinoid X receptor-β	RXR_β	Nucleus	51	9-*cis* retinoic acid
Retinoid X receptor-γ	RXR_γ	Nucleus	51	9-*cis* retinoic acid

Carotenoids

Most provitamin A carotenoids can be cleaved by a carotenoid 15,15′-dioxygenase in the cytosol of the intestinal mucosa, of hepatocytes, and of some other tissue cells β-carotene yields two molecules of retinal, which are in large part reduced and esterified to retinyl ester The cleavage enzyme requires molecular oxygen and apparently contains a metal, possibly iron, at its catalytic site β-carotene and some other carotenoids can also be cleaved asymmetrically to yield β-apocarotenals that, in turn, are converted to retinal, or possibly directly to retinoic acid At the level of retinal, therefore, that is reversibly reduced to retinol by alcohol dehydrogenases in many tissues, the metabolism of carotenoids and that of preformed vitamin A usually coincide β-carotene can also be oxidized to biologically inactive products by lipoxygenase and other oxidative enzymes Much less is known about the metabolism and excretion of carotenoids other

than carotene Each carotenoid, however, seems to show a specific pattern of absorption, metabolism, and transport [30]

The overall metabolism of carotenoids has recently been reviewed [31,32]

Functions of Vitamin A and Carotenoids

Vitamin A

Vitamin A is important for vision, cellular differentiation, morphogenesis, and transmembrane transport (in bacteria) Many other complex physiologic processes in animals, such as growth, reproduction, and the immune response (Chapter 9), seem to be affected by these central functions

Vision

The role of vitamin A in vision is well defined (Chapter 4) [33] In the outer segment of rod cells in the retina, 11-*cis* retinal forms a protonated Schiff base with a specific lysine residue of the membrane-bound protein, opsin, to yield rhodopsin, with an absorption maximum of 498 nm Similar complexes exist in human cone cells to give three specific iodopsins that absorb maximally at 420 nm (blue cones), 534 nm (green cones), and 563 nm (red cones)

When a photon of light strikes the dark-adapted retina, the 11-*cis* bond of retinal in rhodopsin is isomerized to the all-*trans* form This isomerization destabilizes rhodopsin, which passes through a series of different conformational states The light-activated transformation of rhodopsin ultimately results in a reduction in the sodium ion current into the rod outer segment, which induces hyperpolarization of the membrane In the probable sequence of steps in this amplification cascade, light converts rhodopsin to the active intermediate, metarhodopsin II The latter induces the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on a disk protein termed "transducin " Transducin contains three subunits, and the complex of GTP with transducin activates phosphodiesterase, which in turn hydrolyzes cyclic guanosine monophosphate (cGMP) to GMP As the concentration of cGMP falls, the sodium channel closes, leading to membrane hyperpolarization [33]

The cascade is turned off by the time-dependent decay of metarhodopsin II to opsin and all-*trans* retinal, by the conversion of metarhodopsin II to an inactive phosphorylated form, and by the hydrolysis of bound GTP by the inherent GTPase activity of transducin

A number of proteins are involved in this complex cascade besides those already cited rod cell spectrin, the 63-KDa protein and the Na+-Ca++ 220 KDa exchange protein, the Na+ channel, peripherin and the rim protein of the

disc membrane, recovering, phosducin, arrestin, and many others [33] Specific roles in the cascade have been defined for many, but not for all, of the twenty or more characterized protein components involved in the visual cycle [33]

All-*trans* retinal may be isomerized back to the 11-*cis* form in the rod outer segment by light in the presence of certain phospholipids In the dark, however, 11 *cis*-retinol is formed by the action of all-*trans* 11-*cis* retinyl ester isomerase, a membrane-bound enzyme in retinal pigment epithelial cells [34] This enzyme might better be called an isomerohydrolase, in that the energy released in the hydrolysis of the retinyl ester bond is directly coupled to the formation of the energy-rich 11-*cis* retinol The 11-*cis* forms of retinol and retinal are then transported on IRBP to the rod outer segment, whereas all-*trans* retinol is shuttled back on IRBP [3 35]

Both all-*trans* and 11-*cis* retinyl esters are also stored in the retinyl pigment epithelial cells, often in lipid-rich globules Retinyl esters are formed in the eye by a trans-acylation reaction from phospholipids, whereas retinyl ester hydrolases, both in the eye and elsewhere, act preferentially on the cis-isomers [35]

Cellular Differentiation

In vitamin A deficiency, mucus-secreting cells are replaced by keratin-producing cells in many tissues of the body Conversely, the addition of vitamin A to vitamin A-deficient keratinizing cells in tissue culture induces a shift to mucus-producing cells Retinoids also rapidly induce F-9 teratocarcinoma cells, as well as many other cell lines, to differentiate In this process, a number of new proteins appear in the newly differentiated cells Thus, vitamin A and its analogues, both in vivo and in vitro, markedly influence the way in which cells differentiate [3 36]

The mechanism by which retinoids induce cellular differentiation is becoming clear (Fig 8–2) Within tissue cells, all-*trans* retinol, in association with CRBP, can be oxidized to all-*trans* retinoic acid and presumably can also be isomerized to 9-*cis* retinol, which in turn can be oxidized to 9-*cis* retinoic acid Another likely route for the synthesis of 9-*cis* retinal is by oxidative cleavage of 9-*cis* β-carotene All-*trans* or 9-*cis* retinoic acid is transported on CRABP or on other retinoid-binding proteins to the nucleus, where it is tightly bound to one or more of the three (α, β, γ) retinoic acid receptors (RAR) or to one or more of the three (α, β, γ) retinoid X receptors (RXR) respectively [37 38] In the activation of retinoic acid-responsive genes, a heterodimer of RAR and RXR, or in some cases a homodimer of RXR, binds to the response element of the gene to initiate transcription RXR also serves as a coregulator for the expression of genes responsive to triiodothyronine, to calcitriol, and perhaps to other hormones, but not to estrogen [3,37 38] In this latter role, RXR forms a heterodimer with the appropriate nuclear receptor for other hormones, thereby enhancing its affinity for the response element of the gene The quantitative balance among RAR, RXR, and other nuclear proteins may also contribute to gene regulation in vivo

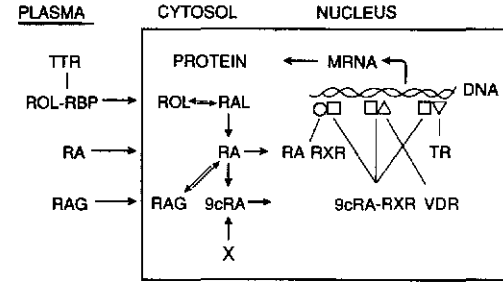

Fig. 8–2. Roles of retinoids in the differentiation of target cells All retinoids are considered to be all-*trans* isomers unless otherwise specified Abbreviations are ROL, retinol, RAL, retinal, RA, retinoic acid, 9cRA, 9-*cis* retinoic acid, RAG, retinoyl β-glucuronide, RBP plasma retinol-binding protein, TTR, transthyretin, RAR, retinoic acid receptor, RXR, retinoic acid receptor that specifically binds 9cRA, VDR, vitamin D receptor, TR, thyroxine receptor, DNA, deoxynucleic acid, mRNA, messenger ribonucleic acid, X, unknown precursors (possibly carotenoids) of 9cRA, ○ □, □△, and □▽, heterodimers of various nuclear receptors

The RAR and RXR receptors, like other nuclear hormone receptors, possess six protein domains with specific functions At the N-terminal end, domains A and B serve as physiologic activators of the receptor, domain C, which is highly conserved, contains zinc-sulfhydryl interactions ("zinc fingers") that bind to DNA, domain D is a hinge region that provides the necessary conformation of the receptor, domain E binds the ligand, and domain F, at the C-terminal end, enhances dimerization All nuclear retinoid receptors contain approximately 460 amino acids and have molecular weights of approximately 50 kDa [3,37,38]

Retinoic acid directly activates the genes for CRBP-I, CRBP-II, RARβ, Hox-1 6, laminin B1, and transglutaminase in diverse types of cells The sequence by which other genes are subsequently activated is not clear Various isoforms of transforming growth factor β (TGFβ) can be induced or suppressed by retinoic acid, depending on conditions Although attention has centered on the activation of gene expression, retinoids can also suppress transcription [3,37,38]

Some retinoids may stimulate differentiation by a different pathway, for example, retinoyl β-glucuronide does not bind to CRBP, CRABP, or nuclear RAR, but nonetheless is highly active biologically [39,40] Similarly, B lymphocytes differentiate in response to 14-hydroxy-retro-retinol, but not to all-*trans* retinoic acid [41] Retinoids also show some physiologic effects in enucleated cells Thus, probably by several mechanisms, retinoids seem to play a central role in the development and maintenance of many tissues [3,37,38]

Morphogenesis

Both a deficiency and an excess of vitamin A and of most other retinoids adversely affect embryogenesis [42,43] In a more physiologic context, pattern formation in the

skin might well be affected by gradients of vitamin A [44] The hypothesis that all-*trans* retinoic acid might well be one of a presumed host of morphogens that control embryologic development originated with the demonstration that an implant containing all-*trans* retinoic acid, when placed in the anterior part of the developing chick limb bud, mimics the activity of the naturally occurring zone of polarizing activity (ZPA) [42 45]

There are two major hypotheses to explain the dramatic effects of retinoic acid and of many other retinoids on the embryo (1) that a morphogenic gradient of retinoic acid exists in the developing limb, which in turn provides signals to cells at a given position in the embryo, thereby inducing them to differentiate in a specific way or to migrate in a given direction, and (2) that retinoic acid induces the differentiation of a specific group of cells the ZPA, which in turn provides as yet unidentified signals to nearby cells, thereby causing them to act in specific ways Although evidence supports both hypotheses, the second suggestion seems to be the most viable.[46]

Both cytosolic retinoid-binding proteins and nuclear retinoic acid receptors appear in different groups of cells at different times in development Retinoic acid also induces the formation of many Hox genes, which have been closely linked to developmental processes [36 42] Further, retinoic acid induces programmed cell death, or apoptosis, in the developing embryo Apoptosis is a normal process in development, but can also cause terata if not regulated [36 42 43] Thus, clarification of the mechanisms of action of retinoids in development, whatever they might be, will enrich our understanding of these complex but important processes

Other Functions

The Immune Response Vitamin A has long been termed the "anti-infective" vitamin based on the increased number of infections noted in vitamin A-deficient animals and humans [1] In vitamin A deficiency, both specific and nonspecific protective mechanisms are impaired the humoral response to bacterial, parasitic, and viral infections, cell-mediated immunity, mucosal immunity, natural killer cell activity, and phagocytosis In addition to vitamin A, both nutritionally active (β—carotene) and nutritionally inactive (canthaxanthin) carotenoids enhance the immune response in animals by as yet undefined mechanisms The roles of vitamin A in these various processes have been recently reviewed [47 48] This important topic is considered in detail in Chapter 9

Intercellular Communication Both retinoids and, at much higher concentrations, carotenoids enhance communication between cells by inducing the formation of connexin 43, a gap-junctional protein [49] These interesting observations may be relevant both to cellular patterns in tissues and to the suppression of neoplastic growth

Transmembrane Transport Bacteriorhodopsin, a light-sensitive, retinal-containing protein similar to rhodopsin, is found in the purple patches on membranes of Halobacterium halobium [50] In response to light, this protein also undergoes a series of conformational changes ultimately linked to the transfer of a proton from the cytosol to the external medium [50] During this cycle, however, the 13-*cis* and all-*trans* isomers of retinal are involved, rather than the 11-*cis* and all-*trans* forms as in the vertebrate visual cycle Several related light-sensitive retinal-binding proteins with different functions have been identified in this and other like organisms

Whether retinoids play somewhat similar roles in animal cells is not clear.

Complex Tissue Involvements

Vitamin A is essential, either directly or indirectly, for the proper functioning of most organs of the body For example, reproductive processes in both males and females and bone development and maintenance are particularly dependent on adequate vitamin A status Whether these complex physiologic processes have unique needs for vitamin A or rely primarily on the action of vitamin A in cellular differentiation is not clear Because vitamin A influences the synthesis and secretion of a variety of cytokines and growth factors, some of its effects may well be induced via the action of such factors on cells [51]

Carotenoids

Carotenoids are necessary for many biological processes, show interesting, usually beneficial effects against abnormal conditions and diseases, and have been associated as possible protective factors against several chronic diseases Their manifold activities can be classified as functions, actions, or associations [52 53]

Functions

Carotenoids function in many ways in nature as accessory pigments in photosynthesis, as protectants against light-induced photo-oxidation of chlorophyll and of other readily peroxidized molecules in plants and bacteria, and as protective coloration in birds, insects, and other species The only well-established function of carotenoids elucidated in humans to date is the formation of vitamin A Only 50 of the approximately 600 characterized carotenoids in nature, however, serve as provitamin A molecules The carotenoid-dependent coloration of many fruits, vegetables, and flowers, which both please the senses and stimulate appetite, might also be considered as a human function, but largely on aesthetic rather than on technical grounds

Actions

Chemical Carotenoids, because of their physical properties, show both chemical and biological actions that are not shown by vitamin A Thus, carotenoids are very effective in quenching singlet oxygen and in serving as antioxidants [54] Carotenoids can quench singlet oxygen without being modified chemically, can consume free radicals and hence serve as antioxidants, or can be autoxidized without a reduction in free-radical concentrations All three of these reactions occur biologically The products of both antioxidant and autoxidant reactions are a set of epoxides, aldehydes and ketones, often produced by carbon-carbon bond cleavage [54–56] Carotenoids are much more effective than vitamin A in these reactions because they possess a much longer conjugated double bond system, e g , eleven in β-carotene versus five in retinol

β-Carotene, and presumably other carotenoids, can also interact with other antioxidants, such as α-tocopherol and ascorbic acid Therefore, β-carotene may serve as part of an antioxidant network within cells [57]

Biological Carotenoids show a variety of protective effects in experimental systems, namely a reduction in free-radicals, in photoinduced neoplasm, in mutagenesis, in sister chromatid exchange, and in cell transformation [58–60] In humans, treatment with β-carotene reduced leukoplakia, the number of micronuclei in the buccal mucosa of betel-nut chewers, and photo-induced skin disorders in inherited light-sensitivity diseases [61 62] β-carotene supplements can also enhance various facets of the immune response in the elderly and can block the immunosuppressive actions of ultraviolet light and of HIV infection in human subjects [61] Thus, carotenoids seem to show moderately beneficial effects in humans with various types of diseases and stresses

Associations In epidemiologic studies, carotenoid ingestion has been associated with protective effects against several types of cancer, and particularly against lung cancer, as well as against senile cataract [61 63] In an intervention trial, the incidence of cardiovascular incidents in a small, high-risk group of physicians treated with β-carotene was reported to be lower than in a similar placebo group [61]

One must bear in mind, however, that these results are preliminary and often depend on the ingestion of pharmacologic rather than physiologic amounts, in quantities that could not be readily approached from dietary sources Potential benefits observed among individuals consuming larger dietary intakes may be related to other differences in their diet, such as being lower in fat than diets predominantly containing animal foods, or other physical activity, and smoking and drinking behavior These potential beneficial effects of carotenoids seem unrelated to their provitamin A activities

Recommended Intakes

In defining dietary requirements for vitamin A, four options are available (A) an average intake that satisfies the needs of 50% of subjects, (B) an intake that satisfies the needs of nearly all subjects, (C) an intake that provides suitable reserves for 50% of subjects, and (D) an intake that provides suitable reserves for nearly all subjects

A reserve of 0 07 μmol/g wet weight of liver, or approximately 160 μmoles for a 70-kg person, serves as the basis for establishing the intake needed under option D Experimentally, the intake of vitamin A needed to satisfy option C is approximately twice that needed for option A Because the coefficient of variation for plasma levels of vitamin A in humans and for dietary requirements in animals is approximately 20%, two standard deviations are usually used to correct option A to option B and option C to option D Thus, the relative intakes needed to satisfy options A, B, C, and D are in the ratio 1 1 4 2 2 8, respectively [64] The recommended nutrient intakes adopted by the FAO/WHO,[65] proposed for the European Community[66] and approved by the National Academy of Sciences in the United States,[67] are given in Table 8–4

The Food and Agriculture Organization and the World Health Organization have selected options B and D in a two-tier system, termed "basal and safe levels" of vitamin A requirements [65] Similarly, the Nordic Committee on Food defined a two-tier system for micronutrients, called "lower limits" and "recommended intakes "[68] Other countries, including the United States, have selected single values for a given age and sex, based mainly on option D

Table 8–4 Recommended and Proposed Dietary Intakes of Vitamin A in μg Equivalents

				European Community[66]			
	Age	*FAO/WHO*[65]		*Lowest*	*Average*	*Population*	*USA*[67]
Category	*(years)*	*Basal*	*Safe*	*Threshold*	*Requirement*	*Reference*	*RDA*[a]
Infants	0–0 5	180	350			—	375
	0 5–1 0	180	350			350	375
Children	1 0–10	200–250	400			400–500	400–700
Males	10–14/15	300–350	500–600			600	1000
	14/15–70+	300–400	600	300	500	700	1000
Females	10–14/15	270–330	500			600	800
	14/15–70+	270	500	250	400	600	800
Pregnancy		+100	+100			+100	+0
Lactation	0–0 5	+180	+350			+350	+500
	> 0 5	+180	+350			+350	+400

[a]Recommended dietary allowance

The proposed values for the European Community are a three-tier system based on statistical considerations The population reference intake (PRI) is similar in nature to the recommended nutrient intakes (RNI, RDA) established in many countries, i e , option D The average requirement (AR) in all likelihood is option C, and the lowest threshold intake (LTI), which is defined as an intake below which nearly all individuals will be unable to maintain metabolic integrity according to the criterion chosen, is closest to option A In any case, values for AR and PRI for European adults, if adjusted for higher reference body weights than those used by FAO/WHO, are both very similar to those adopted by the FAO/WHO[65] and nearly identical to the calculated average requirements and RNI values for vitamin A suggested for use in the United States [69] Both of the latter recommendations, by the way, are based on an adequate total body reserve [65 69]

For infants and children, the selected "safe" (PRI, RNI, RDA) intake is essentially the same in all recommendations 350 μg–375 μg retinol equivalents This recommendation is based, however, on the mean daily volume and the concentration of vitamin A present in the breast milk of well-nourished mothers (0 5 μg retinol equivalents/ml x 700–750 ml milk/day) rather than on a scientific assessment of requirement In this regard, breast-fed Indian infants grow normally on approximately 100 μg retinol equivalents, largely as vitamin A, although they probably possess little or no reserves [65] Human milk also contains carotenoids (0 2 μg/ml–0 3 μg/ml), of which 20%–30% are vitamin A precursors [1] Because the transfer of vitamin A to the fetus is regulated by the placenta, the vitamin A reserves in newborn infants, even from well-nourished mothers, are low Thus, breast milk plays a crucial role in providing sufficient vitamin A for growth, vision, and cell differentiation

The bioavailability of carotenoids can vary greatly, as already mentioned, being highest when given in an oily or detergent solution and lowest when provided in uncooked vegetables [63] The difficulty in correctly assessing the vitamin A value of ingested carotenoids has been thoughtfully discussed [70] In part because of the difficulties involved in accurately assessing both the dietary intake of carotenoids and their utilization, a variety of other assessment indicators has been developed [71 72] The assessment of vitamin A status is specifically considered elsewhere in this volume

Biochemical Consequences of Inadequate Intakes

Vitamin A deficiency ultimately affects most tissues of the body [73] In humans, the eye is affected in two major ways (1) a reduction in the rhodopsin concentration in the retina, which is noted clinically by abnormal dark adaptation (night-blindness), and (2) keratinization of the epithelial layers of the conjunctiva and

cornea, which in severe cases can lead to rupture of the cornea and loss of sight The pathological consequences of these changes, and the specific signs associated with them, are discussed in Chapter 4

Physiologic Dynamics

Vitamin A deficiency does not affect to any major extent the absorption of the vitamin from the intestinal tract The cleavage of provitamin A to vitamin A in the gut, however, may be depressed by accompanying protein-calorie malnutrition In the presence of some dietary fat vitamin A and carotenoids are transported more or less normally in the form of chylomicra in the plasma On the other hand, the efficiency of storage of vitamin A in the liver is markedly depressed when initial liver stores are very low This reduced storage may be due in part to a lack of uptake of vitamin A by hepatocytes as well as to its reduced transfer from hepatocytes to liver stellate cells Humans that suffer from vitamin A deficiency may well show similar defects in the storage of vitamin A For example, a few vitamin A-depleted, malnourished Indonesian children who received 200,000 IU of vitamin A as a single dose developed signs of vitamin A deficiency within a two- to three-month period [73] Thus, by apparently activating the liver storage mechanism by an initial low oral dose of vitamin A, a subsequent large oral dose was better utilized by preschool Indonesian children than was a single large dose alone [74]

During the initial stages of depletion of vitamin A reserves, the vitamin A content of the liver falls, vitamin A concentrations in the plasma and retina remain at normal levels, and in some tissues like the kidney, the concentrations actually increase The excretion of metabolites of vitamin A is reduced, and recycling mechanisms become more efficient Moreover, the turnover rate of plasma retinol becomes slower That vitamin A deficiency is very difficult to induce in adult human volunteers with initially adequate total body reserves attests to the effectiveness of these conservation mechanisms

As depletion becomes more severe, however, homeostatic mechanisms no longer can cope with the situation Plasma retinol values fall, although plasma concentrations of RBP, increasingly in the apo form, are less affected The vitamin A concentration in the saliva also decreases Apo-RBP, however, builds up in parenchymal cells of the liver

Retina

The rods and cones of the retina tenaciously maintain their vitamin A levels until the body is fairly well depleted Nonetheless, an early sign of vitamin A deficiency in both humans and experimental animals is nightblindness, i e , impaired dark adaptation The sensitivity of dark adaptation is directly related to the amount of rhodopsin present in the eye Consequently, as the vitamin A in

the eye falls, the total amount of rhodopsin present also decreases, and then dark adaptation becomes abnormal This defect in rhodopsin formation is further exacerbated by protein-calorie malnutrition and by zinc deficiency In protein-calorie malnutrition, the pigment epithelial cells show abnormalities, and in zinc deficiency, the concentration of opsin seems to be depressed Thus, multiple nutritional deficiencies, which are commonly found in human populations suffering from vitamin A deficiency in animals, enhance these abnormalities in dark adaptation In prolonged, severe vitamin A deficiency, the outer segments of the rod irreversibly deteriorate and blindness results [73]

Cornea and Conjunctiva

Since the cornea and conjunctiva are avascular, the manner in which vitamin A is transferred to corneal cells has aroused considerable interest Possible routes of transfer are (1) the migration of holo-RBP from blood capillaries in the limbus region, (2) the direct uptake of vitamin A from the tear fluid, and (3) the transfer of vitamin A from the aqueous humor

Holo-RBP has been identified in the cornea at about 2% of its concentration in the plasma, and a gradient in holo-RBP concentration from the limbus to the center of the cornea exists Retinol is present in human tears at a concentration of 0 1 μmol/liter, approximately 5% of that in plasma The concentration of retinol in the aqueous humor, however, is barely detectable Thus, diffusion from the limbus and the tear fluid seem to be the major sources of vitamin A for the cornea

In vitamin A deficiency, plasma values of vitamin A are very low, and vitamin A disappears from the tear fluid As a result, the amount of vitamin A delivered to the cornea markedly falls, which gives rise to the histological changes described elsewhere (Chapter 4)

Skin

Vitamin A is essential for the normal differentiation and maintenance of the skin Holo-RBP diffuses into the dermis and then into the epidermis from capillaries in the skin [75] The concentration of RBP in the intercellular fluid of the skin is one-third to one-half that in the serum, whereas the concentration of retinol is somewhat lower Vitamin A and carotenoids are found in higher concentration in the subcutis than in the plasma, although significant amounts are present in the epidermis and dermis as well Of particular interest is the presence in the epidermis of dehydroretinol, an analog not found in the serum Upon entering cells of the skin, retinol presumably is bound by CRBP, and after its oxidation to retinoic acid, by CRABP CRABP has also been identified in the cytosol of the sebaceous follicle [73,75]

Vitamin A has a marked effect on the terminal differentiation of human keratinocytes Under normal conditions, human keratinocytes synthesize keratins with molecular weights of 40,000 and 52,000, as well as many others [76] When vitamin A is absent, these "small" keratins are replaced by larger keratins (molecular weight ≥ 67,000) characteristic of the stratum corneum Retinoids stimulate basal cell proliferation but inhibit the transcription of several epidermal keratins, probably by interacting with RARα or RARγ in the skin [76,77] TGFα and TGFβ also influence skin development, but in different ways Hair follicles, which are particularly sensitive to vitamin A, become obstructed and enlarged in the deficient condition and are replaced by mucus-secreting glands in vitamin A excess In all likelihood, vitamin A deficiency sets in motion a large number of changes in skin structure and metabolism that ultimately lead to the observed pathologic signs [3,75-77]

Because vitamin A is absorbed quite well through the skin, local application yields improvement of specific skin lesions Of course, general improvement in the vitamin A status of an individual also ameliorates skin abnormalities

Other Epithelial Tissues

The trachea, salivary gland, and vaginal epithelium, as well as many other epithelial tissues, are adversely affected by vitamin A deficiency In general, mucus-secreting cells tend to be replaced by squamous and keratinized epithelium This dramatic shift from mucus-secreting to keratinized cells in the trachea and in the vagina has been used as a quantitative biological assay for the effectiveness of retinoids in animals Changes that occur in the tracheal epithelium as a result of vitamin A deficiency have been carefully documented by electron microscopy.[78]

Toxicity

One of the most commonly used strategies for counteracting vitamin A deficiency in less-industrialized countries has been the oral administration of periodic large doses usually 0 21 mmoles (60 mg, or 200,000 IU_a), of retinyl palmitate in oil Transient side effects have been noted in some children at this or at even lower doses (Chapter 15) Thus, safety is an important issue in dealing with intervention strategies, particularly in regard to very young children and to pregnant and lactating women, even perceived toxicity may reduce public acceptance of the program

Carotenoids, even when taken in extremely large doses for long periods, are generally nontoxic The only known exception is canthaxanthin, which can induce retinopathy when it is ingested in large amounts for long periods [79]

Acute

When a single dose of more than 0 7 mmol of vitamin A (> 200 mg, or > 660,000 IU_a) is ingested by adults or when a dose larger than 0 35 mmol (> 100 mg or > 330,000 IU_a) is ingested by children, the results may be nausea, vomiting, headache, increased cerebrospinal pressure, vertigo, blurred (double) vision, muscular incoordination, and (in infants) bulging of the fontanelle Some infants can be adversely affected by single doses of only 0 1 mmol These signs are generally transient and subside within one to two days (Chapter 15) [80,81] When the dose is extremely large, it is soon followed by drowsiness, malaise, inappetence, reduced physical activity, skin exfoliation, itching (particularly around the eyes), and recurrent vomiting

Young monkeys, when given lethal doses by intramuscular injection, fall into a deep coma, often have convulsions and respiratory irregularities, and finally die of either respiratory failure or convulsions [82] The median lethal dose (LD_{50} value) of vitamin A injected intramuscularly in a water-miscible form in the young monkey is 0 6 mmol (168 mg) retinol/kg body weight Extrapolated to a 3-kg child and a 70-kg adult, the total LD_{50} dose would be 1 8 mmol (500 mg) and 41 mmol (11 8 g) respectively A newborn child, who mistakenly was given 0 09 mmol (25 mg) daily, or 28 μmol/kg, for eleven days died of apparent vitamin A toxicity [83] The total dose received was 0 31 mmol/kg, or half of the LD_{50} value for young monkeys Such enormous amounts of vitamin A are present only in high-potency preparations of vitamin A or in large amounts (~500 g) of livers particularly rich in vitamin A (> 0 035 mmol/g or > 10 mg/g)

Chronic

Chronic toxicity is induced by the recurrent intake of vitamin A in amounts at least ten times the RDA, that is, 13 μmol (3 75 mg retinol equivalents or 12,500 IU_a) for an infant or 35 μmol (10 mg retinol equivalents or 33,300 IU_a) for an adult A health-food enthusiast who ingested 26 μmol (25,000 IU_a) of vitamin A as a supplement daily plus a similar amount in food over a prolonged period showed severe signs of toxicity [81]

Approximately 50 signs of chronic toxicity have been reported, of which the most frequent are alopecia, ataxia, bone and muscle pain, cheilitis, conjunctivitis, headache, hepatotoxicity, hyperlipemia, hyperostosis, membrane dryness, pruritus, pseudotumor cerebri, various skin disorders, and visual impairment [80,81] When the supplemental intake of vitamin A is eliminated, these signs usually disappear over a period of weeks to months, but not always

In chronic hypervitaminosis A, holo-RBP in the plasma is not much elevated, whereas retinyl esters are usually increased markedly [39] Factors that enhance toxicity include alcohol ingestion, low protein intake, viral hepatitis, other dis-

eases of the liver and kidney, and possibly tetracycline use Elderly individuals may be more sensitive because of a slower rate of storage in the liver and a reduced plasma clearance of administered vitamin A Tocopherol, taurine, and zinc are protective in tissue culture cells, but they may or may not be effective in vivo [81]

Some individuals seem to suffer from vitamin A intolerance, that is, the appearance of signs of toxicity upon routinely ingesting moderate amounts of vitamin A This relatively rare condition, which seems to be genetic, mainly affects males [84]

Both all-*trans* and 13-*cis* retinoic acid, as well as many synthetic retinoids, induce similar toxic states Indeed, acidic forms of retinoids are more toxic than the alcoholic forms and are much more toxic than some conjugated derivatives [39] Mechanisms of toxicity are ill defined

Teratogenic

Vitamin A and other retinoids are teratogenic, both in experimental animals and in women [81,86,87] In experimental animals, all-*trans* retinoic acid is four times more teratogenic than all-*trans* vitamin A or its ester [85] A single extremely large dose of either retinyl ester or retinoic acid, or exposure for as short as a week on high daily doses of retinoic acid (0 1 mmol to 0 3 mmol, or 30 mg to 90 mg), during early pregnancy can induce spontaneous abortions or major fetal malformations Long-term daily intakes of greater than 26 μmol of retinyl ester (25,000 IU_a or 7,500 RE) have also been associated with birth defects in human fetuses, but causality has not been established [87] Common defects are craniofacial abnormalities, including microcephaly, microtia, and harelip, congenital heart disease, kidney defects, thymic abnormalities, and central nervous system disorders The extent of induction of RARβ2 by retinoids correlates well with their teratogenic actions [88] Permanent learning disabilities have been noted in otherwise normal rat pups whose dams received nonteratogenic doses of vitamin A, as well as in children exposed to large doses of 13-*cis* retinoic acid (Accutane) early in fetal life [89] Synergism between vitamin A and other teratogens, such as alcohol and drugs, at nonteratogenic doses of each is probable Thus, women who are pregnant, or who might become so, should carefully control their intake of vitamin A, in regard both to rich foods sources, such as liver, and to vitamin A supplements

Healthy women who routinely ingest diets containing adequate fruits and green leafy vegetables do not require supplements of vitamin A during pregnancy [81] In cases where supplementation is advisable, the total daily intake should not exceed 10 μmol (approximately 3 mg or 10,000 IU) of vitamin A [39,81,90] It should be noted that vitamin A deficiency, just as its excess, adversely affects the reproductive process

Conclusions

Vitamin A and carotenoids are essential for basic physiologic processes in many living organisms As a consequence, nature has devised ingenious ways to protect us both from an inadequacy and from an excess of vitamin A The biochemistry of vitamin A and carotenoids in mammals deals largely with nature's clever devices the efficient absorption of preformed vitamin A, its transport, in large part, on protein chaperones within the body, special mechanisms for its storage, efficient recycling, effective homeostasis, and a regulated catabolic and excretory system Because carotenoids, until recently, have served as major dietary sources of vitamin A, their bioconversion to vitamin A has evolved as a slow and regulated process, thereby protecting us in large part from the toxic effects of dietary excess

But vitamin A inadequacy, ironically, remains a major nutritional problem in the world, despite nature's care in trying to prevent it The reasons underlying this important problem, and some of the strategies designed to address it, are the major focus of this book

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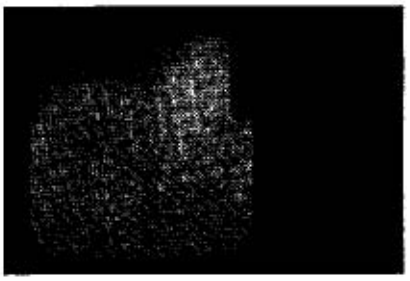

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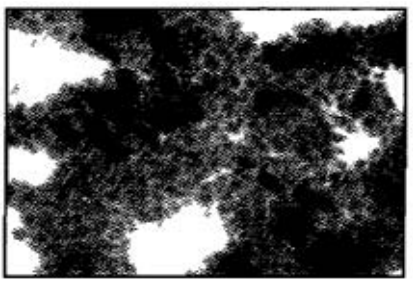
Plate 1. Xerophthalmic retinopathy in a 24-year-old otherwise well-nourished Indonesian woman who presented with nightblindness, constricted visual fields, and severe conjunctival and corneal xerosis

Plate 2. Two months after vitamin A therapy the small yellowish-white retinal lesions seen in the patient in Plate 1 have largely disappeared



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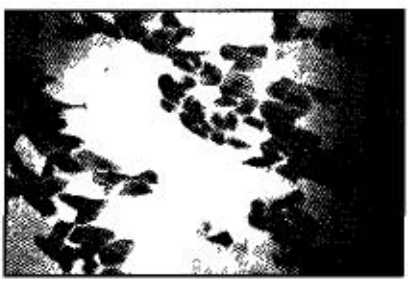

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Plate 3 Impression cytology specimen (CIC-A) of normal conjunctiva revealing abundant PAS-positive mucus-secreting goblet cells amid a sheet of small, regular epithelial cells (PAS and Harris' hematoxylin, ×400)

Plate 4 CIC-A specimen of abnormal conjuctiva from a vitamin A–deficient child Epithelial cells are large and irregular (though not yet keratinized) and lack goblet cells (PAS and Harris' hematoxylin, ×400)

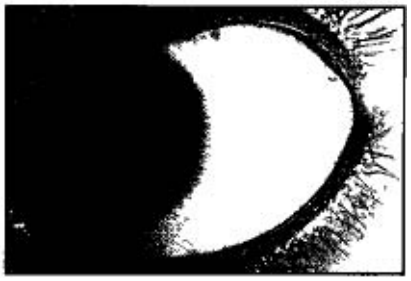

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Plate 5. Localized area of dry, granular conjunctival xerosis temporally in a 5-year-old Indonesian boy

Plate 6 Tiny, localized areas of foam or bubbles of conjunctival xerosis in a 4-year-old Indonesian boy

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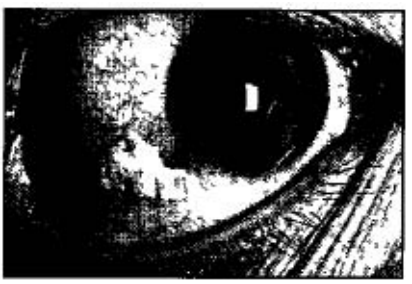

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Plate 7 Classical foamy Bitot's spot in a 29-year-old Indonesian man that largely resolved within 2 months of vitamin A therapy

Plate 8. Classical Bitot s spot in a 20-year-old Indonesian man The superior aspect is foamy in character, the inferior aspect is denser and cheesy in appearance There was no history of nightblindness and the Bitot's spot failed to respond to vitamin A therapy

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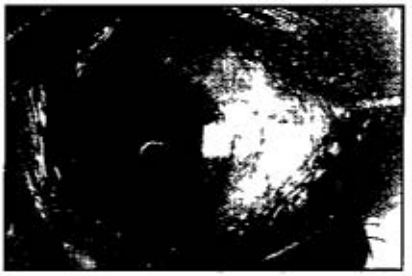

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Plate 9 Foamy Bitot's spot in a 3-year-old girl from South India Note the heavily pigmented appearance of the conjunctiva

Plate 10. Thick, stiffened temporal conjunctiva in a 4-year-old Indonesian girl with advanced conjunctival xerosis Haziness of the inferior cornea represents mild corneal xerosis

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Plate 11 Thickened skinlike temporal conjunctiva in a 1-year-old Indonesian boy with early corneal xerosis The eye had been stained with Rose Bengal

Plate 12. Extensive punctate keratopathy in a 10-month-old Indonesian boy The cornea appeared crystal clear to handlight examination, but fluorescein-staining punctate epithelial lesions were apparent on examination with the slit-lamp biomicroscope The cornea of the other eye was entirely necrotic (Plate 13)

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Plate 13. Fellow eye of subject described in Plate 12 The cornea was entirely necrotic and bulging forward Dark, pigmented iris is visible in areas where most of the stroma had already sloughed

Plate 14. Inferior corneal xerosis in a severely malnourished 3-year-old Indonesian boy The keratinized surface is sharply demarcated The cornea of the other eye was entirely necrotic (Plate 15)

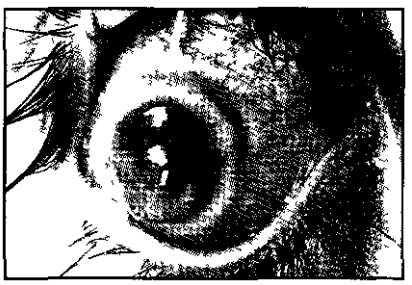

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Plate 15 Fellow eye of case described in Plate 14 Except for the peripheral 1 mm the cornea is entirely necrotic, most having already sloughed resulting in a large central descemetocele The conjunctiva is moderately inflamed and without evident xerosis

Plate 16. Heavily keratinized corneal surface in the eye of a 2-year-old Indonesian girl two days after initiation of therapy A portion of the keratinized surface has already peeled off inferiorly forming a scroll below The clear area is now devoid of its keratinized layer At the initial examination the identical area of the other eye contained a typical ulcer (Plate 17)

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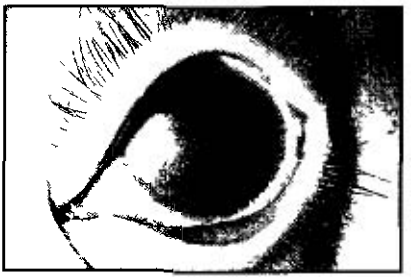

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Plate 17. Fellow eye of case described in Plate 16. A typical, sharp-margined oval ulcer is present inferiorly. The conjunctiva is not inflamed and contains extensive xerosis which is most apparent in the inferior quadrant adjacent to the cornea.

Plate 18. Extensive conjunctival xerosis and heavily keratinized corneal surface in a 3-year-old Indonesian boy.

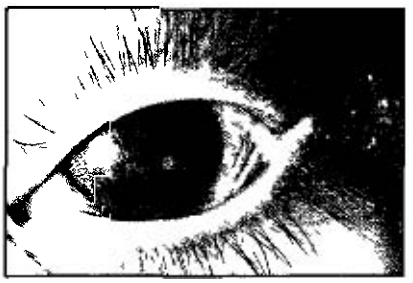

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Plate 19. Same eye shown in Plate 18, two days following initiation of therapy. The heavily keratinized corneal plaque inferiorly has sloughed, leaving a small, round, sharply demarcated superficial ulcer in its place. Crevices of the surrounding keratinized surface converge on the ulcer, most apparently above the inferior limbus. Keratinized plaques are still present in the interpalpebral zone.

Plate 20. External appearance of one eye of the case described in Plate 1. Marked xerosis of both the conjunctiva and cornea is present.

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Plate 21. Heavily keratinized corneal plaque in a 2-year-old Indonesian girl with poor lid closure and severe malnutrition Conjunctival and corneal xerosis is extensive

Plate 22 Right eye of a 3-year-old Indonesian girl A classical punched-out, shallow round ulcer was present anteriorly, with pigment (presumably adherent iris surface left behind following dilitation) posteriorly, and normal-appearing stroma in between Two pinpoint ulcers are present superiorly The left eye is shown in Plate 23

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Plate 23 Left eye of case described in Plate 22 A large, circumscribed area of cornea is missing centrally with iris bulging through the defect The surrounding cornea was hazy but viable The conjunctiva was xerotic and not inflamed

Plate 24. The left eye of a 4-year-old Indonesian boy Corneal xerosis is apparent inferiorly, and a classically sharp, round, punched-out three-fourths depth ulcer is evident superonasally The ulcer reached Descemet's membrane

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Plate 25. A characteristically fluorescein-staining oval, inferior corneal ulcer in a 2-year-old Indonesian girl A small hypopyon is present and the conjunctiva is inflamed

Plate 26. A 19-month-old Indonesian boy The right eye contained extensive conjunctival xerosis and punctate keratopathy The left eye, shown here one day following initiation of vitamin A therapy contained three areas of surface abnormality One area was irregular, composed of mounds (clear epithelial or superficial stromal vesicles) alternating with dellen-like depressions Temporal to this area was a depression In its center was a sharp, deeper, cylindrical ulcer, the only part that stained with fluorescein A second, larger depression with a centrally staining ulcer was present more temporally The bases of the two ulcers, originally clear were by now moderately opaque A small hypopyon was present and the conjunctiva was inflamed

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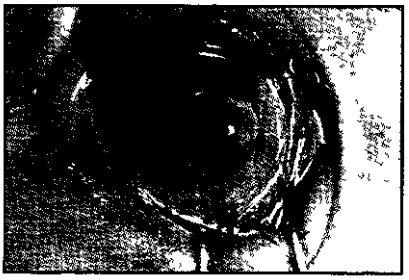
Plate 27 Same eye shown in Plate 26, three days after presentation, stained with fluorescein and observed through a blue filter The superonasal area is less irregular and the adjacent ulcer has largely healed But now the most temporal ulcer has enlarged, the entire area of depression being deeper and staining edge-to-edge The hypopyon has increased

Plate 28 Opaque, necrotic cornea in a 2-year-old Indonesian boy The conjunctiva is both xerotic and inflamed The fellow eye contained an area of inferior focal, corneal necrosis



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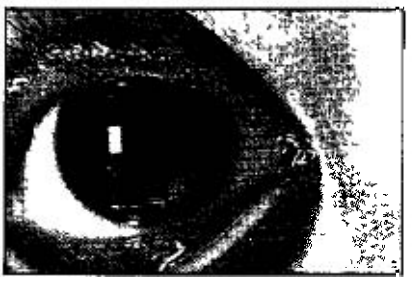

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Plate 29. Sharply focal necrotic lesion in the right eye of a 2-year-old Indonesian boy The stroma has largely sloughed, a bulging descemetocele filling the defect The rest of the cornea is hazy and xerotic The conjunctiva is inflamed The other eye had moderately severe corneal xerosis

Plate 30 Same eye shown in Plate 29 two months later The defect has healed as a remarkably small, peripheral adherent leukoma, leaving a clear pupillary zone

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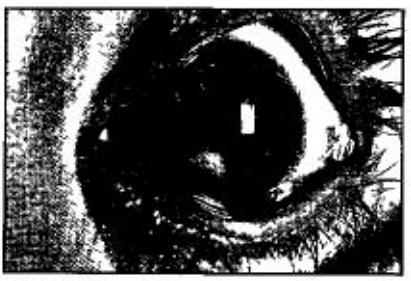

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Plate 31. Left eye of an otherwise well-nourished 2-year-old Indonesian boy A large, nonstaining, focally necrotic bulging, opaque corneal lesion is present inferiorly The conjunctiva is both inflamed and xerotic Abnormalities in the other eye were limited to dense punctate keratopathy

Plate 32. Same eye shown in Plate 31, one month later The previous lesion has healed as a surprisingly small adherent leukoma preserving a clear central, pupillary zone

9

The Relationship between Immunocompetence and Vitamin A Status

A. CATHARINE ROSS

A role for vitamin A in maintaining the structure and function of the immune system was recognized in very general terms as early as the 1920s Based on a comprehensive light-microscopic study of vitamin A-deficient rats, Wolbach and Howe[1] concluded that deficiency is associated with marked morphological alterations in the epithelia of numerous organs (e g , the trachea and cornea) The tissues of vitamin A-deficient rats showed abnormal keratinization in place of the normal mucus-secreting phenotype, and abnormal flattening (squamous metaplasia) in place of the normal columnar or cuboidal form In the lymphoid organs, thymic atrophy was observed with advanced vitamin A deficiency, and cellular changes indicative of accumulation of debris were described in the lymph nodes Wolbach and Howe commented that these epithelial changes occurred quite apart from evidence of infection A few years later, Green and Mellanby[2] reported that vitamin A-deficient rats often died with histopathologic evidence of infection, in contrast to their well-nourished counterparts From a study of morbidity and mortality in rats infected with a specific pathogen (paratyphoid bacillus), Lassen[3] concluded that both vitamin A-deficient and control rats became infected, but that few of the vitamin A-deficient rats recovered from their infection

Over the years, numerous observations in a variety of experimental animals have made it clear that resistance to infection is often reduced by chronic vitamin A deficiency (Table 9–1) The concept that improving the vitamin A status of

Table 9–1 Some Observations on Resistance to Infection in Vitamin A-Deficient Animals

Observations	*Species*	*Reference*
Histopathologic evidence of infection	rat	Green[2]
↑ Resistance to typhoid, anthrax, and paratyphoid bacilli	rat	Lassen[3]
↑ Resistance to endogenous infection	rat	Ongsakul[78]
↑ Resistance to *E. coli* infection	chick	Friedman[79]
Longer survival of vitamin A-deficient animals when germ-free or given antibiotics	rat	Bieri[80] Raica[81] Anzano[82]
	chick	Rodgers[83]
↑ Corneal inflammation, ulceration, and necrosis after herpes virus infection	rat	Nauss[84]
↑ Leukocyte infiltration and corneal ulceration after *P. aeruginosa* infection	rabbit	DeCarlo[85]
Impaired clearance of parasites and ↑ worm burden	rat, mouse	Parent[86]
Intestinal villus destruction in vitamin A deficiency combined with rotovirus infection	mouse	Ahmed[28,87]
↓ Clearance of *E. coli* and in vitro phagocytic activity	rat	Ongsakul[78]
↓ Antibody response to *Schistosoma mansoni* infection	rat	Parent[86]
↓ Antibody response to Newcastle disease virus or *Salmonella pullorum* antigen	chick	Sijtsma[27] Panda[88]
No difference in worm burden or replication following infection with *Trichinella spiralis* but ↓ anti-IgG response	mouse	Carman[58]

From Proceedings of Public Health Significance of Vitamin A Deficiency and Its Control [146]

humans may be an effective way to decrease infectious morbidity and mortality has gained strength from recent controlled community trials (Chapters 2, 3), and particularly from hospital-based studies of vitamin A supplementation in children with severe measles,[4–6] an infection known to produce a rapid and profound immunosuppression.[7] In addition, randomized, controlled community studies designed to examine morbidity have shown few differences in the prevalence of infectious diseases, but a reduction in the severity of infection (particularly diarrheal disease), in vitamin A-supplemented children (Chapters 2, 3).[8,9,10]

How does vitamin A maintain health, specifically as related to infectious disease? Two plausible hypotheses are suggested in Table 9–2, one focusing on epithelial barriers and the other on immunological functions. It is possible that vitamin A reduces mortality by maintaining epithelial barriers and, hence, reduces the rate of infection. This hypothesis (which has its origins in the work of Wolbach and Howe) puts the major emphasis on the role of vitamin A in maintaining structural integrity, i.e., normal tissue differentiation. Although it is often said that epithelial barriers provide a "first line of defense" in resistance to infection, it is more precise to define this role as an *offensive* one, related in

Table 9–2 Hypotheses Concerning the Protective Role of Vitamin A

Epithelial Barrier Hypothesis	*Immunologic Response Hypothesis*
Principally *offensive* protects against initial infection	Principally *defensive* responds to pathogens or non-self antigens
Structural integrity paramount	Functional integrity emphasized, but cell differentiation also important
Predicts resistance to infection will be reduced by vitamin A deficiency	Predicts response to infections will be reduced by vitamin A
Predicted major effect decreased incidence of infection	Predicted major effect decreased duration or severity of infection

part to mucus secretion and the movement of cilia, which serve to entrap and disable pathogens A reasonable expectation from this hypothesis is that providing vitamin A to deficient individuals will reduce the incidence of infections compared with equally deficient, but untreated, controls However, a consistent reduction in the incidence of infection after vitamin A supplementation has not been detected (Chapter 3) From these data, it should not be concluded that vitamin A supplementation in at-risk children has no beneficial effects on the epithelia of the corneal, gastrointestinal, and respiratory tracts, but rather that supplementation does not appear to be a major explanation for the dramatic effects of vitamin A on child survival

It is also plausible that the main action of vitamin A is to maintain immunocompetence The immune system is primarily a *defensive* network that has evolved to make highly specific responses to nonself antigens and pathogens and to generate long-lived protection through antigen-specific memory Immunocompetence requires both structural maintenance in terms of a continual renewal of lymphoid cells (lymphopoiesis in bone marrow and subsequent differentiation of lymphoid cells in the primary lymphoid organs) and functional maintenance in terms of cell-cell signaling required to generate appropriate responses to microbial infections or other immunogenic stimuli A requirement for vitamin A in immunocompetence might be revealed as a decrease in the incidence of infection following supplementation, but would more likely be seen as a reduction in either the duration or severity of illness Most of the community-based studies were not designed to examine duration or severity of disease in detail and, therefore, little data are available However, as noted above, a reduction in the severity of infection (particularly diarrheal disease) has now been reported in vitamin A-supplemented children (Chapters 2, 3) [8,9] These data, and especially the therapeutic effect of vitamin A on severe measles, suggest that improvements in immunocompetence and the response to infection may explain the ability of vitamin A to decrease mortality without having an observable effect on the incidence of infection

Over the years, experimental studies, mostly with animal models, have provided insight regarding the requirements for vitamin A in the immune system

This chapter will focus on six main issues concerning vitamin A and immunocompetence First, the consequences of vitamin A deficiency will be considered as they relate to three aspects of immunity lymphoid organs and hematopoiesis, "cell-mediated immunity," and humoral immunity How vitamin A administration affects immune responses will be considered next This subject has been studied from two viewpoints (1) whether vitamin A is effective in rehabilitating the immune responses of vitamin A-deficient animals or humans, and (2) whether vitamin A has any immunostimulatory activity in individuals whose vitamin A status is normal Finally, two issues will be explored that may or may not have practical implications but are nonetheless of interest for understanding the nature of the requirement for vitamin A First, which forms of vitamin A have immunoactivity, and second, is vitamin A always essential, or are there circumstances in which other immune factors can circumvent the requirement for this nutrient?

Lymphoid Organs and Hematopoiesis

Does vitamin A deficiency have a major effect on the organs or cells of the immune system? Changes in the size, weight, and cellularity of lymphoid organs have been reported in experimental models of vitamin A deficiency[11,12], however, the consistency of these findings is not strong because, depending on the species studied, both increases and decreases in spleen and lymph node weight or leukocyte number have been reported For example, severely vitamin A-deficient mice were reported to have significantly enlarged spleens and lymph nodes,[13] whereas vitamin A-depleted rats either showed no change in organ mass or cell number prior to the onset of symptoms of vitamin A deficiency, or the thymus and spleen weights and cellularity were reduced after symptoms had developed [14–16] Lymph node weights of severely vitamin A-deficient rats, like mice, were reported to be elevated [14] In the gut-associated lymphoid tissues of the guinea pig, vitamin A deficiency was associated with fewer intestinal Peyer's patches and immunoglobulin-bearing cells [17]

Total leukocyte and differential counts have been determined in some animal models of vitamin A deficiency, and specific lymphocyte populations have been enumerated by flow cytometry after staining with cell type-specific monoclonal antibodies Although in the aggregate the results point to disturbances in hematopoiesis, the results of these investigations have also varied considerably among different models of vitamin A deficiency A study of vitamin A-deficient mice showed no organ-specific changes in lymphocytes, B cells, T cells or macrophages, but a calculation of the number of cells per mouse revealed significant increases in B cells and macrophages [13] T cell subsets, determined by expression of surface glycoproteins, did not differ between vitamin A-sufficient and -deficient mice [13] In contrast, studies of rats with a single nutrient deficiency of vitamin A have shown a reduction in circulating lymphocytes and an elevation in granulo-

cytes [14,18,19] Within the lymphocyte population, the balance between B and T cells and between $CD4^+$ (helper) and $CD8^+$ (cytotoxic/suppressor) T cells was normal in two reports [14,19] Recently we completed a study of the numbers and proportions of lymphocytes and subsets including B and T lymphocytes and natural killer (NK) cells (Zhao and Ross, unpublished results) The number of peripheral blood lymphocytes of vitamin A-deficient rats was consistently reduced, while the number of granulocytes was elevated Among lymphocyte populations, decreases on the order of ~20%–25% were found for B cells in blood and the spleen respectively, as well as for total T cells and $CD4^+$ T cells of the spleen The largest relative differences in the cell populations of vitamin A-deficient rats were observed for the numbers of NK cells, which were reduced by 36% and 57% in the spleen and peripheral blood respectively However, after deficient rats were repleted with retinoic acid for 4 5 days, their blood lymphocyte counts and the numbers of B, T, and NK cells increased significantly to values that equaled or exceeded those of vitamin A-sufficient controls

In humans, vitamin A deficiency appears to be associated with low lymphocyte counts In a study of African children (four months to twenty-four months old) with complicated measles, blood lymphocyte counts were depressed relative to those of noninfected children [20] In measles-infected children who received vitamin A (three doses equivalent to 30 mg or 60 mg of retinol), the total lymphocyte counts increased significantly [20] In a study of the relationship of low vitamin A status (manifested as xerophthalmia) to immune function in preschool Indonesian children, the ratio of T cells bearing $CD4^+$ and $CD8^+$ antigens was marginally lower in the peripheral blood lymphocytes of thirty xerophthalmic children, as compared with twenty-five non-xerophthalmic controls [21] However, five weeks after both groups of children were supplemented with vitamin A (equal to 60 mg of retinol), the proportion of $CD4^+$ to $CD8^+$ T cells and the percentage of naive $CD4^+$ T lymphocytes increased in comparison to the children given a placebo [21]

As these data indicate, the results from animal and human studies vary considerably, and yet nearly all studies support the general hypothesis that lymphopoiesis and/or maturation of lymphocytes are altered (generally, reduced) by a lack of vitamin A It is questionable whether the magnitude of cellular depletion, which often has been modest when observed at all, is great enough to account for some of the very dramatic defects in immune function However, in addition to the observations regarding vitamin A deficiency, there are other reasons to believe that vitamin A plays an important role in hematopoiesis retinoids have been effective in controlling the differentiation of cell lines of hematopoietic and nonhematopoietic origin in vitro [22] The recent success of all-*trans*-retinoic acid in the "differentiation therapy" of patients with acute promyelocytic leukemia has dramatically illustrated the ability of retinoids to regulate at least some aspects of leukocyte maturation [23] Further investigations of the role of vitamin A in hematopoiesis, with discrimination among lymphocytic,

monocytic, and granulocytic lineages, should be revealing in understanding the requirements for vitamin A and the mechanisms of action of various retinoids

Cell-Mediated Immunity

The term "cell-mediated immunity" (CMI) was originally used to describe localized reactions to pathogens that are mediated principally by lymphocytes and macrophages, and in which antibodies play a subsidiary role [24] However, this terminology is imprecise due to the numerous overlaps between CMI and humoral immunity For example, $CD4^+$ T cells provide help to cytotoxic T cells in CMI and to B cells in the humoral response, while interleukins or cytokines released by T cells or macrophages play regulatory roles in both cell-mediated reactions and antibody production, and antibodies are intimately involved in some mechanisms of cytotoxicity The major effector cells in CMI are the cytotoxic ($CD8^+$) T lymphocytes, NK cells, and macrophages ("professional phagocytes"), which destroy infected or foreign cells through direct contact or via secreted, soluble factors

Experimental vitamin A deficiency has been reported to affect several measures of CMI, including the blastogenic transformation of lymphocytes in response to mitogens, T cell cytotoxic responses, and delayed-type hypersensitivity (DTH) responses [11 18] Both decreases and increases in mitogen-induced lymphocyte transformation have been reported (with differences apparently related to the lymphoid organ source of cells, the type of mitogen, and the duration or stage of vitamin A deficiency) [14] In most cases, the blastogenic response of spleen cells to T cell mitogens has been reduced [14 25] The response of Peyer's patch lymphocytes to B and T cell mitogens was also reduced [26] The cytotoxicity of T lymphocytes was low in vitamin A-deficient chicks challenged with Newcastle disease virus [27] Similarly, DTH responses, often used to assess CMI, were significantly reduced in vitamin A-deficient mice and chicks [13 28] In contrast, however, a study of children in Bangladesh reported no change in the DTH response before and after vitamin A supplementation [29] With the latter exception, the studies to date are consistent with the hypothesis that CMI responses, shown or presumed to be mediated by T cells, depend on adequate vitamin A

Vitamin A deficiency has also been observed to reduce the "natural cytotoxicity" expressed by NK cells NK cells function in immune defenses through cytotoxic killing of virus-infected (or tumor) cells, and are also thought to play a broader immunoregulatory role by secreting soluble factors (e g , interferon [IFN]-gamma and tumor necrosis factor [TNF]-alpha) that modulate antibacterial responses [30] Vitamin A deficiency in rats was associated with large decreases in the NK cell cytotoxic activity of spleen,[31 32] and was correlated with a significant reduction in the release of IFN from mitogen-stimulated spleen cells of the same animals [31] It may be relevant that the NK cytotoxicity of peripheral blood was

low in young children with acute measles [33] Although this observation was not shown to be correlated with a lack of vitamin A, reduction in the plasma concentration of retinol during acute infection is well documented (Chapters 9, 11) [34,35]

Recently, the cause of low NK cell activity in vitamin A-deficient rats was investigated using a specific monoclonal antibody to determine NK cell number [36] The cytotoxic activity of NK cells in blood and spleen was reduced by ~56% compared with controls, and most of the decrease was accounted for by low NK cell numbers (reductions of 57% in blood and 36% in spleen) After correction for low NK cell number, the NK cell lytic efficiency (cytoxicity per NK cell) was normal (in blood) or only marginally reduced (in spleen), even in the vitamin A-deficient state Moreover, the NK cells of vitamin A-deficient rats retained their ability to be activated by lymphokines in vivo and in vitro [36] Thus, in the case of NK cell cytotoxicity the predominant defect appears to be a reduction in hematopoiesis (whether due to changes in bone marrow or subsequent maturational steps is still unknown), rather than a functional impairment in the cytolytic capability or activation potential of mature NK cells

Humoral Immunity

Antibody responses are integral to successful vaccination and to recovery from natural infections such as measles The relationship of vitamin A deficiency to antibody production has been addressed in detail recently [11,12] This discussion of individual studies will be limited, instead, an attempt will be made to draw some generalizations from current experimental studies of the antibody response

Vitamin A deficiency compromises the antibody response to some types of antigens, but not to others. The primary antibody response has been investigated in vitamin A-deficient rats, mice, or chicks immunized with a number of T cell-dependent (TD) antigens (e g , proteins such as tetanus toxoid, hemocyanin, and albumin, or heterologous red blood cells) [11] The primary antibody response has been consistently low compared with the response of vitamin A-sufficient, pair-fed, or age-matched controls In studies that included previously vitamin A-deficient animals after repletion with vitamin A, antibody production was normal or even somewhat increased,[37-39] even when retinol was not provided until the day after immunization [39] These observations are consistent with the idea that the initial interactions between antigen, antigen-presenting cells, and other cells does not depend on vitamin A In addition, antigen-presenting cells of vitamin A-deficient mice functioned as well in vitro as those from vitamin A-sufficient controls [40] From these depletion and repletion studies it seems clear that a single nutrient deficiency of vitamin A is responsible for the reduced specific antibody response, and that retinol by itself is able to reverse this immunodeficiency These data do not diminish the importance of general nutrition, including adequate protein and calories, but they do illustrate that vitamin A-deficient animals,

even compared with pair-fed controls that may themselves be somewhat undernourished, respond poorly to TD antigens and that restoration of retinol alone reverses their immunodeficiency

These results from animal studies are borne out by recent studies of the response to vaccination in young children Indonesian children who received a single 60 mg dose of vitamin A two weeks before immunization with DPT vaccine produced anti-tetanus titers that were higher than pre-vitamin A levels, whether or not they had preexisting signs of vitamin A deficiency (mild xerophthalmia) It is noteworthy that before treatment with vitamin A, approximately half of the children, regardless of ocular status, had mean low plasma retinol concentrations[41] ($\leq$ 0 7 μmol/liter or 200 μg/liter), and that these levels increased significantly in the vitamin A-supplemented groups Thus, vitamin A status and the response to TD antigens are also correlated in humans

An investigation of the antibody response of vitamin A-deficient rats to two capsular polysaccharides (from *S pneumoniae* and *N meningitidis*) also revealed marked immunosuppression ($\leq$ 20% of control), similar to the results described above for TD antigens [15,16] A significant reduction in the antibody response to pneumococcal polysaccharide was detected as early as two weeks after young rats were weaned onto a vitamin A-free diet, well before any physical signs of vitamin A deficiency would have been apparent [19] Conversely, antigen-specific antibody concentrations were normal after repletion with retinol, even in animals whose previous vitamin A deficiency had been severe [16] Although polysaccharide antigens such as pneumococcal polysaccharide have been characterized as T cell-independent (TI) antigens (based on the ability of athymic nude mice to respond), additional studies of euthymic animals showed that the magnitude of the antibody response to pneumococcal polysaccharide is regulated by T cells [42] These antigens have now been subclassified as TI type 2 (TI-*2*) antigens [16,43] The similar depressions in antibody responses to both TD and TI-*2* antigens in vitamin A-deficient animals may, therefore, be consistent with impairments in T cell functions and/or T cell–B cell interactions

In marked contrast, the ability of vitamin A-deficient rats to produce an antigen-specific response to two lipopolysaccharides (both TI *type 1* [TI-*1*]) antigens that can stimulate B cells directly) was normal, even after rats had progressed to the stage of frank, symptomatic vitamin A deficiency [16] These contrasting results with experimental immunizations lead to at least two conclusions First, it is clear that at least certain populations of B cells from vitamin A-deficient animals retain the ability to respond to antigen and to synthesize and secrete antigen-specific antibody Therefore, the basic "machinery" of antibody production must be intact, even in severe deficiency Second, the contrasting results between TD and TI-*2* antigens versus TI-*1* antigens imply that the requirement for vitamin A in the antibody response to natural infections may also depend strongly on particular features of a pathogen's antigenic components or the type of response it elicits One might reasonably predict that, in the case of natural

infections, there also will be situations in which the immune response is compromised by a lack of vitamin A deficiency and other situations in which vitamin A deficiency, or its reversal by supplemental vitamin A, will have little impact on the course of infection or the formation of protective antibodies or immune cells

The generalizations in Table 9–3 are proposed based on the similar results with TD and TI-*2* antigens in experimental animals and the opposite findings with TI-*1* antigens The underlying reasons for the differences in response among these types of antigens are not yet understood It is known, however, that bacterial lipopolysaccharides strongly elicit the secretion of certain soluble factors, such as TNF-alpha from macrophages Therefore, it is possible that these TI-*1* antigens trigger a pathway that is relatively insensitive to vitamin A deficiency, or that the particular factors released after stimulation with TI-*1* antigens are functional even in the absence of vitamin A Additional studies with other antigens will be of interest to further test these generalizations and to clarify which particular features of the immune response actually require vitamin A The immunogenicity of lipopolysaccharide antigens in vitamin A-deficient animals also raises the possibility that factors elicited by such antigens might have immune enhancing (adjuvant) activity toward other antigens (such as TD or TI-*2* antigens), even during vitamin A deficiency

There is a dichotomy between the levels of total IgG and antigen-specific IgG in vitamin A-deficient animals It is interesting and seemingly paradoxical that, despite the low antigen-specific responses observed for TD or TI-*2* antigens, the total IgM or IgG concentrations were elevated in vitamin A-deficient rats, mice, or chicks [11] For example, young chicks exposed to Newcastle disease virus produced a low virus-specific antibody titer even though nonspecific IgM and

Table 9–3 Possible Generalizations from Studies of Antibody Production in Vitamin A-Deficient Animals

RETINOL IS REQUIRED FOR THE ANTIBODY RESPONSE TO T CELL DEPENDENT (TD) AND T CELL-INDEPENDENT TYPE *2* (TI *2*) ANTIGENS
TD antigens
Tetanus toxoid
Heterologous red blood cells
Proteins (e g , hemocyanin, *albumin*)
Viral infections
Parasitic infections
TI-*2* antigens
pneumococcal polysaccharide
meningococcal polysaccharide
RETINOL IS NOT ESSENTIAL FOR THE ANTIBODY RESPONSE TO TI TYPE *1* (TI *1*) ANTIGENS
Pseudomonas aeruginosa lipopolysaccharide
Serratia marcescens lipopolysaccharide
Brucella abortus

Summarized from results described in detail in Ross et al [11]

IgG were increased.[27,44] In rats, total (nonspecific) IgG increased in an age-dependent manner from twenty days to seventy days of age regardless of whether rats were fed a vitamin A-free or -adequate diet, however, concomitant with the appearance of signs of vitamin A deficiency at about fifty-five to sixty days of age, the concentration of total IgG was significantly elevated compared with vitamin A-sufficient controls.[39,45] These data further support the conclusion that the B cells of vitamin A-deficient animals retained their ability to synthesize and secrete immunoglobulin, even though antigen-specific responses to TD and TI-*2* antigens were very low in the same animals.

There are qualitative as well as quantitative differences in the antibody response of vitamin A-deficient animals. The immunoglobulin G class in humans and rodents commonly used in immunological research comprises four distinct subclasses,* each regulated by a specific gene within the heavy chain gene cluster. Current evidence supports the view that cytokines, especially IL-4, IFN-gamma, and transforming growth factor-beta, regulate the expression of the constant heavy genes that encode the Ig classes.[46] For example, in the mouse, IL-4 increases the magnitude of IgG1 responses to TD, TI-*1* and TI-*2* antigens,[30,46] while IFN-gamma increases IgG2a and often IgG3 responses.[30,47] Thus, the cytokines released by T lymphocytes, NK cells, or other cells during the response to antigen, together with direct cell-cell interactions, determine the qualitative as well as quantitative characteristics of the antibody response. A retrospective examination of the Ig classes formed during a particular antibody response may sometimes provide clues as to the cytokines that have regulated it.

There is evidence, although from a small number of experiments, for subclass-specific impairment in the antibody response to TD antigens. When vitamin A-deficient mice were immunized with TD protein antigens, their IgG responses decreased to a greater extent than IgM responses[48] and, among IgG subclasses, reductions were observed for both antigen-specific IgG1 and IgG3.[48] The low IgG1 may imply a reduction of IL-4, while the low IgG3 would be consistent with a reduction in IFN-gamma.[46] The low primary anti-tetanus IgG concentration in vitamin A-deficient rats (whose IgG subclass nomenclature differs from that of the mouse [footnote]) was due almost entirely to a low level of anti-tetanus IgG2b, in contrast, the concentrations of anti-tetanus IgG1 and IgG2a were both equal to those of the control group.[45] These data may imply that IL-4 production and/or signaling was not significantly impaired, but a reduction in IFN could

*In humans, mice, and rats, IgG is made up of four subclasses with distinct heavy chain constant regions. The four IgG subclasses of humans, mice and rats have apparently diverged during gene evolution[46,76,77] and, although similar nomenclature has been adopted, the IgG subclasses with similar names in different species are not necessarily homologous. In humans, the four subclasses (isotypes) are designated as IgG1, IgG2, IgG3, and IgG4, in mice, as IgG1, IgG2a, IgG2b, and IgG3, and in rats, as IgG1, IgG2a, IgG2b, and IgG2c. Among rodents, mouse IgG2a/2b and rat IgG2b have been shown to be similar in structure and function.[77] Whereas IFN-gamma increased murine IgG2a responses,[46,47] IFN was implicated in rat anti-IgG2b production.[45]

underlie the low IgG2b response In support of this, spleen cells from vitamin A-deficient rats were observed to release less antiviral IFN in response to mitogenic stimulation [31] Conversely, preliminary studies have implicated an elevation of IFN in the increased production of rat anti-tetanus IgG2b [45] Abnormalities in IFN production are suggested by results from both vitamin A-deficient rats[31] and mice,[49,50] although the type of change seems to differ substantially between the two models Dysregulation of cytokine production or secretion is also suggested from experiments in vitamin A-deficient mice in which T helper cells of the Th1 type (which mainly secrete lymphokines [Il-2, IFN-gamma] that stimulate cell-mediated immunity) predominated early in the antibody response[51], this shift away from the Th2 clonotype (associated more with antibody responses) may be important in the poor antibody response in the vitamin A-deficient state

Immunologic memory may be formed, even when the humoral antibody response is low The results of studies with TD antigens have revealed a dichotomy between the primary antibody response, which is low in the vitamin A-deficient condition, as previously described, and the formation of immunologic memory, which appears to be normal [39,40] A study was designed to examine the secondary anti-tetanus response of previously vitamin A-deficient rats that were repleted with retinol before reimmunization The secondary response of these retinol-repleted rats displayed normal quantitative and qualitative characteristics (higher anti-tetanus IgG titers[39] and normal IgG subclass proportions in the secondary response of retinol-repleted rats[45]) The inference drawn from these studies, illustrated as a model in Figure 9–1, was that immunologic memory had

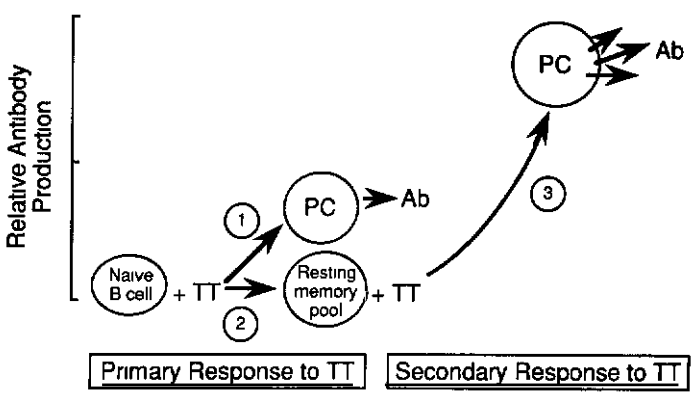

Fig. 9–1. Model of the consequences of vitamin A deficiency on the formation of memory B and T cells and antibody secreting plasma cells (PC) in the primary and secondary response to tetanus toxoid (TT) 1 = reduced in vitamin A deficiency (specific for anti-TT IgG2b), 2 = normal in vitamin A deficiency, 3 = reduced in vitamin A deficiency but normal or increased in previously deficient rats after repletion with retinol (Modified from M Kinoshita and A C Ross [45])

been established and retained in the vitamin A-deficient condition, and that memory could be activated later, provided that retinol status had improved, to produce the secondary response From these data one might speculate that if vitamin A-deficient humans, like rats, also form immunologic memory when they are vaccinated (even if their initial antibody response is low), then vaccination may still provide protection if the individual's vitamin A status is subsequently improved

Thus, the overall picture of changes in the humoral immune response of vitamin A-deficient animals and humans points to dysregulation in T cell signaling or T cell–B cell interactions, but not to a general impairment in antibody production The data also indicate that assays of the primary antibody response do not necessarily correlate with the formation of antigen-specific memory Finally, the idea that vitamin A may be important for the production of particular classes or subclasses of antibody fits conceptually with dysregulation in the synthesis, release, or signaling pathways of certain immunoregulatory cytokines such as IFN

Effect of Vitamin A Administration on Immune Response

Animals or humans have been treated with retinol for at least two purposes first, to test the ability of the vitamin to reverse immunodeficiencies and, second, to determine whether retinol (or other retinoids) has immunostimulatory activity in individuals whose vitamin A status is thought to be normal

Repletion in the Vitamin A-Deficient State

As noted previously, in essentially all situations of immunocompromise due to vitamin A deficiency, immune responses were normal after animals were repleted with vitamin A Human studies also support the ability of orally administered vitamin A to increase immune functions Following administration of a bolus (oral) dose of vitamin A, plasma levels of retinol rise within a few hours as newly absorbed chylomicron retinyl esters enter the circulation, are cleared into liver, and retinol is released in association with retinol-binding protein (RBP) (Chapter 8) [52,53] Kinetics studies have been conducted to determine the rate of tissue repletion in vitamin A-deficient animals given vitamin A as large or small oral boluses[53] or in the diet [54] In vitamin A-deficient animals, provision of even small doses of retinyl ester rapidly restored plasma retinol to normal levels, whereas repletion of liver vitamin A depended on the vitamin A dose [53,54] Following administration of vitamin A in quantities sufficient to cause deposition in liver, a state of vitamin A sufficiency may be maintained for several months After treating vitamin A-deficient rats with a single, large oral dose of retinyl ester,

there was an elevation of vitamin A in the spleen and thymus during the post-absorptive phase,* followed by a decline after ~24 hours [53]

There is limited evidence that immune responses can be stimulated, even in comparison to the vitamin A-sufficient state, when vitamin A-deficient animals are repleted with retinol near the time of immunization For example, when vitamin A-deficient rats were immunized with tetanus toxoid and repleted with a single oral dose (footnote) of vitamin A the next day, their anti-tetanus response not only increased significantly compared with vitamin A-deficient rats, but was approximately twice that of the vitamin A-sufficient control group [39] The mechanism(s) through which retinol might cause immune enhancement have not been identified, but they may be related to the uptake of retinol by macrophages[55] (or other cells participating in immune responses) It is conceivable that during vitamin A deficiency there are compensatory increases in the expression of certain receptors or other molecules involved in vitamin A-regulated responses, and that greater-than-normal signaling might occur shortly after vitamin A is available but before such receptors have been readjusted to normal levels

Adjuvant Properties in Vitamin A-Sufficient Animals and Cells

Some situations have been described in which retinol or retinoic acid appear to function as immune stimulants (adjuvants), increasing the responses to specific antigens or tumors [56] The adjuvant properties of retinol in the antibody response were first reported by Dresser,[57] who immunized mice with soluble bovine gamma-globulin (a nonimmunogenic protein in the mouse) and observed that specific antibody production was induced in a dose-dependent manner when large quantities of retinol (1 mg–10 mg per mouse) were administered before immunization However, Dresser did not administer retinol orally and it is unclear whether this stimulation of antibody production by retinol was due to its action as the vitamin or as an oily vehicle, similar in effect to mineral oil In addition to stimulating antibody production,[11,12] measures of CMI have also been reported to increase following treatment with retinoids in vitro or in vivo, including lymphocyte proliferation,[58] expression of IL-2 receptors on T cells and NK cells,[59] and production of IL-2 and IFN-gamma [60] However, it should be noted that in nearly all of these studies, retinoids were administered by nonphysiological routes

Animal studies also support the idea that high doses of vitamin A may stimulate phagocytosis and cell-mediated killing of pathogens, examples of the functions performed by the nonspecific arm of the immune system [11] The clearance of gram-negative and gram-positive bacteria or fungus was greater in normal

*A dose of 1 5 mg of retinol used in studies of rats weighing approximately 200 g[39] is roughly comparable, based on either straight body weight or metabolic body weight ($Wt^{0.75}$), to administration of 200,000 IU of vitamin A (60 mg retinol) to a 10-kg human

mice pretreated with high doses of vitamin A palmitate, injected intraperitoneally,[61,62] as was macrophage phagocytosis after vitamin A was added to the diet [63] Semiweekly administration of vitamin A palmitate (totaling 16 mg, intraperitoneally, over five weeks) enhanced the ability of rats to clear a sublethal dose of *S typhimurium* from blood and tissues,[64] as did pretreatment with a diet very high in vitamin A [65] These treatments, however, also resulted in an elevation of serum retinol [64] An intriguing connection between normal chylomicron vitamin A metabolism and macrophage function is suggested by the observation that macrophage-like cells of bone marrow assimilated and then released chylomicron vitamin A [55,66] Since the quantity of chylomicron vitamin A in the circulation is related directly to recent ingestion of the vitamin, the amount of vitamin A available to chylomicron-metabolizing cells could be great following administration of large oral doses of the vitamin

Regarding the therapeutic action of vitamin A in children with severe measles, it is unclear whether it involves the adjuvant properties or the nutritional activity of the vitamin, or both Indeed, the pharmacological and physiological modes of action may be very difficult to distinguish in the vitamin A-deficient individual Based on studies of both vitamin A-deficient and -sufficient animals, it seems plausible that high doses of vitamin A not only provide nutritional rehabilitation (resulting in release of retinol and its binding protein from liver, delivery of retinol to tissues, and subsequent repair of mucosae), but may also activate phagocytes and/or T cells in an adjuvant-like fashion, as observed in vitamin A-sufficient animals Each of these mechanisms has the potential to contribute to clinical improvement

Identification of and Requirements for Immunoregulatory Retinoids

Which vitamin A metabolites regulate immune functions? Isomers of retinoic acid (all-*trans*, 9-*cis*) have been shown to regulate gene expression or cell differentiation both in vivo and in vitro,[22] and the concept is now well accepted that this oxidized metabolite of retinol (Chapter 8) is the principal activated form of vitamin A Therefore, it was surprising when Buck et al [67] reported that a metabolite of retinol may have regulatory functions in the immune system In in vitro assays of the growth of virus-transformed human B cells[67] and the proliferative response of thymocytes or T cell lines,[68] retinol-containing serum or exogenous retinol was required, in contrast, retinoic acid had little activity A new metabolite of retinol produced by the transformed B cell cultures was isolated, identified as 14-hydroxy-4,14-*retro*-retinol (14-HRR), and shown to have growth-promoting properties on these B cells [69] From these studies, Buck, Hammerling and coworkers[68,69] proposed that retinoids at the oxidation level of retinol (e g , 14-HRR) (Fig 9–2) function as regulators or costimulatory molecules in the immune system

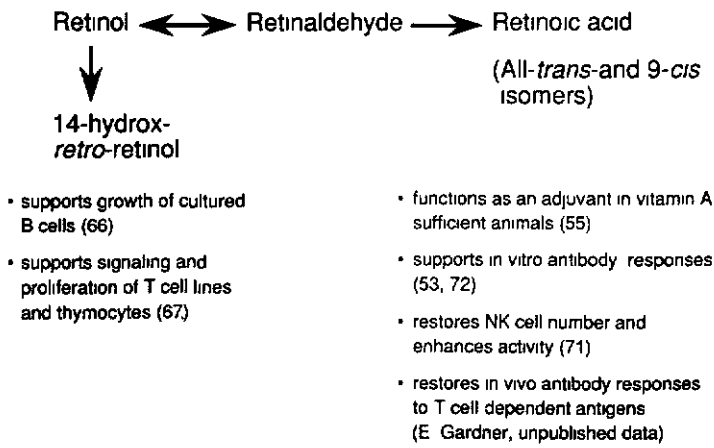

Fig 9–2. Potential role of retinoids as regulators of the immune system

In contrast, other results from in vitro and in vivo experiments support a role for retinoic acid Retinoic acid has not been shown to be reduced to retinol in vivo (Fig 9–2), and therefore it is not expected to function as a precursor for retinol metabolites The growth-promoting activity of retinoic acid in vitamin A-deficient animals is well known [70] The hematocrit, white blood cell count, and the lymphocyte transformation response, all reduced in vitamin A deficiency, were increased toward normal after administration of retinoic acid [71] The number of immune cells, including lymphocytes and NK cells,[71,72] was also normalized in previously vitamin A-deficient rats fed a retinoic acid-supplemented diet Retinoic acid also appears to adequately support the antibody response to TD antigens in vitro and in vivo When retinoic acid was added to TD antigen-primed cells from vitamin A-deficient mice, the antigen-specific IgG1 response was restored [54] Compared with retinol, retinoic acid was approximately 100 times more effective, leading Chun et al [54] to conclude that "retinoic acid is probably the physiological metabolite for sustaining IgG immune responses in vivo " Other in vitro studies showed increased antibody production by mitogen-stimulated B cells in the presence of retinoic acid-treated T cells [73] In vivo, repletion of vitamin A-deficient rats with oral retinoic acid increased their anti-tetanus IgG response to a level comparable to retinol-repleted rats (E Gardner, D Arora, and A C Ross, unpublished data)

As these contrasting results indicate, exactly which retinoids have immunoregulatory properties under physiological conditions is not yet clear Whether one or more metabolites of retinol, other than retinoic acid, is essential for immune responses in vivo deserves further consideration, but this idea requires reconciliation with the ability of retinoic acid to restore complex immune responses in retinol-depleted animals

Is the role of retinoids obligatory? Certain immune responses do not seem to require that the host's vitamin A status be normal For other immune responses that are low during vitamin A deficiency, the requirement for vitamin A apparently can be obviated by a high level of certain cytokines As noted previously, the antibody response to lipopolysaccharides (TI-*1* antigens) was normal in vitamin A-deficient rats [16] This observation, and evidence that lipopolysaccharides may function as adjuvants toward TD responses, prompted us to ask whether the antibody response to TD or TI-*2* antigens could be increased in vitamin A-deficient rats through simultaneous immunization with lipopolysaccharide When vitamin A-deficient animals were immunized with tetanus toxoid and *P aeruginosa* lipopolysaccharide, or with pneumococcal polysaccharide and lipopolysaccharide, their specific anti-tetanus[74] or anti-capsular polysaccharide responses[75] were markedly elevated Because lipopolysaccharides are known to elicit the release from macrophages of a number of cytokines (among which TNF and IL-1-beta are predominant), these data led us to question whether administration of purified cytokines might also stimulate immunity in the absence of retinol Indeed, when vitamin A-deficient rats were immunized with tetanus toxoid and treated with recombinant TNF, their anti-tetanus IgG response was increased more than eightfold [74] Similarly, the cytotoxicity of NK cells of vitamin A-deficient animals was activated, similar to normal NK cells, after induction of IFN release in vivo or by the addition of IL-2 or IFN to leukocytes in vitro.[36] Nevertheless, the combination of stimulation with TNF and provision of oral retinol at the time of immunization resulted in an even greater anti-tetanus response in previously vitamin A-deficient rats (D Arora and A C Ross, unpublished results) One interpretation of these results is that, although TNF is stimulatory by itself, the full activity of this cytokine in the anti-tetanus response requires the presence of vitamin A Similarly, the addition of IFN-gamma to anti-CD3-stimulated T cells in vitro circumvented the requirement for retinol in T cell proliferation, but the combination of IFN-gamma and retinol produced the largest stimulation [68]

Together, the studies illustrate that antigen-specific responses to both TD and TI-*2* antigens are possible during vitamin A deficiency as long as additional strong stimuli are provided Further, these observations raise the possibility that purified cytokines, or other biological response modifiers that elicit cytokine production in vivo, may be useful as adjuvants to vaccination and would be effective even in the vitamin A-deficient condition

Conclusions

Although the exact role of vitamin A in maintaining immunocompetence has yet to be made clear, a few broad generalizations can be drawn from the results described in the preceding section First, vitamin A plays an important part in maintaining the lymphocyte pool Whereas lymphocyte counts are generally low during vitamin A deficiency, administration of retinol to vitamin A-deficient

animals or humans, or of retinoic acid to retinol-depleted animals, increases the number and/or proportion of lymphocytes in blood and spleen This change, occurring over several days, would be consistent with a likely role for vitamin A in the proliferation and differentiation of progenitor cells of the lymphocytic lineage In animals treated with retinoic acid, lymphocytes of both B and T lineages and subsets of both $CD4^+$ and $CD8^+$ cells increased concomitantly, in normal proportions Similarly, the number of NK cells, an immunoregulatory subpopulation of lymphocytes, was substantially decreased by vitamin A deficiency, but increased to normal values following retinoid administration Although lymphocytes are among the best characterized of cells with regard to their surface receptors and adhesion molecules, knowledge of lymphocyte subsets and their functions is still incomplete It is clear that surface features can change rapidly, for example, during the course of cell activation, and it is possible that future studies will reveal subpopulations of cells that are particularly dependent on vitamin A

Second, there is strong and consistent evidence that vitamin A functions in T cell-mediated responses This requirement is made evident in vitamin A deficiency by low cell-mediated immune responses measured by a number of different assays, and by low antibody responses to a variety of T cell-dependent and T cell-regulated antigens Where supplementation with vitamin A has been studied, responses have almost uniformly been restored It is remarkable that some immune functions are well preserved in the vitamin A-deficient state, for example, that total antibody levels are not reduced The discrepancy between antigen-specific and total antibody production implies that vitamin A is not fundamental per se to the synthesis and secretion of immunoglobulins Similarly, the ability of vitamin A-deficient animals to mount a strong cytokine response after appropriate stimulation also leads to the conclusion that at least portions of the "response machinery" are not seriously impaired The low level of antigen-specific, T cell-dependent responses supports the contention that vitamin A plays some type of signaling role unique to the activation or propagation of antigen-specific responses, perhaps related to the proliferation and clonal expansion of antigen-specific cells

Another paradox—the failure to produce a normal antibody response and, at the same time, the formation of long-term memory—suggests that there may be some particular defects involving signaling or interactions between subpopulations of T and B cells Despite the formation of memory to antigen in vitamin A-deficient animals,[39,40] a normal secondary response occurred only after deficient animals were repleted with vitamin A before reimmunization [39] Thus, even though memory T cells appear to have been generated, they required retinol (or retinoic acid) to activate the B cells' antibody production in the secondary response, similar to the requirement observed for the primary antibody response

There is evidence from a small number of studies that the requirement for vitamin A in T cell-dependent responses may be circumvented when the production or availability of certain cytokines is high Normal antibody responses and

NK cell activation have been observed in vitamin A-deficient animals following cytokine elicitation in vivo, and after addition of IFN-gamma to T cells cultured in the absence of retinoids in vitro A new hypothesis may be suggested from these data that vitamin A and cytokines function as costimulatory molecules, or as coregulators in the same signaling pathways [12] It is possible that vitamin A reduces the cytokine dose-dependency of certain immune responses, but that vitamin A produces no additional effect when high, saturating concentrations of cytokines are present

Third, there is reasonably good evidence that vitamin A can stimulate nonspecific immunity, such as the activation of macrophages Stimulation of phagocytosis and bacterial clearance has been observed in vitamin A-sufficient animals after administration of quantities of vitamin A that are clearly supra-physiologic The mechanism of this stimulation is unknown Regarding the use of vitamin A in humans, it is unclear whether the improvement seen, for example, in children with severe measles is due at least in part to the pharmacological properties of high doses of vitamin A

Now that the basic mechanism of action of retinoic acid on gene expression has been identified, it should be possible to make rapid progress in understanding which genes are responsive to retinoids, and whether regulation is tissue-specific In terms of the immune system, it will be important to learn which retinoids are formed within it, or taken up from plasma, and their mode of action Future investigations of these basic issues in retinoid metabolism should help clarify the specific biochemical and molecular actions of the retinoids, while clinical studies on the efficacy of vitamin A toward different types of infections should improve our understanding of this essential nutrient's therapeutic potential

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IV

Treatment

10

Treatment of Vitamin A Deficiency and Xerophthalmia

> I have no experience treating nightblindness by incarcerating sufferers in a darkened room, as advised by Wharton and Netter nobody can be astonished however that patients declare themselves [cured] after a longer or shorter confinement in places of the sort
>
> —S Stephenson, 1896[1]

> The effect [of cod liver oil on nightblindness and conjunctival xerosis] is very marked it appears, in fact, almost specific
>
> —S Snell, 1881[2]

> Virtual elimination of vitamin A deficiency and its consequences,
>
> *—Nutrition Goals for the Year 2000, World Summit for Children*

The distinction between "treatment" and "prevention" is somewhat arbitrary since *treatment* is always meant to *prevent* a more serious event As a practical matter, however, it is helpful to distinguish between the two

Optimal health requires the maintenance of normal vitamin A status, including adequate stores to withstand acute insults and seasonal dietary deprivation Prevention programs aim to sustain normal vitamin A nutriture among populations that would otherwise be deficient, particularly the most vulnerable groups (traditionally infants and young children from poorer socioeconomic strata) The initial consequence of such broad, population-based programs will be improvement in the vitamin A status of the population and a decline in the prevalence and severity of vitamin A deficiency Ideally, vitamin A deficiency will disappear entirely except for rare, isolated events that can be recognized and effectively

treated within existing health care services This might well serve as an operative definition of the stated goal of the International Conference on Nutrition [3]

Treatment differs from prevention in focusing on the *urgent* need of an *individual* for *prompt* improvement in vitamin A status to prevent imminent risks to sight and life (classic instances being xerophthalmia and measles)

Only a decade ago, "treatment" referred solely to the management of xerophthalmia [4–6] Admonitions to provide high dose vitamin A to children with severe measles, protein-energy malnutrition (PEM), or diarrhea were considered a form of "targeted prevention" for those at particularly high risk of further deterioration of vitamin A status [7] Newer knowledge concerning the risks that milder vitamin A deficiency poses to life and sight has expanded the scope and indications for "treatment "

Vitamin A status can be improved,[8] and mild xerophthalmia cured, within one to three weeks of instituting the frequent feeding of inexpensive foods rich in provitamin A carotenoids [9 10] But when sight and life are at imminent risk, as they are in xerophthalmia and measles, improvement in vitamin A status is a medical emergency requiring prompt administration of large-dose vitamin A supplements A recent study from Senegal indicated that supplementation with high dose beta carotene (1,200,000 IU) was equivalent to 200,000 IU retinyl palmitate in reversing abnormal impression cytology (within seven weeks of administration) [11] In theory, large dose beta-carotene has fewer side effects than vitamin A, but whether it produces the rapid clinical response needed to prevent further corneal destruction or measles mortality remains to be determined Even more questionable are the biologic availability and physiologic impact of beta-carotene provided as food rather than pure concentrate (Chapter 13)

Use of Systemic Vitamin A

As discussed earlier (Chapters 1, 8), regulation of circulating levels of physiologically active vitamin A is a complex affair governed, in part, by the availability of both retinyl esters and retinol-binding protein (RBP) In the rat, vitamin A deficiency results in a loss of liver stores, but accumulation of RBP to four times normal levels [12 13] Administration of vitamin A causes a prompt outpouring of holo-RBP and rise in serum levels in both animals and humans Severe protein deficiency can limit the availability of RBP and blunt the response [14 15] Regulation of vitamin A status of target tissues is probably even more complex (Chapter 8) Recent data suggest retinoic acid and even retinyl esters may enter cells directly without the need for transport proteins

Large-dose vitamin A is meant to correct systemic deficiency as rapidly as possible, inducing a positive clinical response Early recommendations advised intramuscular injection of water-miscible vitamin[7 16] to circumvent potential problems of malabsorption, particularly in children with intestinal parasites and

diarrhea, and because parenteral administration appeared to result in a greater increase in (total) serum A (retinol plus retinyl ester) It is now accepted, however, that oral vitamin A, whether water- or oil-miscible, is as effective as parenteral vitamin A Given its greater availability, safety of administration, and lower cost, oral vitamin A is generally the treatment of choice [17–19] Pirie has suggested that oral therapy is also less likely to produce side effects since the vitamin A is presented to the liver within its natural vehicle (chylomicra) and, being more slowly absorbed, tends not to produce toxic levels of circulating esters [20]

A randomized controlled clinical trial studied the relative effectiveness of intramuscular, water-miscible vitamin A (100,000 IU), followed the next day by an oral dose (200,000 IU), compared with purely oral dosing (200,000 IU on two successive days) The subjects were children with corneal xerophthalmia (severe vitamin A deficiency), many of whom suffered concomitant diarrhea, pneumonia, and PEM [4,14,21,22] The clinical response in the two groups was virtually identical, indicating they achieved comparable degrees of physiologically satisfactory nutriture at the level of the target tissues (Table 10–1) [14] This was supported by the biochemical response (Table 10–2)

As anticipated, total serum vitamin A (including esters) reached greater levels among parenteral recipients, though never the heights reported by Srikantia for non-xerophthalmic children [23] More important, the holo-RBP response (the form in which physiologically active vitamin A is transported) was virtually identical in the two groups These results are similar to those reported following parenteral dosing of severely malnourished xerophthalmic Indian children,[24] and a controlled trial of oral versus parenteral vitamin A dosing of non-xerophthalmic, vitamin A-deficient children with varying forms and severity of PEM [15]

At baseline, 90% of non-xerophthalmic controls had holo-RBP levels above 3 μg/ml, 90% of the children with corneal xerophthalmia (X2/X3) had holo-

Table 10–1 Clinical Response of Corneal Lesions among Double-Dose Recipients[a]

Days Since Therapy	*Worse*		*Static*		*Improved*		*Cured*		*Total*	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%[b]
1 Oral ×2	3	(6)	29	(62)	15	(32)	—	—	47	(53)
IM + Oral	7	(23)	16	(53)	7	(23)	—	—	30	(52)
2–4 Oral ×2	7	(10)	11	(16)	39	(57)	12	(17)	69	(78)
IM + Oral	3	(7)	14	(30)	23	(50)	6	(13)	46	(79)
6–8 Oral ×2	—	(—)	4	(6)	34	(51)	29	(43)	67	(76)
IM + Oral	2	(4)	—	(—)	29	(55)	22	(42)	53	(91)
12–16 Oral ×2	—	(—)	1	(2)	12	(24)	36	(73)	49	(56)
IM + Oral	2	(5)	0	(0)	11	(27)	28	(68)	41	(71)

[a]Oral therapy—200,000 IU oil miscible vitamin A PO on two successive days, IM—100 000 IU water-miscible vitamin A intramuscularly on day 1, followed by the oral dose the next day

[b]Percent of all eyes in series examined in the interval

From A Sommer [4]

Table 10–2 Serum Vitamin A and Holo-RBP Response among Double-Dose Vitamin A Recipients

		Vitamin A (μg/dl)		*Holo-RBP (μg/ml)*	
Time Since Initial Dose		*Oral*	*IM*	*Oral*	*IM*
0 h	$\bar{x}$	6 8	6 1	1 2	1 3
	SD	3 9	3 8	1 4	1 8
	n	35	16	35	29
4 h	$\bar{x}$	33 6	47 6	19 5	15 1
	SD	26 9	36 4	15 1	10 2
	n	36	16	23	11
1 day	$\bar{x}$	22 1[b]	107 7	12 1[a]	20 0
	SD	24 2	46 2	10 0	9 8
	n	35	10	22	13
3 days	$\bar{x}$	28 4[b]	152 8	18 6	21 6
	SD	34 1	86 5	12 4	9 8
	n	37	17	18	15
7 days	$\bar{x}$	22 9[b]	53 0	24 7	27 4
	SD	8 0	39 8	10 1	16 3
	n	37	18	20	16

Limited to cases of corneal involvement included in the analysis of clinical response, on whom at least two values were available Oral 200 000 IU oil-miscible vitamin A at 0 hours and 1 day IM 100,000 IU water-miscible vitamin A intramuscularly at 0 hours, plus 200,000 IU oil-miscible vitamin A orally at 1 day

[a]Difference between oral and parenteral (IM) group statistically significant at $p < 05$

[b]Difference between oral and parenteral (IM) group statistically significant at $p < 001$

From A Sommer et al[4,14]

RBP levels below 3 μg/ml[4,22] Within four hours of the first oral dose, 90% of the xerophthalmic children had levels ≥ 5 μg/ml The only difference in the pattern of biochemical response was the mild drop in serum vitamin A and holo-RBP among oral recipients preceding the second oral dose (T_4–T_{24}) As noted, this had no effect on the clinical response, which is hardly surprising even at T_{24}, over 90% of oral recipients had holo-RBP values > 3 μg/ml[22]

Diarrhea reduces absorption of orally administered vitamin A[25,26] Recent studies with tritium labeled vitamin A indicate children without diarrhea absorbed 94% of a dose of 100,000 IU water-miscible vitamin A administered orally, and retained 87% Children with diarrhea (five to ten loose stools per day) absorbed 74% and retained roughly 70%[27] Hence, while absorption of a large oral dose is reduced in acute diarrhea, a substantial proportion is still absorbed, sufficient to cause a rise in serum vitamin A[27,28] This is to be expected The liver of a healthy, 7 5-kg child between the ages of two and three contains roughly 30,000 IU vitamin A[20,29,30] Even if absorption were reduced 70%[31] from the use of oil-

miscible preparations[32,33] and the presence of concomitant diarrhea and intestinal parasites,[25,34–36] a 200,000 IU dose would still deliver more vitamin A than the liver normally contains As would be anticipated, children in the corneal xerophthalmia clinical trial[14] who had diarrhea not only healed as quickly on oral (PO-PO) as on parenteral (IM-PO) vitamin A, but almost as quickly as children without diarrhea [4,37]

Because protein deficiency reduces synthesis and release of RBP from the liver,[38] it can blunt the response to a massive dose of vitamin A [39–41] Smith and co-workers reported that 100,000 IU parenteral vitamin A failed to elicit an RBP response in Thai children with xerophthalmia and PEM [42] However, most studies have found some response, even if it is suboptimal [15,22,24]

As reported elsewhere,[15] the holo-RBP response in the Indonesian randomized treatment trial was closely related to baseline protein status (Table 10–3) [4,22] The lower the baseline serum albumin, the lower the initial holo-RBP response, regardless of the route of administration But a statistically significant holo-RBP response occurred even among children with severe PEM This rise in physiologically active holo-RBP can be masked by the presence of incompetent, denatured apo-RBP if only total (immunologically detectable) RBP is measured [24,42,43]

Although protein deficiency may limit the retinol and RBP response,[38,43–46] it clearly does not eliminate it [15,22] The initial clinical response of corneal xerophthalmia in children with PEM (albumin < 3 g/dl, transferrin < 50 mg/dl, weight-for-height < 70% or pedal edema) was almost as good as in better-nourished children,[4,37] indicating that their holo-RBP response, even if blunted, was nonetheless clinically effective However, the clinical response was not as well maintained

One case (200/011) was particularly illustrative A severely malnourished two-year-old with corneal xerosis and ulceration refused hospitalization and received only a single oral dose of vitamin A [4] Despite persistently depressed albumin (1 5 g/dl–1 7 g/dl) and transferrin (≤ 20 mg/dl–25 mg/dl), he mounted a brisk (if short-lived) holo-RBP response (Table 10–4) and was free of all clinical evidence of active xerophthalmia by day 9 By day 15 he developed punctate keratopathy and by day 23, bilateral corneal ulceration Holo-RBP had dropped to 0 A second oral dose was given, along with a high protein diet and liquids Both corneas improved by the following day Four other severely malnourished children suffered clinical relapses three to four weeks after initial treatment and a favorable response (two had received PO-PO and two had received IM-PO vitamin A) [4]

The three modern measles intervention trials support the effectiveness of large-dose oral vitamin A One trial employed oil-miscible vitamin A[47], the other two, water-miscible vitamin A [48–50] In all three trials, the children suffered from moderate to severe measles, and many also had diarrhea, pneumonia, and significant PEM The beneficial impact of vitamin A was profound in each study (Chapters 2, 3)

Table 10–3 Holo-RBP Response in Cases of Corneal Xerophthalmia (μg/dl)

Time Since Dose (hours)		*Serum Albumin (g/100 ml)* ≥ 3.5	3.4–3.0	2.9–2.5	< 2.5
ORAL DOSE[a]					
0	n	9	6	6	6
	$\bar{X}$	1.7	1.3	0.3	1.3
	SD	1.4	1.2	0.5	1.0
0–4	n	9	6	6	4
	$\bar{\Delta}$	+26.2[c]	+14.5[c]	+8.7[c]	+5.8[c]
	SD	17.2	3.4	5.0	1.7
4–24	n	10	4	7	3
	$\bar{\Delta}$	−10.7[b]	−8.0[b]	−3.6	+0.3
	SD	15.2	4.2	6.0	3.2
IM DOSE					
0	n	2	6	9	—
	$\bar{X}$	0.0	1.3	0.9	—
	SD	0.0	0.8	0.9	—
0–4	n	2	6	9	—
	$\bar{\Delta}$	+26.5[c]	+12.3[c]	+6.7[b]	—
	SD	7.8	6.3	8.2	—
4–24	n	1	8	6	—
	$\bar{\Delta}$	−3.0	+1.1	+9.2[b]	—
	SD	—	7.0	8.9	—

[a]Oral 200,000 IU oil-miscible vitamin A PO at time 0 IM 100,000 IU water-miscible vitamin A intramuscularly at time 0 $\bar{\Delta}$ are all paired comparisons Cases at 0 h are those available for paired comparison at 4 h $\bar{\Delta}$ correlated with serum albumin category

Oral change at 0–4 h, $p < .02$, change at 4–24 h, $p < .001$ IM change at 0–4 h, $p < .01$, change at 4–24 h, $p < .05$

[b]Net change ($\bar{\Delta}$), $p < .05$

[c]Net change ($\bar{\Delta}$), $p < .01$

From A Sommer et al[4,27]

Not only is high-dose oral vitamin A as effective as parenteral, water-miscible vitamin A, but it is also cheaper, safer, more widely available, and does not require needles and syringes (with their attendant risks of blood-borne infections) Oral vitamin A is therefore the systemic preparation of choice Although parenteral vitamin A is considered an option for children unable to swallow or with severe, repeated vomiting, such instances are extremely rare It was not necessary to use injectable preparations in any of the measles and corneal xerophthalmia treatment trials, though occasionally nasogastric tubes facilitated hydration, feeding, and administration of vitamin A

If parenteral therapy is contemplated, only *water-miscible* preparations should be employed Oil-miscible injections can occasionally reverse xerophthalmia, but the clinical response is delayed and the serum retinol response, even to 200,000

Table 10–4 Serial Blood Chemistries Case 200/011

Time Since Therapy[a]	*Serum Vitamin A (μg/dl)*	*Serum Holo-RBP (μg/ml)*	*Serum Carotene (μg/dl)*
0 h	3	0	9
4 h	16	6	6
1 day	9	4	10
2 days	16	—	14
4 days	31	7	9
7 days	11	12	12
23 days	—	0	—

[a]200 000 IU vitamin A in oil orally

From A Sommer [4]

IU in divided doses, is negligible (Table 10–5) [4] If systemic vitamin A therapy is necessary and the only available preparation is oil-miscible parenteral vitamin A, it should be administered by mouth[1]

The potential side effects of large, multiple doses of vitamin A, in appropriate amounts, are negligible compared with the benefits of treatment None of the measles or corneal xerophthalmia trials recorded significant or persistent toxicity

Table 10–5 Serum Vitamin A among Single-Dose Vitamin A Recipients

Time Since Initial Dose[a]		*Treatment Regimen*[b] *Oral*	*IM (water)*	*IM (oil)*
0 h	$\bar{x}$	8 0	9 7	5 8
	SD	4 7	5 1	4 2
	n	10	11	12
4 h	$\bar{x}$	41 4	70 0	6 0
	SD	32 6	44 1	4 5
	n	7	11	12
1 day	$\bar{x}$	28 0	108 7	7 6
	SD	13 6	47 3	10 0
	n	9	11	9
3 days	$\bar{x}$	30 5	83 5	9 4
	SD	33 5	46 7	6 1
	n	6	11	10
7 days	$\bar{x}$	24 4	28 0	15 9
	SD	10 3	6 7	13 4
	n	5	5	7

[a]Limited to cases of corneal involvement included in the analysis of clinical response Holo-RBP response omitted because numbers very small Serum vitamin A in μg/dl

[b]Oral 200 000 IU oil-miscible vitamin A PO IM (water) 100,000 IU water-miscible vitamin A intramuscularly IM (oil) 200 000 IU oil-miscible vitamin A intramuscularly

From A Sommer [4]

Of thirty xerophthalmia cases in which the posterior pole of the eye could be seen within two days of initial dosing, only two children (6 7%) developed transient, low-grade papilledema [4] A malnourished three-month-old was inadvertently given 200,000 IU on two successive days He developed a bulging fontanelle but not papilledema,[4] supporting the evidence from Doppler studies that bulging fontanelles in young infants in response to large-dose prophylactic vitamin A is evidence of increased intracranial fluid, but *not* necessarily significantly increased intracranial pressure (Chapter 15) [51 52] Rosales and Kjolhede reported young children who received multiple massive doses over a brief period of time (one, a twelve-month-old, received 1 6 million IU in divided doses over three weeks) without evidence of toxicity [53]

Treating Xerophthalmia

Xerophthalmia is a manifestation of moderate to severe vitamin A deficiency It is therefore a medical emergency, as much to prevent death as to prevent blindness

Systemic Vitamin A

For millennia, nightblindness has been treated with substances rich in vitamin A Ancient Egyptians, Hippocrates, Paul of Aegina,[54] and Galen[55] all recommended liver, a popular folk remedy in India,[56] Japan,[57] and Europe Wolf[55] observed that the Papyrus Ebers (c 1520 B C) did not mention eating the liver, but rather, recommended applying it directly to the eye In speculating on how the vitamin A made its way from the conjunctival sack in the front of the eye to the retina behind, he suggested that it might trickle down the lacrimal duct and eventually be absorbed Topical application of liver juices remains a popular remedy in rural Indonesia,[58] where the mode of action is more obvious after completing the prescribed topical "treatment" as in ancient Egypt, the patient is fed the remaining organ Although the Indonesian villager does not consider the latter action an essential part of therapy, Aetius[59] prescribed exactly this sequence

During the nineteenth and early twentieth centuries, clinicians became increasingly familiar with the therapeutic value of fish liver oil, especially that of the cod [2 57 60–63] Schiele[64] discovered that he could cure xerophthalmia in breast-fed children by feeding cod liver oil to their mothers

Both Mori[57] and Blegvad (cited by Bloch)[65] administered cod liver oil parenterally with allegedly beneficial results Bloch[65] was convinced that the addition of parenteral to oral therapy speeded clinical recovery, and recommended its use in cases of corneal involvement Oily injections were once widely used and are capable of temporarily curing xerophthalmia,[66–70] but as we've seen (Table 10–5), biochemical studies indicate that relatively little vitamin A is actually released from the injection site [4 23 69]

Oral vitamin A is clearly the clinical preparation of choice [17–19] The first large dose should be administered immediately upon diagnosis or suspicion of xerophthalmia, and an additional dose administered the following day (Table 10–6) This appears to reduce the potential relapse rate A third, "prophylactic dose" is provided within one to four weeks to further "top-up" liver reserves While data are scarce that directly compare the protective value of two versus three doses, recent studies suggest that a single dose of 200,000 IU protects xerophthalmic children longer than a 100,000 IU dose[71 72], and that administration of two sequential doses, one week apart, is superior to a single dose, even if the first dose is relatively small (25,000 IU) [73] Presumably the first, "priming" dose improves absorption and retention of the second, larger dose (Chapter 15)

Active xerophthalmia begins to respond almost immediately, regardless of whether or not the child had diarrhea or significant PEM In closely monitored controlled trials,[4 14 37] 70% of corneal lesions (X2/X3) improved within four days, and 95% within one week (Table 10–1) The vast majority were entirely healed within two weeks The early response among single-dose recipients was virtually identical to those who received doses on two successive days, however, it was not as prolonged, particularly among children with significant PEM the relapse rate among single-dose recipients was nearly four times as great as among children given two doses (38% versus 10%) [4,22]

Case 200/003 A four-year-old boy with an initial albumin of 2 7 g/dl presented with localized necrosis in the right eye He received 100,000 IU water-miscible vitamin A IM, both eyes were healing well by day 5 Serum vitamin A was 8 μg/dl on admission, rose to 145 μg/dl by day 1, and declined to 30 μg/dl by day 4 On day 6, however, the tissue covering the perforation in the right eye began to thin, by day 12, the perforation was twice its original size Active pulmonary tuberculosis was confirmed radiologically and classical varicella skin lesions appeared Serum vitamin A had fallen to 17 μg/dl He was given 200,000 IU vitamin A orally By day 19, serum vitamin A had risen to 32 μg/dl, and healing of the perforation had resumed When seen on day 54, serum vitamin A was 29 μg/dl

Table 10–6 Recommended Xerophthalmia Treatment Schedule—Oil-Miscible Oral Vitamin A

	< 1 Year of Age	*≥ 1 Year of Age*[a]
Immediately	100,000 IU	200 000 IU
Next day	100,000 IU	200,000 IU
2–4 wk later	100,000 IU	200,000 IU
Severe Protein Energy Malnutrition (PEM)		
Monthly until PEM resolves	100,000 IU	200,000 IU

[a]Women of reproductive age require special attention because of the potential teratogenic effects of very high dose retinol early in pregnancy As corneal lesions are a severe, medical emergency, apply the full therapeutic regimen described above If the woman is otherwise healthy and only suffering from nightblindness or Bitot's spots treat instead with daily doses of 10,000 IU orally for two weeks [19]

and the right eye entirely healed, with an inferonasal adherent leukoma and clear pupillary zone [4]

It seems likely that the original, single IM dose provided insufficient vitamin A to withstand the added stress of chickenpox[74] exacerbated by active tuberculosis [75] However, double dosing does not entirely eliminate the problem of early relapse, particularly in severely ill, malnourished children Corneal relapses were observed in four such children, three of whom died shortly thereafter Others[60 76 77] have reported cases in which the corneas responded rapidly to systemic vitamin A even though the children died soon afterward Osborne[78] recalled the same phenomenon in animals

The single most important determinant of mortality among treated corneal cases was severe PEM (Table 10–7) A quarter of these children died, most within the first month Only 4% of better-nourished children died (usually more than a month after initial treatment) [4]

Protein and Calories

As has been seen, xerophthalmia will respond to isolated administration of vitamin A even in the presence of significant PEM [4] Mori noted clinical improvement "soon after" administering cod liver oil to children with kwashiorkor Reddy[79] described the disappearance of corneal xerosis after giving vitamin A to children with PEM who refused hospitalization Community-based prophylaxis trials result in marked reductions in the incidence of xerophthalmia,[80] including

Table 10–7 Mortality among Treated Cases of Active Corneal Disease

Duration of Follow-Up	*Not Severely Malnourished*				*Severely Malnourished*[a]				*Total Cases*			
		Deaths				*Deaths*				*Deaths*		
	Total	*N*	*(%)*	*Cum %*	*Total*	*N*	*(%)*	*Cum %*	*Total*	*N*	*(%)*	*Cum %*
1 wk	96	0			65	4	(6 2)	6 2	161	4	(2 5)	
2 wk	92	0			59	2	(3 4)	9 6	151	2	(1 3)	3 8
1 mo	90	1	(1 1)	1 1	57	4	(7 0)	16 6	147	5	(3 4)	7 2
2 mo	81	0		1 1	50	2	(4 0)	20 6	131	2	(1 5)	8 7
3–4 mo	74	2	(2 7)	3 8	48	1	(2 1)	22 7	122	3	(2 5)	11 2
5–6 mo	55	0		3 8	45	0		22 7	100	0		11 2
7–8 mo	41	0		3 8	33	1	(3 0)	25 7	74	1	(1 4)	12 6
9–10 mo	28	0		3 8	24	0		25 7	52	0		12 6
11–12 mo	24	0		3 8	18	0		25 7	42	0		12 6
13–14 mo	14	0		3 8	11	0		25 7	25	0		12 6
Total		3		3 8		14		25 7		17		12 6

[a]*Severe malnutrition* defined as any of the following edema, albumin < 2 5 g/dl, or weight-for-height < 70% of standard

Difference in mortality between the two groups $p < 01$

From A Sommer [4]

keratomalacia [81] A substantial proportion of the children who benefitted, particularly in the slums of Hyderabad, must have been significantly malnourished

While the xerophthalmic response can be attributed to the small but definite rise in holo-RBP, retinol[82] and retinoic acid can apparently enter at least some cell lines even when free of the RBP transport protein

Treating PEM is an important component of xerophthalmia therapy, both to prolong the response and to reduce associated morbidity and mortality

Case 200/147 A two-year-old presented with complete necrosis of the right cornea and a 2 mm ulcer in the left She was severely malnourished, with frank anasarca, serum albumin of 1 9 g/dl and transferrin ≤ 20 μg/ml She was given 200,000 IU vitamin A orally on two successive days The ulcer was definitely improved by day 3 and essentially healed by day 5 At two weeks the ulcer appeared to be deteriorating, but the mother insisted the child be discharged When next seen, at three weeks, serum albumen was still only 1 8 g/dl and a central ulcer occupied 75% of the corneal surface An additional oral dose of vitamin A was given A week later, the ulcer had completely healed, leaving a dense leukoma

Repeated massive dosing with vitamin A every two to four weeks is advisable until protein status improves [18 19,22]

Physicians, like patients, have a fascination with injectable preparations They must be repeatedly reminded of the advantages (in terms of safety, practicality and efficacy) of oral vitamin A [83] Otherwise, they run the risk of inadvertently using oil-miscible preparations parenterally [84]

One unusual circumstance forms an exception, largely relevant only to wealthy countries the poor vitamin A stores of ventilator-dependent very low-birth-weight infants (< 1300 g) may be more effectively raised during the first seven days of life by frequent, small, periodic doses of water-miscible vitamin A by IM injection rather than by mouth, though not necessarily to a clinically significant degree [85] As already indicted, some individuals with severe malabsorption, usually from cystic fibrosis, may require periodic parenteral supplementation, though most can be adequately managed with large-dose oral therapy (with or without enzyme replacement)

Topical Vitamin A

Despite its long popularity as a folk remedy,[55 58] topical administration of vitamin A is neither recommended nor regularly employed Systemic administration, required to reach distant sites and build up liver stores, also induces corneal healing [57 68 70 86 87] Unfortunately, the corneal response to systemic therapy is often delayed two to four days, during which time the cornea may deteriorate further,[69,87–90] especially among children with severe PEM Pirie[91] suggested that PEM-associated depression of RBP synthesis might be responsible for this delay,

and demonstrated that topically applied retinoic acid had a beneficial effect on the cornea of vitamin A depleted rats Topical retinoic acid speeds reversal of corneal keratinization in vitamin A-deficient rabbits, whether administered alone[92,93] or in conjunction with systemic vitamin A[94] Retinoic acid supports normal epithelial differentiation[95] and apparently does not circulate in association with a specific transport protein[96,97]

Shortly after the initial report by Pirie, a controlled clinical trial of topical retinoic acid in conjunction with systemic vitamin A was carried out in Indonesia[89,98] Topical retinoic acid clearly speeded corneal healing among eyes randomized to receive it, however, more rapid healing rarely made a clinically significant difference and ulcerated eyes treated with topical retinoic acid occasionally healed with denser scarring[98]

Subsequent animal studies suggest that weaker preparations of retinoic acid[93] or retinol itself[92,99,100] might be less irritating and equally effective in reversing keratinizing metaplasia of the cornea Given the relatively small practical advantage of adjunct topical vitamin A therapy, the potential for increased scarring (especially problematic in centrally ulcerated corneas), and the difficulty of maintaining sterility and adequate supervision in the field, topical retinoic acid (or retinol) probably has a limited role until further studies better define appropriate formulations and dosage Whether retinoic acid offers any advantages over retinol is unclear in addition to the animal studies noted above, Bors[86] reported that vitamin A drops (150,000 IU/ml) every two hours provided symptomatic improvement in an adult case (but so did topical chloramphenicol[101], suggesting a local lubricant effect of the vehicle) Recent studies indicate that vitamin A is present in tears of humans and animals,[102,103] is actively secreted by the lacrimal gland,[104] and that oral supplementation of mildly deficient children results in increased levels of retinol in tears[105] Considering the prevalence of reduced tearing in xerophthalmia (Chapter 4) and the possibility that retinol can directly enter some cell lines, tears may play an important role in the normal delivery of vitamin A to the ocular surface If so, topical delivery of an appropriate vitamin A formulation might one day prove a useful *addition* to our therapeutic armamentarium Since vitamin A deficiency is a systemic condition, with widespread systemic implications, topical vitamin A will never be more than adjunct therapy to mandatory systemic administration

Adjunct Therapy

A variety of other therapeutic modalities can prove decisive[18,106] In addition to the correction of vitamin A deficiency and PEM, children require appropriate management of concomitant systemic infections (e g , gastroenteritis, respiratory infections, malaria, tuberculosis) and dehydration. Adequate lid closure is necessary to prevent corneal exposure in semistuporous or comatose patients, and can be achieved by temporarily taping the lids shut While it is unlikely that

bacterial infections initiate xerophthalmic ulceration, they can certainly exacerbate it (Chapter 4) Careful, sterile techniques should be used when approaching the globe and consideration given to the use of broad spectrum topical antibiotics to prevent secondary bacterial infections Any evidence of corneal infection should be vigorously treated with appropriate topical and systemic antibiotics

Surgical Intervention

In general, active xerophthalmia does not lend itself to surgical intervention Ben-Sira et al [107] reported successful use of "covering grafts" in children with active xerophthalmic corneal ulceration In 48/50 eyes, the anterior chamber was eventually restored In 41/50 eyes with eccentric ulcers, the corneas eventually healed with clear pupillary zones These results are no different from those of our (entirely nonsurgical) series The vast majority of ulcers are eccentric and become plugged with iris, thereby preserving the anterior chamber They subsequently heal rapidly on vitamin A therapy

Complete corneal necrosis precludes any possibility of corneal grafting In those few instances in which localized ulcers are too large or central for spontaneous maintenance or restoration of the anterior chamber, the angle is usually already lost by the time the child first presents

Treating Measles

As has been made clear, measles can be a devastating disease and high-dose vitamin A is an effective means of reducing attendant morbidity and mortality (Chapters 2–4) The World Health Organization and UNICEF recommend immediate administration of a large dose of vitamin A to all children with measles from communities where vitamin A deficiency is a "recognized problem" or where measles case-fatality rates are 1% or greater [108,109] While a single large dose may be adequate, all three controlled measles intervention trials administered a large dose on two successive days[47,48], the Durban trial provided additional doses on days 8 and 42 [49,50] Until controlled trials demonstrate that a single dose is just as effective as a dose on two successive days, it is probably prudent to follow the double-dose schedule

Since children with complicated measles often come from vitamin A-deficient communities (to which they return), the full xerophthalmic treatment schedule should probably be administered (Table 10–6) [19]

Because of the recent resurgence of measles and measles deaths in Europe and the United States,[110] consideration should be given to the judicious use of supplemental large-dose vitamin A in the treatment of measles among individuals from high-risk communities, as recommended by the American Academy of Pediatrics [111]

Treating Other Infections

There is as yet no consensus on treating other illnesses with vitamin A (other than as a convenient means of targeting prophylaxis to children likely to be[112] or to become deficient) On the other hand, since a host of data suggest a close and potentially causal relationship between vitamin A status and the severity and chronicity of some infections (Chapter 3), high-dose vitamin A therapy might be considered potentially therapeutic in selected cases The strongest evidence exists for *chronic* dehydrating diarrhea Community intervention trials regularly reduced diarrhea-specific mortality[113–116] (Table 2–12) and morbidity[116–118] Whether high-dose treatment has the same effect once diarrhea sets in, as reported decades ago by Ramalingaswami,[119] remains to be seen Considerable attention is being given to combining vitamin A supplementation with oral rehydration[27] One study reported that vitamin A treatment had no impact on the course of non-cholera, watery diarrhea[120] This is hardly surprising since the episodes terminated among placebo recipients within two days, leaving little opportunity for demonstrating an effect, and serum vitamin A levels were unchanged by supplementation Nonetheless, the results are consistent with the apparent absence of any significant impact of community prophylaxis on the incidence of frequent, trivial diarrhea (Chapter 3)

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Assessment and Prevention

11

Assessment of Vitamin A Status

> We know too much to take the older views as to the criteria of [vitamin A] malnutrition seriously, and we know too little to lay down specific rules as to what criteria should be used according to recent knowledge
>
> —E Mellanby, 1934[1]

Faced with the challenge of "virtually eliminating vitamin A deficiency and its consequences,"[2] health officials must determine the existence, severity and extent of vitamin A deficiency in the population Approaches range from accepting findings from similar countries or environments[3] to preliminary "case-finding" and, where warranted, detailed population-based investigations of vitamin A status and its determinants These are essential in identifying a problem and its cause, and in establishing a baseline against which to assess the impact of subsequent control measures [3] The reliability of such assessments ultimately depends on the validity and interpretation of the measures of vitamin A "status" employed

Vitamin A Deficiency as a Public Health Problem

In 1974, specific prevalence rates of xerophthalmia (among preschool-age children) were recommended as criteria for the existence of a "public health problem "[4] These were revised in 1982[5] to reflect the relative ratio of prevalence rates actually observed in a large-scale field survey (Table 11–1) [6] The choice of criteria arose from concerns of the time

- The main issue was identifying countries in which potentially *blinding* vitamin A deficiency was a significant problem

Table 11-1 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 (1982 Revision)

Criterion	*Minimum Prevalence*
CLINICAL (PRIMARY)	
Nightblindness (XN)	1.0%
Bitot's spots (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/liter (10 μg/dl)	5.0%

From WHO Technical Report Series 672, 1982.

- Since clinical prevalence rates spoke directly to this principal concern, they seemed the most appropriate criteria for establishing policy and evaluating progress
- Xerophthalmia was the only "pathognomonic" manifestation of vitamin A deficiency, and was unlikely to be confused with other etiologies
- Where vitamin A deficiency was a serious problem, xerophthalmia prevalence rates were anticipated to be relatively high
- Biochemical criteria were only of secondary value because of their tenuous relationship to the main public health concern and to continuing issues of reliability and analysis
- Dietary intake data were considered too imprecise to serve as the basis for major policy initiatives, and for that reason, they were not included as acceptable criteria

We now need new approaches. Since more children die than are blinded each year as a consequence of vitamin A deficiency, while still others are at increased risk of serious infection and other health problems, more sensitive indices are required to recognize communities where these broader public health problems may exist.

Practical, reliable, field-based techniques for assessing vitamin A status are increasingly in demand. Not so much to determine whether particular *individuals* need vitamin A—at 4¢ for 200,000 IU it will always be easier, cheaper, and safer to assume that they do—but as a way of identifying deficient *populations* that require community-based intervention (Chapters 12–15). Despite this focus, the validity of any assessment technique ultimately rests on its ability to distinguish between populations of differing vitamin A nutritional status.

Vitamin A Status

Olson divides vitamin A status into five categories[7] "deficient," "marginal," "satisfactory," "excessive," and "toxic" As we are principally concerned with the more massive and compelling problem of *inadequate* vitamin A, we will restrict this discussion to that end of the spectrum

Traditional views of vitamin A status no longer apply "Deficiency" has been characterized as 'the presence of xerophthalmia," and "marginal" as "inadequate *reserves* of vitamin A in the absence of xerophthalmia "[7] However, it is now clear that children suffer consequences of inadequate vitamin A nutriture long before they ever become xerophthalmic increased rates of severe infection, anemia, mortality, and quite possibly growth retardation (Fig 11–1)[8]

Xerophthalmia represents relatively *severe* deficiency If "marginal" has any precise meaning, it is the absence of deleterious effects of vitamin A deficiency under ordinary conditions, but a degree of adequacy so precarious that relatively mild events (e g , prolonged diarrhea, childhood exanthems, and an unusually extended interval between harvests) can sufficiently undermine vitamin A status as to result in functional disturbances secondary to deficiency A *sufficient* state is one in which there are adequate reserves to protect against deficiency during unusual periods of stress or transient reductions in vitamin A intake Most countries' recommended nutrient intakes (RNI) (variously named recommended dietary allowance [RDA], recommended dietary intakes [RDI], reference nutrient intakes [RNI]) seek to provide a nutrient status well within the "satisfactory"

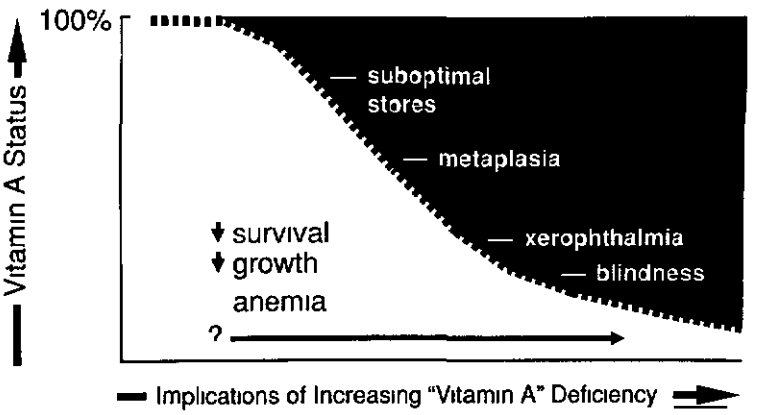

Fig. 11–1 The effect of declining vitamin A nutriture on vitamin A-dependent clinical status The shape of the curve is illustrative only Reduced survival, growth, and hematopoesis appear before the onset of xerophthalmia, however, the point at which they first begin to be affected, and the quantitative relationship between their severity and vitamin A status, have not been established (From A Sommer[8])

(sufficient) range [9] Olson has provided a detailed and convincing argument that "satisfactory vitamin A status, meeting all physiologic needs plus a four-month reserve, [is] achieved at liver concentrations of 20 μg vitamin A/g liver (0 07 μmol/g), which is readily achieved by consuming 600 to 700 retinal equivalents (RE) per day by women and men respectively, and 400 RE by preschool-age children" (Chapter 8) [10]

It is easier to define status than to demonstrate it! Recognition that deficient children (without xerophthalmia) are at increased risk of life-threatening infections and death comes mostly from observations on the response of large groups of children to vitamin A supplements—not from special tests identifying increased susceptibility to disease While a large number and variety of tests of vitamin A "status" exist, none precisely and accurately detects when a particular child has passed from the state of physiologic "adequacy" to "deficiency " Increased knowledge gained from molecular biology might some day change that Vitamin A has already been shown to influence the expression of over 300 genes Administration of oral vitamin A to deficient rats can, within one to four hours, measurably alter a wide variety of gene products [11] Once we recognize which gene products are affected early in "deficiency," at a time when vitamin A nutriture first becomes a limiting factor to normal metabolic functions essential for health and survival, we will be in a position to define the exact threshold of "deficiency " It may well be that other metabolic alterations will prove even more sensitive, providing a means for defining "marginal" status either expressed by alterations in gene products or metabolic function *not* essential to health and survival, or by some form of "stress test" that predicts the severity of insult (either the degree and/or duration of increased metabolic demand, or decline and duration of vitamin A intake) necessary to precipitate such changes Olson, on the other hand, would define status on the basis of total vitamin A stores rather than on measure of functions (James A Olson, personal communication, October 1994)

Meanwhile, until we gain more knowledge we remain dependent on relatively gross, indirect indices such as vitamin A ' intake" (which by itself is rarely an indicator of status), retinol concentration of a variety of tissues (e g , blood and liver), and vitamin A-dependent functions altered relatively late in the course of deficiency All such indices are far removed from the ideal threshold determinants described above, and each has serious practical limitations

It is perhaps easiest to think of the available indices in relation to one another and to some hypothetical state of "satisfactory" vitamin A nutriture (Fig 11–2) But that is all it is—an "idealized" conceptual framework Actual comparisons of the prevalence of the various indicators do not always demonstrate higher rates for those thought to be affected earliest [12] Some of the variance from the "ideal" no doubt reflects confounding factors that affect the individual indicator (e g , infection on serum retinol),[7] homeostatic mechanisms that tend to maintain normal function (and to a lesser degree, serum retinol) in the face of falling

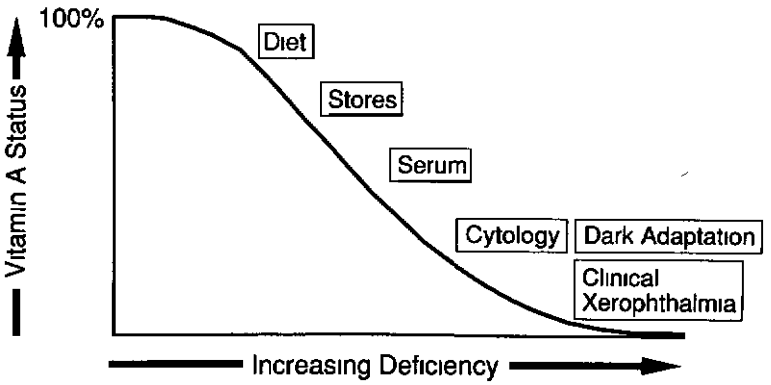

Fig. 11–2. Schematic relationship between the decline in vitamin A status and the onset of potentially relevant indicators of inadequate vitamin A nutriture The shape of the curve is illustrative only

vitamin A reserves, and the idiosyncracies of various investigations, cut-off criteria, and environmental variables

Dietary Intake

In theory, vitamin A intake should be a leading indicator of vitamin A status Since consuming too little vitamin A is bound to result in vitamin A deficiency, this fact forms the basis for the variety of officially sanctioned dietary recommendations [9] While they provide guidance for government food policies, these recommendations fall short in that they assume the needs of an "average" population and are not intended for assessing individual status The major problem is the imprecision that pervades the entire system (one reason they are not called "requirements") The kernel of human data upon which the recommendations rest was largely obtained on small numbers of healthy adults under controlled conditions [10 13 14] Such information is likely to differ significantly from the needs of children in developing countries, varying as they do in metabolic demand, protein-energy malnutrition (PEM), and in the influence of repeated infections, parasitic infestations, the form of the dietary sources and the impact of other food items (especially oils) on bioavailability, absorption, storage and transport of the vitamin Therefore, even in the absence of other confounding issues, there is little data that directly and precisely relate food intake to *requirements* of different Third World populations

A number of practical issues further compound the problem the vitamin A content of particular foods varies with the local form of that food, its degree of maturation (ripeness), and how it is prepared for consumption For example, at least three carefully controlled studies have recently failed to demonstrate that

supplements of cooked green leafy vegetables or carrots materially affect serum levels of either beta-carotene or retinol (Chapter 13),[15–17] although the same is not true of purified beta-carotene [15,16] Food composition tables, moreover, are often inaccurate (Chapter 13) Finally, and perhaps most difficult and imprecise, is establishing exactly how much vitamin A-containing food individuals actually ingest over clinically relevant periods of time under routine field conditions [18]

Several vitamin A dietary-assessment techniques have been advocated to identify individuals and populations at risk of vitamin A deficiency[19–21], but few have been extensively correlated with indices of vitamin A *status* The simplest and most practical food frequency assessment (used in the Indonesian studies of 1976–1979[6,22]) identifies relative differences in dietary pattern *between groups* of children shown by other indices (xerophthalmia, serum retinol) to differ in their vitamin A status [6 22–29] There is less evidence that practical field tools exist for identifying differences in vitamin A intake (let alone status) of populations in *absolute* terms

One widely used prototype[19] has sometimes proved difficult, time-consuming,[30] and poorly correlated with other measures of vitamin A status [31–33] Yet, in an Indonesian study, three food items alone, not necessarily those that provided the most vitamin A, were best correlated with maternal vitamin A status,[34] providing useful clues to directions in which dietary intervention should move Careful development of locally appropriate tools may suggest simpler approaches

Dietary data can certainly be predictive in extreme situations (such as famines, absence of breast-feeding, dietary exclusion of vitamin and provitamin A-rich foods) Dietary assessment might also prove helpful in educating populations about proper foods and designing effective intervention strategies,[19 20] but these are other issues

Indices of Vitamin A Status

Tissue Concentrations of Vitamin A

Tissue concentrations provide a more direct measure of bodily vitamin A content than do estimates of dietary intake

Serum Vitamin

As already noted, serum retinol has the longest history and probably the widest use as a measure of vitamin A "status."[35–39] It correlates well with the prevalence and severity of xerophthalmia (Table 1–3) and reflects changes in vitamin A status secondary to intervention [40–46] For over three decades specific labels have equated serum retinol levels with vitamin A "status" (Table 1–3), though given

biologic variation they are more appropriately applied to populations than to the classification of individual subjects

While these traditional labels can provide useful guidelines, they can also be misleading The earliest manifestations of *clinical* dysfunction secondary to vitamin A deficiency can occur at levels above 30 μg/dl,[14] though these are uncommon and mild Traditional hallmarks of xerophthalmia (XN, X1B) occur at levels above 20 μg/dl, though increasingly so below Severe (corneal) xerophthalmia begins to occur at levels under 15 μg/dl, particularly below 10 μg/dl, a range at which xerophthalmia is common (Table 1–3) In counterpoint, the *average* serum retinol level among Indonesian children *without* xerophthalmia in 1978 was 20 μg/dl (Table 1–3), yet at that level vitamin A supplementation can have a profound impact on childhood morbidity and mortality (Chapters 2, 3)

Considering the health significance of milder degrees of "inadequacy," the classification scheme needs to be revised, perhaps along the following lines < 15 μg/dl, "severe" deficiency (high risk of blinding keratomalacia and death), 15 μg/dl–25 μg/dl, "moderate" deficiency (increased risk of milder xerophthalmia, serious infection and death), 25 μg/dl–40 μg/dl, "marginal" deficiency, ≥ 40 μg/dl, "adequate/sufficient " While serious dysfunction is probably rare at levels above 30 μg/dl, any overt stress (e g , measles) or prolonged dietary deprivation can result in frank and potentially serious physiologic and clinical consequences [47] Local characteristics, particularly the prevalence of chronic infections like malaria, might require modest adjustments in these classifications

Since we are interested in criteria for identifying *populations* at risk, we need to choose prevalence rates of specific serum concentrations that place the population at risk of vitamin A deficiency-related disturbance (ill health) sufficient to justify comunity intervention Currently, the only traditional and widely accepted biochemical criterion is a 5% prevalence of serum retinol levels < 10 μg/dl—an infrequent rate at the extreme range of deficiency As this commonly accompanies a 40% or greater prevalence of serum retinol levels < 20 μg/dl, the existing index could be made more sensitive and the field test more practical a 40% or greater prevalence of serum retinol levels < 20 μg/dl needs only one-eighth the sample size required to establish the presence of a public health problem identified by the current 10 μg/dl criterion However, a number of caveats apply The first is that the distribution of vitamin A values in the pediatric population will not always follow the same curve For example, many children may have values at or just below 20 μg/dl, with few if any below 10 μg/dl This may not be important, as we now know 20 μg/dl is itself "inadequate" and associated with significant health risks On the other hand, the Ghana VAST trial[46] achieved a 20% reduction in mortality with a relatively modest increase in mean serum retinol (~10%–18%), but a sharp decline in prevalence of the lowest levels (Fig 11–3) (Ghana VAST Survival Study Team, personal communication, September 1994) If serum retinol levels can be obtained and accurately determined, their distribution in the community can probably differentiate popu-

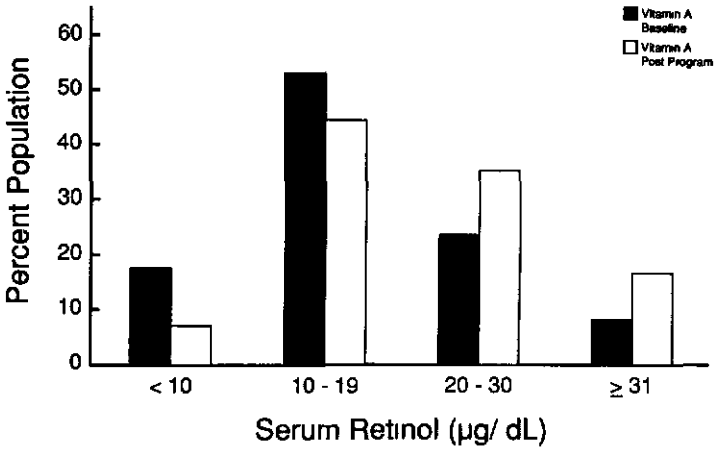

Fig. 11–3. Serum retinol distribution of study population of preschool children at baseline and at the end of vitamin A intervention program The figure includes only those children randomized to receive vitamin A supplements, once every four months (From the Ghana VAST Survival Trial [personal communication, Ghana VAST Survival Study Team, September 1994])

lations at high and low risk of "inadequate" vitamin A status, a shift in their distribution can be used to assess the potential impact of an intervention program (Chapter 14) [48]

Flores et al [49] suggest that an appropriate criterion for a "safe" serum retinol distribution is provided by their experience with a population of deprived Brazilian children following supplementation with vitamin A Supplementation eliminated the lowest values and all relative dose-response (RDR) positive tests without changing the basic shape of the distribution curve This is an interesting finding If it can be generalized, one might obtain the same insights by simply using the prevalence of serum values < 30 µg/dl However, the "deprived" children in Flores's study had serum retinol levels equal to or greater than those of the general U S childhood population *before* they received their vitamin A supplement [50] This unlikely scenario highlights the vagaries of biochemical measurements even by highly respected laboratories

An important issue relates to the "stability" and validity of serum retinol as an index of vitamin A status After all, blood is merely a medium for transporting vitamin A from (largely liver) stores to target cells A variety of factors influence serum retinol concentrations independent of the size of those stores or the adequacy of vitamin A-dependent function In general, as vitamin A stores are depleted to maintain delivery to target cells, circulating levels of vitamin A are sustained disproportionate to the decrease in liver reserves [51–54] By the same token, as stores become seriously depleted, the rate of vitamin A metabolism declines (at least in rats), and hepatic release slows [55] A variety of other factors

influence the rate of holo-RBP (retinol-binding protein) release from the liver, including liver function,[56] other organ disease,[57] protein status,[58,59] the adequacy of other nutrients (like zinc[60]), and infection and other metabolic insults.[61,62] Further, cellular metabolism affects the rate at which vitamin A is "consumed." Fever, infection (measles is a classic example), and other factors can all cause transient changes in serum concentration unrelated to any change in vitamin A "stores."[62,63] Filteau et al.[61] estimate malaria eradication could have a dramatic impact on serum retinol distribution in Ghanian children by influencing serum concentration regardless of vitamin A "status" (or liver stores). In Papua, New Guinea, where xerophthalmia is rare but malaria widespread, half the preschool-age children allegedly have serum retinol levels below 10 μg/dl.[64] Whether this is true or laboratory artifact, it raises serious questions concerning the value of serum retinol biochemical determinations and their derivatives. The only certain relationship between serum retinol levels and body reserves is that *high* circulating levels of vitamin A preclude significantly depressed stores.[65]

None of these caveats necessarily precludes the use of serum retinol as an important (perhaps currently the ideal) index of vitamin A status given existing alternatives. One might, for example, be able to achieve more precision by adjusting for confounding systemic morbidity through simultaneously measuring acute phase proteins.[61] Nevertheless, major logistical issues still arise. These include the feasibility of collecting and handling blood samples under representative field conditions, particularly given growing concerns about transmitting HIV infection and hepatitis, and the reliability of the determinations. Serum analyses are notoriously susceptible to variations in collection, transport, and storage of specimens, and the choice of laboratory procedures.[66] A new system for collecting and transporting a "blood spot," subsequently analyzed for its holo-RBP content, might well minimize some of these logistical impediments.[67] Results correlated well with serum retinol determined by high performance liquid chromatography (HPLC) ($r = 0.9$).

- What has been measured and reported as "vitamin A" varies widely. Modern laboratories strive to report levels of serum "retinol." Too often, "total" vitamin A (including retinyl esters) are included in the measurement, and laboratory methods are themselves finicky. Several techniques are used for measuring serum vitamin A.[66,68–71] Older laboratories still rely on the Neeld-Pearson technique and its variants. At most modern laboratories, high performance liquid chromatography (HPLC) has become the method of choice. Still, results from different laboratories often vary significantly. (Variability of 25 laboratories measuring vitamin A levels of human sera by HPLC reported by NIST. Personal communication, L. Riva, February 14, 1990.) The problem is compounded in developing countries where sophisticated training and facilities are unavailable or difficult to maintain.

- Despite seemingly reasonable stability under carefully controlled laboratory conditions,[72] the manner in which samples are collected, transported, and stored in the field seems to have a marked impact on the measured results

Thus, variations in techniques result in wide variations between even the most dedicated laboratories, less sophisticated laboratories, or instances in which determinations are conducted at considerable distance from the site of sample collection, pose additional problems As a result, criteria for "normal" serum levels are best adjusted to local standards and validated by whatever means is most suitable to local conditions (e g , compare samples drawn two weeks apart from vitamin A-supplemented and -nonsupplemented children)

An alternative, simpler method for gauging serum "vitamin A" concentrations is the immunologic assay of circulating levels of RBP [57 73] RBP analyses are methodologically simpler than those for retinol, but can be less meaningful Immuno-assay of RBP does not distinguish between holo-RBP (RBP to which a molecule of retinol is attached) and the "bare" apo-RBP carrier The latter continues to circulate in significant amounts, even in the presence of severe vitamin A deficiency [6 74 75] Nonetheless, as we now seek more sensitive indices of inadequacy than in the past, the failure of RBP levels to track retinol into the lowest ranges may no longer pose the same handicap it once did, and higher thresholds (reflecting "mild deficiency" or "marginal" to "satisfactory" status) may prove more revealing But criteria for such levels have yet to be determined, and circulating RBP is depressed in protein malnutrition and other transient metabolic insults (like infection) [6 58 75]

A specialized technique for specifically assaying holo-RBP was developed by Glover and colleagues [74] While accurate and precise, it proved complex HPLC determination of serum retinol (essentially all of which is bound to RBP) is simpler and has since replaced Glover's method, although the new "blood spot" technique might revise its value [67]

Liver Vitamin A (including RDR and MRDR)

Under normal circumstances, 90% of vitamin A reserves are stored in the liver[10 65 76 77], in severely deficient individuals it may account for as little as 50% of total stores [7] Liver vitamin A should therefore directly reflect the availability of vitamin A to meet bodily needs As long as stores are high, there is sufficient vitamin A to meet any reasonable contingency, from increased metabolic demand to prolonged dietary deprivation As such, the liver is perhaps the best measure of "adequacy" of stores as they relate to "satisfactory" status, though an inability to transport and use those stores might result in functional "deficiency" at the level of target tissues

High liver stores can coexist with depressed serum levels, presumably because protein deficiency limits the synthesis of RBP and release of holo-RBP [78–80] Similar phenomena may explain depressed serum retinol in the face of elevated acute phase proteins [61] High liver stores and low serum levels also exist in primary biliary cirrhosis [56] Whether these secondarily depressed serum levels have functional consequences for target cells is unknown

Correlations between serum and liver vitamin A concentrations in animals and humans and comparisons with total vitamin A stores determined by isotope dilution suggest liver vitamin A concentrations of $\geq$ 20 μg/g (0 07 μmol/g) represent "satisfactory" reserves [10,53 66 81] All the healthy adults in Furr's isotope dilution studies exceeded this level, most by three to five times [81]

In recommending liver vitamin A concentration of 20 μg/g as a criterion for "satisfactory" status, Olson[10] points out that at this level

- clinical signs of deficiency have not been encountered,
- RDR tests are negative, suggesting sufficient stores to maintain steady-state plasma retinol levels,
- retinol kinetics indicate this is sufficient to protect an adult from deficiency during transient periods of metabolic stress or over an extended period (four months) of a vitamin A-free diet

Liver samples are generally obtained from the central portion of the right lobe, either at the time of surgery or autopsy [51 53 82–86] Despite considerable variation in the distribution of vitamin A in the liver with age and vitamin A content,[87] macro-samples[87] and even micro (needle) biopsy of as little as 7 mg of liver tissue[88] reportedly provide reasonably accurate estimates of overall liver concentrations

The problem is that people autopsied, even those who died in accidents, are not necessarily representative of the target population Exactly who they represent is often unclear Unless one accepts laboratory error, it is hard to explain why 35% of apparently well-nourished and previously healthy New York adults would have liver vitamin A concentrations below 40 μg/g, and 8%, below 20 μg/g[51]—while in Recife, Brazil, where subclinical deficiency is common, all but one accident victim had normal stores [86]

Relative Dose-Response (RDR) The RDR is a novel approach to estimating "adequacy" of hepatic vitamin A reserves indirectly, without need for a liver sample [89] As already described, both apo-RBP and vitamin A are needed for the liver to release holo-RBP (the active transport form of retinol) into the serum When inadequate hepatic vitamin A is the limiting factor, apo-RBP accumulates in the liver, an exogenous bolus of vitamin A will result in a surge in holo-RBP release and a rise in serum retinol [90 91] Underwood and colleagues proposed to quantify this relationship to distinguish animals (and possibly hu-

mans) with low serum retinol secondary to inadequate hepatic reserves from those with adequate reserves in whom holo-RBP release was depressed for other reasons [89] The RDR is calculated as the ratio of the difference between baseline serum retinol (A_0) and serum retinol five hours after the test bolus of retinyl ester (A_5)

$$RDR = \frac{A_5 - A_0}{A_5} \times 100$$

Initial animal experiments suggested a correlation between RDR and liver vitamin A concentrations, but not a simple one RDR values > 40% were almost always associated with liver concentrations below 8 μg/g–10 μg/g, but some animals with lower liver concentrations had smaller RDRs Hence the "specificity" seemed reasonably good but the sensitivity poor One confounding variable was the baseline (pre-dose) plasma level Where these were low (< 30 μg/dl) the correlation was better Whether the problem is inherent in the use of a *relative* change or is intrinsic to attempts to simplify, standardize, and quantify a more complex and variable metabolic relationship (or both) remains unclear

While the test has received wide currency and use in special studies of human populations,[92] there are little consistent data to validate its assumptions or to identify its particular niche in the arsenal of population assessment techniques Most investigators accept RDR as a more sensitive index of suboptimal ("inadequate") vitamin A status than serum retinol, principally because subjects may have relatively "normal" serum retinol levels (according to existing nomenclature, ~20 μg/dl) while their liver reserves are low (< 20 μg/g) The problem in proving this, as in validating some of the functional tests of vitamin A adequacy discussed below, is the absence of a "gold standard" for vitamin A "status" against which tests (of function and reserves) can be compared Over one-third of pregnant women with a "normal" RDR (< 20%) had serum retinol levels < 20 μg/dl,[93] while more than half of Indonesian children with concomitant nightblindness and vitamin A-responsive Bitot's spots, as well as children with serum retinol levels < 10 μg/dl, had "normal" RDRs [94 95]

The only direct comparison between oral RDR and liver stores was conducted on twelve well-nourished adults,[96] only one of whom actually had a liver concentration < 20 μg/g (it was 14 μg/g), his RDR was 28% The next lowest reserves were 30 μg/g, yielding an RDR of 15% Both individuals were given vitamin A supplements, their RDRs subsequently dropped below 10% The authors suggested an abnormal RDR corresponds to 20% As in the previous rat experiments,[89] there was poor correlation between hepatic reserves and lower RDR levels an RDR of 11% occurred with an hepatic concentration of 58 μg/g, 0%, with 94 μg/g, and 8% and 12%, with 114 μg/g (Table 11–2)

A second comparison by the same group applied an intravenous bolus of vitamin A to children with liver disease [97] Six of the twelve children had initial

Table 11-2 Baseline Serum Retinol, Liver Vitamin A and RDR

Subject	*Initial Serum Retinol* μg/dl	*Liver Vitamin A* μg/g	*RDR (%)*
1	40	14	28
2	46	30	15
3	56	58	11
4	37	85	0
5	77	94	0
6	39	107	4
7	72	112	0
8	59	114	8
9	47	114	12
10	53	137	2
11	38	160	5
12	38	434	0

From O Amedee-Manesme et al [*]

liver concentrations < 20 μg/g, five of the six had positive RDRs ranging from 21% to 90% (median 43%) (The sixth "deficient" subject, with a liver level of 15 μg/g, had an RDR of 0%) None of the children with "adequate" liver concentrations had an RDR > 20% (somewhat surprisingly, the highest was only 4% and the median was 0%) This suggested that a liver concentration of 20 μg/g, recommended as a criterion for the lower limit of "safe" (therefore "adequate") status,[10] was actually a fixed, absolute, definitive threshold with little if any individual variation, tightly tied to an RDR of 20% Somewhat disconcertingly, however, RDR values among children with "inadequate" stores correlated poorly with their liver concentrations

While the above data provide a strong theoretical basis for the RDR test, the best correlative data, particularly under field conditions, comes from Brazil [47] Flores's meticulous comparison of serum retinol and RDR at baseline and following large-dose supplementation is perhaps the most convincing evidence of the reliability of the test when carefully performed and considered The RDR cutoff (20%) was customized to the variance observed in their own laboratory, and serum for baseline levels and RDR were collected on fasting children After the initial RDR, all children received 200,000 IU vitamin A and were retested at 30, 120, and 180 days In this instance, baseline RDR displayed an impressive monotonic ("dose-dependent") relationship between baseline serum retinol and the proportion of RDR positive children (Table 11-3)

All children with serum levels < 20μg/dl had an abnormal RDR, as did a majority of children with levels between 21 μg/dl and 29 μg/dl Thirty days after the large dose, mean and median serum retinol had risen, the distribution of serum retinol shifted to the right, reducing the number with low values (an

Table 11-3 Relationship Between Serum Retinol and RDR at Baseline and 180 Days Following Vitamin A Supplementation

	Day 0		*Day 180*	
Serum Retinol (μg/dl)	*RDR+ (≥ 20%)*	*Children (number)*	*RDR+ (≥ 20%)*	*Children (Number)*
≤ 20	100%	12	100%	8
21–29	86%	21	84%	19
30–40	26%	19	9%	22
> 40	3%	39	0%	11

From H Flores et al [47]

important outcome for intervention programs[44,98]), and the RDR response became normal in all subjects Abnormal RDRs did not return in significant numbers until 180 days, in roughly the same relationship to serum levels observed at baseline If this close relationship can be confirmed in other laboratories and under other field conditions, it would suggest the following

- RDR is indeed a useful surrogate for some predetermined level of "adequate" liver reserves, whether they correspond to 20 μg/g liver or not This would be a powerful tool for special studies requiring precise determination of the status of vitamin A reserves in individual subjects with otherwise "adequate" serum retinol levels
- The distribution of serum retinol in the target *population* (or the prevalence of values below 20 μg/dl or 30 μg/dl) may be as useful (and more practical) for identifying *populations* at risk of inadequate liver reserves and vitamin A deficiency The RDR adds little additional information on either individuals or populations when serum retinol values are low [99]

The same investigators made use of the unexpected occurrence of chickenpox in some of their subjects to demonstrate the dramatic impact of infection on serum retinol levels and RDR (Chapter 7) [100]

A number of issues remain to be resolved in interpreting and using RDR more widely the impact of protein status and cirrhosis on choice of an appropriate threshold[101–105], intra-individual variability (a critical issue if it is to be valuable in special investigations of individuals subjects, but one that is also dependent on the reliability of the laboratory performing the assay[106,107]), and the range of baseline serum levels at which it becomes a valid measure As Usha[99] points out, very low levels of A_0 (e g , 5 μg/dl–10 μg/dl) almost guarantee a large proportion of positive RDR values secondary to the impact of biologic and methodologic variation [108]

Exactly what RDR actually measures in terms of "liver adequacy" remains uncertain Nonetheless, results support clinical data and studies of liver kinetics that suggest retinol levels between 20 μg/dl and 40 μg/dl do not necessarily

denote "safe" or "adequate" vitamin A nutriture Both the RDR and its variants, particularly MRDR, are areas of intense investigation They will be plagued by the practical problems of obtaining valid and reproducible serum retinol values, and the cooperation of children who must remain under controlled conditions at the test site for the five-hour interval required

Modified Relative Dose-Response (MRDR) The RDR has some practical disadvantages apart from its reliance on potentially difficult serum retinol determinations children must be tested in a fasting state and two bloods must be drawn, separated by a five-hour interval To address at least one of these drawbacks, Tanumihardjo, Olson and colleagues developed a modified approach requiring only a single serum specimen [109 110] Orally administered 3,5 didehydroretinyl acetate is converted only slowly, if at all, to retinol (R) and will therefore not affect serum retinol values As dehydroretinol (DR), it will combine with apo-RBP stored in the liver, if the apo-RBP levels are elevated secondary to retinol deficiency, a surge of DR will appear in the serum The ratio of DR to R serves the same purpose as the RDR ratio

The test was easily applied to young, well-nourished children in the United States[111] and deficient children in Indonesia [112] Among apparently healthy American children, the DR-R peak plateau occurred between five and ten hours after their DR dose, only three of the twenty-four subjects had DR-R ratios > 0 03 at 5 hours [111] Two of the children with elevated ratios received vitamin A treatment and were retested two weeks later, their DR-R fell below 02 A criterion of > 0 03 at five hours suggests that 62% of children studied in West Java had less than adequate vitamin A reserves, a proportion only slightly higher than the prevalence of serum retinol values < 20 μg/dl [112] As with RDR,[47] the proportion of children with a positive MRDR was monotonically related to baseline serum retinol

Subsequent comparisons with abnormal conjunctival impression cytology (CIC) led the investigators to provisionally revise the DR-R threshold to ≥ 0 060 [20 59 113] This appears to be reasonably appropriate for lactating women with serum levels below 20 μg/dl [114] However, other studies from Indonesia,[115 116] India,[117] and Bangladesh[118 119] suggest further revisions may be in order The Bangladesh studies indicate that for the criterion chosen, an abnormal MRDR was far less prevalent than either an abnormal RDR or serum retinol (< 20 μg/dl) Since the response may depend in part on body size (and/or age), the originators of the MRDR test have suggested that "cut-off" criteria may be affected by the dose of DR-acetate employed [114]

Reproducibility (of individual classification) can be high [114]

Both RDR and MRDR are yet to be validated in severely malnourished populations But that is not necessarily where their value would lie Both may prove most useful in investigating vitamin A nutriture in individual subjects, where serum retinol alone may be a misleading index of vitamin A "adequacy"

(particularly when in the marginal range) Even with the simplicity of the MRDR test, however, it is unlikely either of these approaches will prove suitable for routine field use, where the major operational challenge lies in identifying *populations* likely to be vitamin A deficient

Isotope Dilution

The most precise assessment of vitamin A "reserves" should be the direct measure of total body stores This can be safely accomplished by the use of stable isotope dilution Unfortunately, the deuterium labeled isotope needed for the test is not readily available, sophisticated and expensive laboratory instrumentation (HPLC and gas chromatography–mass spectrometry) is required, and is not usually accessible in Third World countries, and several weeks are needed between administering the isotope and collecting blood samples in order for equilibrium to be achieved [120] Isotope dilution has not been used in field studies and is unlikely to prove practical under most routine field conditions

Functional Tests of Vitamin A Status

While vitamin A is essential for a host of bodily functions, two abnormalities in particular have attracted interest as potentially practical indices of vitamin A deficiency impaired dark adaptation and keratinizing metaplasia Both are relatively early abnormalities in relation to xerophthalmia, but both probably *follow* the decline in body stores because they represent vitamin A-delimited physiological disturbance of target tissues

Vitamin A is an essential component of rhodopsin, the visual pigment that composes the outer segments of rods These are photosensitive cells responsible for vision under low levels of illumination Deficiency results in impaired dark adaptation, at a clinically significant degree it is manifest as nightblindness (XN), which can often be recognized in children by questioning adults familiar with their behavior (Chapter 4)

A variety of new techniques have been employed to detect milder, "preclinical" evidence of impaired dark adaptation (longer duration until full adaptation and/or an elevated light threshold) Traditional techniques require sensitive and sophisticated equipment, 20–30 minutes of observation and recording, and a very cooperative subject Such tests are simply not practical under field conditions, particularly among young preschool-age children

Vision Restoration Time (VRT)

One new approach relies on the ability of a bleached eye to recognize a letter under low levels of illumination [41] After two minutes of exposure to 10,000

candela/mm^2, the time to identify a letter illuminated with 0 15 candela/mm^2 is recorded Trials in well-nourished schoolchildren suggested a normal value (±SD) of 122 ± 46 seconds A VRT of > 180 seconds (90th percentile) was provisionally suggested as abnormal (Note the inherent specificity will be, at best, 90%)

School-age children in Thailand with minimal to marginal vitamin A deficiency (and perhaps more significantly, zinc deficiency), a high prevalence of abnormal CIC (see below), and prolonged VRT received vitamin A and zinc alone and in combination, or placebo Vitamin A with or without zinc reduced CIC abnormality, but only zinc normalized the VRT [41] While it appears zinc is an important determinant of VRT, the value of the test for establishing vitamin A deficiency, particularly in preschool children, remains unclear

Scotopic Vision Tester

In his 1867 account of nightblindness in the Confederate army, Hicks[121] recognized that soldiers thought to be malingering were actually nightblind, based on the inability of their pupils to constrict normally at night when examined by candlelight Modern psychophysical studies indicate the weakest threshold of light visible in the dark-adapted state is roughly the same intensity needed to cause pupillary contraction (as recognized by a sophisticated pupillometer)[122,123], further, the two pupils normally respond together when only one is exposed to a light stimulus (direct and consensual response) These two observations led to the development of a simple instrument that briefly illuminates the retina of one eye (Ganzfield response) while the observer visually monitors the response of the other pupil under low levels of illumination [124] Studies on normal American children as young as one year established a "normal" threshold curve for children with brown irides [124] Studies on Indonesian[124] and Indian children[125] demonstrated a close correlation between vitamin A status and the light threshold that elicited a pupillary response or a verbal recognition that the stimulus was observed (Table 11–4)

Table 11–4 Pupillary Response to Threshold Light Stimulus

Population	*Mean Serum Retinol (μg/dl)*	*Mean Light Threshold (log candela/m^2)*[b]
Healthy American children	—[a]	− 1 335
Indonesian children, 6 mo following 200,000 IU vitamin A	24 3	− 0 985
Indian children with mild xerophthalmia (XN and/or X1B)	16 9	− 0 622

[a]Not measured, but healthy American children generally run levels > 35 μg/dl

[b]The lower the threshold, the more light-sensitive the dark-adapted retina Results determined from pupillary response Test for trend $p < 0001$

From N Congdon and A Sanchez et al [124,125]

Greater sensitivity (lower light thresholds) was needed for children to recognize a stimulus than for observers to record a pupillary response The greater amount of light required to cause a pupillary response than a visual one reflects the insensitivity of reproducibly recognizing a pupillary response without sophisticated pupillometric equipment Even though the visual thresholds were more sensitive than the pupillary thresholds, the two were closely correlated (children with the lowest light sensitivity by visual response tended to have the lowest light sensitivity measured by the pupillary response) As expected, pupillary responses could be reproducibly performed on younger children, often only one year of age, the more subjective, visual response required children three to four years of age Under field conditions (forty different villages), 90% of Indian children could be effectively tested for their pupillary response, over 72% of them as young as two years of age [125] There was a close relationship between the mean serum retinol and pupillary score (the higher the score, the brighter the light threshold and lower the retinal sensitivity) (Fig 11–4) [125]

Indonesian and Indian children with abnormal thresholds had higher RDR ($p < 01$) and lower serum retinol values ($p < 05$) than children with normal thresholds, thresholds significantly improved following large-dose vitamin A but not following placebo supplementation (Fig 11–5) [124]

Further testing is under way in other vitamin A-deprived populations using special night vision devices to better view the consensual pupillary response If these confirm the results in Indonesia and India, consensual pupillary threshold light response might well prove a valuable, practical field technique for identifying populations in need of a vitamin A intervention program, as well as a quantitative and convenient method for evaluating the impact of intervention A change in distribution of light thresholds, with a decline in the least sensitive (most vitamin

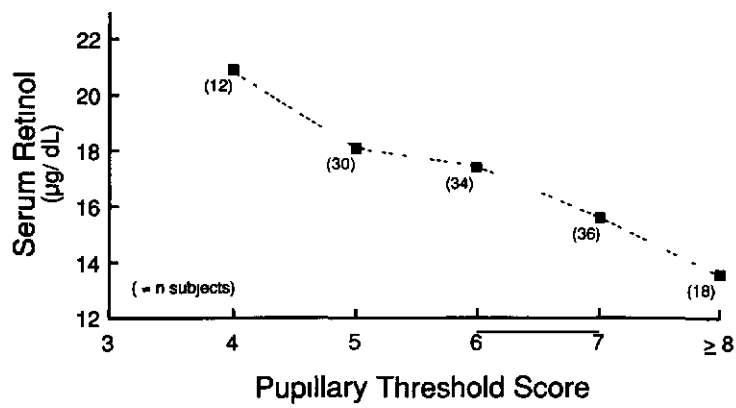

Fig. 11–4. Comparison of serum retinol level and pupillary threshold The lower the serum retinol, the higher the pupillary score—that is the brighter the light needed to elicit a response ($p < 01$) (From A Sanchez et al [125])

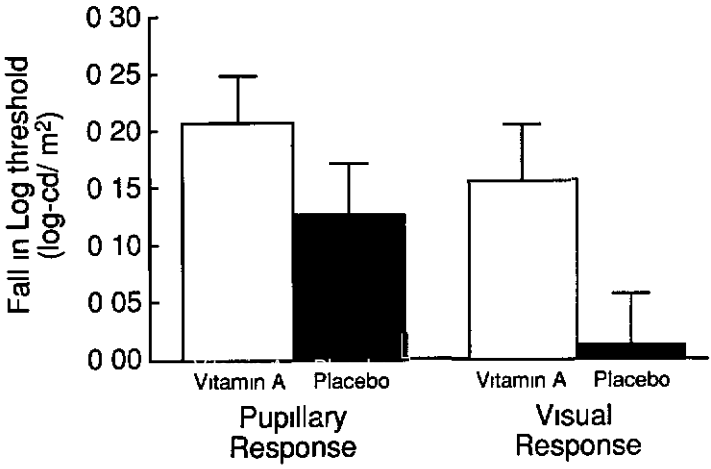

Fig. 11–5. Light threshold fell (e g , retinal sensitivity to light rose) after study children were supplemented with vitamin A, whether measured by the consensual pupillary response or by the subject's verbal indication of ability to distinguish a light stimulus Placebo supplementation was associated with a smaller, and statistically non-significant, change (From N Congdon et al [124])

A-deficient) part of the curve, would be even more meaningful and informative than simply a change in mean values

Conjunctival Impression Cytology (CIC)

Another approach to recognizing mild physiologic disturbance seeks evidence of keratinizing metaplasia, an early consequence of vitamin A deficiency in animals and humans (Chapters 1–4)

The concept is not new Alterations in vaginal smear cytology have long been used as an animal assay for vitamin A status, as well as for determining vitamin A "activity" of pharmacologic agents [126] In their first series of clinico-histopathologic correlations of vitamin A deficiency in children, Blackfan and Wolbach recommended the presence of keratinized epithelial cells in the scrapings of cornea, nose, mouth, and vagina as a diagnostic tool [127] Their suggestion was echoed by Sweet and K'ang,[128] and later by Agarwal [129] None of these studies, however, were quantitative in nature or validated against other indices of vitamin A status, none seem to have persisted beyond their original description

More detailed knowledge about the normal and deficient nature of conjunctival epithelium, particularly the geographic distribution of goblet cells[130] and their response to vitamin A deficiency,[131] has created renewed interest in this approach Unresponsive and responsive Bitot's spots[132] have an identical biologic appearance principally the absence of mucous-secreting goblet cells and the presence

of larger, irregular, keratinized cells (Chapter 4) [6,131] Of particular interest, eyes with localized but responsive lesions have histopathologic abnormalities that involve *all* of the bulbar conjunctiva [131,133] In contrast, the pathologic abnormalities in eyes with unresponsive lesions are limited to the area of the Bitot's spot itself Following oral vitamin A, the conjunctiva in eyes with responsive Bitot's spots return to normal [131,134] While this finding suggested a new approach to identify active vitamin A deficiency, the biopsies necessary to document these changes were clearly not practical under field conditions Preliminary trials with "scrapings" were unsuccessful, given contamination by keratinized cells of the lid margin and (potentially unresponsive) Bitot's spots

Egbert and Lauber,[135] Nelson,[136] and others described a system of 'simple conjunctival biopsy " A cellulose acetate filter briefly applied to the conjunctival surface will remove the superficial layers of the conjunctival epithelium By 1984, this surface "biopsy" technique (subsequently named "conjunctival impression cytology" [CIC]) had been applied to vitamin A-deficient rabbits *before* they became clinically xerophthalmic [137] The proportion of microscopic fields containing enlarged epithelial cells increased while the proportion containing goblet cells decreased in linear fashion as the animals became increasingly vitamin A-depleted, beginning at least four to six weeks before clinical changes first became apparent (Fig 11–6)

Initial trials demonstrated markedly abnormal CIC from clinically uninvolved conjunctival areas of children with mild xerophthalmia (XN, X1B) when compared with comparable specimens obtained from normal children or after vitamin A treatment (Plates 3–4) [138,139] A larger follow-up study revealed a close, monotonic relationship between the degree of CIC abnormality and serum vitamin A levels [139] The mean retinol among children with normal CIC specimens was 22 2

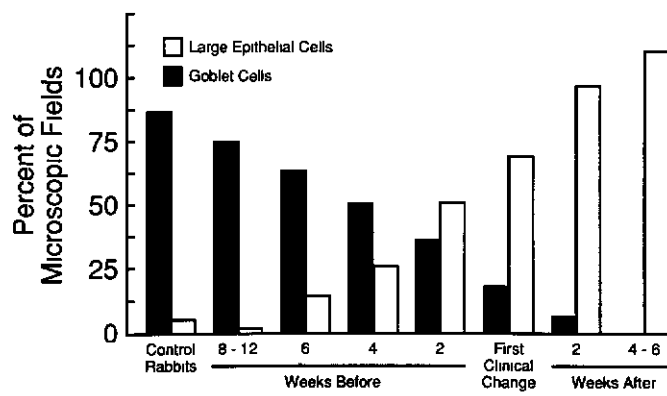

Fig. 11–6. As rabbits become increasingly vitamin A-deficient, the percentage of microscopic fields of conjunctival biopsies containing epithelial goblet cells declines and the percentage of fields containing large, irregular epithelial cells increases, even before the appearance of clinical abnormalities (From D L Hatchell et al [137])

± 5 5 μg/dl, versus 15 2 ± 4 5 μg/dl, among those with any degree of abnormality These data were subsequently analyzed to assess the prevalence of abnormal specimens in relation to the subjects, "true" vitamin A status [140] The analysis faced the same problems as the RDR test "validation" the absence of a reliable standard for vitamin A "status " In this instance, "status" was approached by stratifying all subjects according to the likelihood of severity of vitamin A deficiency as suggested by a combination of clinical and biochemical criteria (Table 11–5)

There is a strikingly close correlation between the prevalence of abnormal CIC and the severity of deficiency suggested by the composite indices of *relative* vitamin A status 93% of children with clinically responsive xerophthalmia and low vitamin A levels (further confirming active deficiency) had abnormal cytology At the other extreme, only 6% of children with normal eyes and serum levels (largely between 25 μg/dl and 29 μg/dl) had abnormal CIC, suggesting a specificity of approximately 5% to 10% This range was confirmed by samples taken from 100 healthy young women in the United States (J Humphrey and D Hazelwood, personal communication, 1995) The prevalence of abnormal CIC among children of less definite status fell between these extremes An important finding was that almost half the children without xerophthalmia but with serum levels < 20 μg/dl had abnormal CIC, suggesting a definite functional correlate to their "low" serum retinol levels Since half the children in Indonesia

Table 11–5 Prevalence of Abnormal CIC Versus Vitamin A "Status"

		Prevalence of Abnormal CIC	
Vitamin A Status	*Patient Characteristics*	*Number*	*Abnormal (%)*
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[a]	18	6

[a]Level > 30 μg/dl would have been preferred, but there were too few such subjects

From G Natadisastra et al [140]

(Table 1–3) and many other developing countries have serum levels in this range, it is likely that at least 25% of all children in these countries suffer demonstrable functional consequences of vitamin A deficiency (at the level of CIC) in the absence of clinically detectable xerophthalmia (Chapter 2)

A similar study was conducted by Coutsoudis and coworkers[141] (A Coutsoudis, personal communication, 1992) in South Africa The prevalence of abnormal CIC ranged from 78% among clinically normal children with serum retinol < 10 μg/dl to 6% among "normal" children with levels > 25 μg/dl—results extraordinarily similar to those from Indonesia

The validity of CIC has been further confirmed by the following comparison of liver concentrations and RDR in French children with normal eyes but hepatic disease[142], comparisons of abnormal CIC rates in xerophthalmic and non-xerophthalmic children in India[143,144] and Micronesia[145,146], correlation of CIC with serum and breast-milk retinol in Indonesian women and the response of all three parameters to vitamin A therapy[147], an equivalent (> 50%) reversion to normal within seven weeks among Senegalese children receiving high-dose vitamin A or beta-carotene[148], and by correlation of CIC with other parameters of vitamin A status[59,149–151] and manifestations of functional impairment commonly associated with vitamin A deficiency (anemia, otitis, stunting, chronic diarrhea, etc) [99,145,146,152] In one particularly revealing study, Usha and colleagues performed CIC and RDR on twenty-three children under five years of age who suffered persistent diarrhea but did not have xerophthalmia [99] CIC, RDR, and serum vitamin A were correlated with one another (Table 11–6) (Serum retinol levels seem extraordinarily low for children without xerophthalmia, reinforcing the likely variability of retinol determinations between laboratories or an extraordinary, if often transient, impact of infection on serum retinol, confounding the use of serum retinol in establishing the state of an individual's vitamin A nutriture)

While CIC is now widely employed around the world, a variety of modifications have been proposed to simplify procedures and/or improve the quality of the specimens obtained [153] This has been partially accomplished by substituting a small circular disk held by a suction device instead of the rectangular paper, making handling easier and more precise [154] It correlates as well or better than the strip technique with other indices of vitamin A status (Fig 11–7) [20]

Table 11–6 CIC, Serum Retinol and RDR in Persistent Diarrhea

Group	*Number*	*CIC*	*Serum Retinol (μg/dl)*	*RDR (%)*
1—Persistent diarrhea	17	abnormal	1 ± 1	88 ± 14
2—Persistent diarrhea	6	normal	8 ± 4	16 ± 12 (median = 21)
Normal controls	23	normal	19 ± 8	—

From N Usha et al [99] Subjects were all free of ' xerophthalmia "

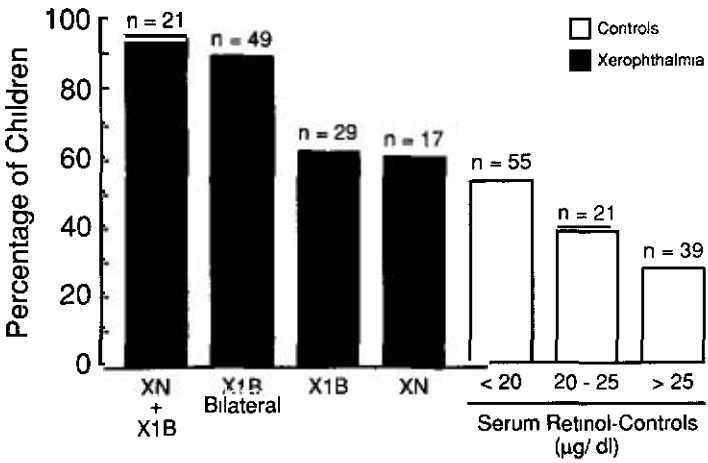

Fig. 11–7. Proportion of subjects with abnormal conjunctival impression cytology (CIC) Specimens obtained by the suction disk applicator technique from children of varying vitamin A "status" Essentially all children with combined nightblindness and Bitot's spots yielded abnormal specimens (From D G Keenum et al [154])

Another modification, introduced by Amedee-Manesme and co-workers, simplifies the subsequent processing of specimens by immediately transferring the collected epithelium from the acetate filter to a glass slide (impression cytology with transfer [ICT]) [155,156] While Amedee-Manesme and colleagues have found the technique useful and highly specific,[157,158] they have reported losing 7% to 20% of their specimens during the transfer process [159,160] As in CIC,[154] results have been affected by the presence of active conjunctivitis trachoma apparently increases the (false) positive rate [160,161]

Regardless of CIC technique, procurement of specimens is simple, even under field conditions,[153,154,162,163] as is the handling, storage, and transport of specimens They keep indefinitely in preservative Processing of specimens needs no more than running water and appropriate glassware and stains Interpretation of the specimens, however, remains nettlesome, requiring considerable training and standardization, with two to four days of "hands-on" experience A detailed manual,[153] while helpful, is no substitute for practical experience Reproducibility is high,[163–166] but variations in criteria, particularly of specimens intermediate in appearance (not clearly abnormal or normal), can have a significant impact on the presumed prevalence of deficiency

Despite the plethora of reports indicating a close correlation between CIC and other parameters of vitamin A status, and its response in controlled trials to vitamin A or beta-carotene (dietary) supplementation,[41,167] several reports have questioned its validity and/or interpretation [168] Just over 200 children in Guatemala were studied by CIC and RDR Significantly, perhaps, the population was more vitamin A "sufficient" than most others that have been investigated

only 8% had a positive RDR, 18% had a serum retinol < 20 μg/dl, and 13% had an abnormal CIC Even more important, the prevalence of RDR abnormality was lower than the prevalence of retinol deficiency, raising doubts about the reliability of the biochemical determinations This would explain the relatively small difference found between the serum vitamin A levels of children with normal and abnormal CIC (an experience inconsistent with most other reports) Finally, study analysis describes RDR as the "gold standard" for determining "sensitivity, specificity and positive predictive value" of CIC, which is as inappropriate as using CIC as the "gold standard" for calculating similar indices for RDR Not only does RDR have its own shortcomings, but even more significant, RDR is an indirect surrogate for liver (hence body) stores, while CIC is a measure of *functional* status at the tissue level What *is* important is whether these different indices distinguish *populations* at risk of vitamin A "deficiency" and its consequences, regardless of their correlation with one another on specific *individuals* In aggregate, most studies have found remarkably similar prevalence rates for abnormal CIC, serum retinol, and RDR for the *population* as a whole, even when the tests were abnormal in different *individuals* [113 124 166.169]

Essentially all studies that have followed CIC status serially report that abnormalities resolve within two weeks to two months after vitamin A supplementation, longer follow-up reveals CIC abnormalities frequently return by six months In some (if not most) instances this probably reflects an actual change in vitamin A status It must be remembered, however, that abnormal CIC is basically a microscopic Bitot's spot As such, some will persist even after vitamin A status has been normalized, while others will disappear but subsequently return for reasons that are poorly understood (Chapter 4)

Clinical Xerophthalmia

Xerophthalmia is the classical, but late, clinically recognizable expression of gross functional disturbance secondary to severe vitamin A deficiency Conjunctival xerosis (X1) is the visible equivalent of abnormal CIC Corneal xerosis and keratomalacia are more devastating consequences A history of XN, where a suitable, local term exists, is generally the most practical and prevalent manifestation of vitamin A deficiency The clinical manifestations of xerophthalmia are described in detail in Chapter 4 They are far less prevalent than subclinical functional abnormalities and biochemical evidence of deficiency and marginal stores [92 140]

Interpretation

As noted in the introduction to this chapter, our current understanding of the significance of vitamin A deficiency for health, sight, and survival requires that

we utilize better tools for identifying *populations* in need of community-based intervention programs

More sensitive indices of deficiency must be employed [170] This will have a dual effect by detecting earlier, milder deficiency, populations will be identified in which frank xerophthalmia does not occur, or occurs so rarely it is impractical to detect by clinically related prevalence surveys, the associated consequence is that a more sensitive parameter will also be more prevalent, hence sample size requirements for representative surveys will be much smaller For example, a prevalence of CIC abnormality of 50% may be *equivalent* to the currently accepted serum retinol criterion of < 10 μg/dl in $\geq 5\%$ of children, while its documentation would be far simpler and less costly

Of course, a CIC abnormality rate of 50% may not be the relevant criterion CIC, scotopic vision, and other sensitive indices must be correlated with the population's risk of health consequences from vitamin A deficiency To set policy, decision makers need to know the health consequences of vitamin A status of different degrees established by alternative indices As with all resource-based policy decisions, concerns for cost versus benefit and competing priorities will need to be taken into account and are likely to differ from one country to another Olson,[7] a WHO "consultation,"[171] and others have tentatively suggested criteria for a variety of parameters, these can serve as useful points of departure for future discussion and evaluation

None of the available indices are ideal All are surrogates for a more basic expression of vitamin A status They therefore suffer from considerable variability and "background noise " Clinical criteria are relatively rare, particularly when seeking more sensitive measures of mild deficiency The various biochemical indices of vitamin A stores (actually measures of tissue concentration) are handicapped by the vulnerability of tissue samples (primarily blood) to sometimes transient systemic confounders and laboratory variability The growing prevalence of HIV infection (not to mention the more common and persistent issue of viral hepatitis) poses additional concerns Nonetheless, there have been considerable advances in what these tests can offer, which may make their vicissitudes more acceptable Future advances are likely to make tissue-related tests more practical and reliable under field conditions

For the moment, measures of functional impairment seem to carry the most promise CIC, for example,[170] does not require sophisticated laboratories, storage or transport However, it is not without its limitations In theory (though not necessarily in practice) an ideal criterion would detect deficiency *before* it has occurred, when *stores* are below "safe" levels capable of withstanding a sudden insult or prolonged deprivation but prior to physiologic disturbance By definition, a functional abnormality is evidence that physiologically significant deficiency already exists From a public health perspective, this is not necessarily a problem since our concern is with identifying populations at risk, rather than individuals, a less prevalent criterion could be employed for the population at

large, thus catching most subjects *before* they suffer serious consequences of deficiency This is analogous to equating a rate of X3B (keratomalacia) of 1/10,000 (a terrible individual outcome but a rare event) to a rate of XN (nightblindness) of 2% (a mild, reversible, but prevalent abnormality) Aside from CIC, which still requires further standardization, other functional tests have yet to be validated by widespread field experience The ideal test would be entirely objective, free of samples requiring laboratory analysis, and capable of being performed on the youngest children under the most primitive circumstances Considerable progress toward this goal has been made in the past ten years—we can expect acceleration of the process A brief review of several existing tests and those in various stages of preliminary development and testing has recently been published [20]

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12

Epidemiology of Deficiency

Prevention, at the community level, seeks to improve and sustain adequate vitamin A nutriture among individuals who would otherwise be deficient, thereby blocking the morbid consequences of vitamin A deficiency Successful prevention would reduce the extent and severity of vitamin A deficiency so as to minimize or eliminate entirely its health consequences

A number of fundamental epidemiologic characteristics of xerophthalmia and vitamin A deficiency help to identify high-risk groups and aid in understanding its genesis These include its magnitude and distribution by age, sex, socioeconomic status, location, and season (periodicity), and associated dietary patterns and infectious disease risk factors (addressed in Chapter 3) These characteristics provide a basis for selecting and designing appropriate intervention strategies Effective prevention policy and action depend on a solid knowledge of occurrence of deficiency (by place, time, and person) and an understanding of its determinants (diet, morbidity [Chapters 2–3], and related factors)

Magnitude

The global dimension of vitamin A deficiency is evident in Figure 12–1: it persists, as xerophthalmia and subclinically, as an international public health problem, especially in the southern and periequatorial regions of the world Xerophthalmia is known to occur in some seventy-three countries, which can be categorized by likely severity based on existing evidence [1,2] The list of high-risk countries remains remarkably similar to that drawn up by Oomen, McLaren and Escapini three decades ago [3] "Blank" (and poor) countries on the map usually represent an absence of data rather than an absence of risk

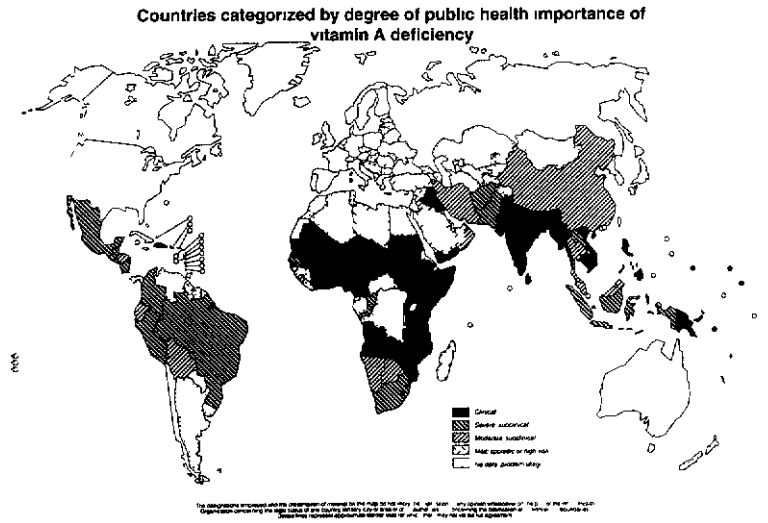

Fig. 12–1. Global distribution of countries by known (or presumed) severity of xerophthalmia and vitamin A deficiency as a public health problem (From the World Health Organization, March 1995)

Population-based data suggest that 5 million to 10 million children develop xerophthalmia each year, a half million of whom go blind [4] Estimates of the extent of all stages of vitamin A deficiency vary widely due to imprecise data and definition of terms As many as 190 million[5] to 240 million children have been estimated to be "at risk of subclinical vitamin A deficiency" (B Underwood, personal communication, 1994) although there is little agreement about what "at risk" means [2] From existing population-based survey, community trial, and standard demographic data, it seems likely that about 125 million children of preschool age are vitamin A-deficient, 1 million to 2 5 million of whom die annually [6] The magnitude of this problem and its consequences for child survival give us a clear imperative for understanding the population characteristics of vitamin A deficiency and seeking its prevention

Age

Strategies to prevent vitamin A deficiency normally target children in the preschool years Deficiency, particularly severe deficiency, is most prevalent among this age group and only compounds the greater risk of infectious morbidity and mortality that affects them (Chapter 2)

The incidence of corneal xerophthalmia traditionally peaks after weaning which, in many cultures, occurs from the second to fourth years of life,[7–9] an age at which dietary intake of vitamin A may be low[10,11] and risk of precipitating

infections high [7,12-15] In two large series of patients with corneal xerophthalmia (X2,X3) presenting at referral eye centers over twenty-four-month periods in Indonesia (n = 162)[7] and Nepal (n = 295),[8] 85%–90% of children were below age five, another 5%–7% of cases were five years old This indicates that effective control of vitamin A deficiency in the preschool years would practically eliminate nutritional blindness (Fig 12–2) Although corneal disease is relatively rare under twelve months of age, keratomalacia may becoming more frequent among young infants living in poor, transitional populations it has been suggested infants of marginal status are being weaned at an earlier age onto milk products or other weaning foods that have little vitamin A value [16,17]

The prevalence of mild xerophthalmia (XN and/or X1B) typically increases with age through the preschool years [7,8,18-29] This relationship appears to hold across different cultures, regardless of age-specific rates of xerophthalmia (Fig 12–3, A-D), providing a clear rationale for supplementing children through the sixth year of life For example, the prevalence of xerophthalmia declined in Indonesia from 1978 to 1992 following a sustained, national prevention program, but the classical demographic association persisted (Fig 12–3, D) [27] The increased risk of moderate-to-severe vitamin A deficiency with age during early childhood is a likely consequence of chronic dietary inadequacy (discussed later in this chapter) combined with nutritional demands of continued growth and repeated infections Subclinical vitamin A deficiency (reflected by low serum retinol or abnormal CIC) would be expected to increase through early childhood, but this has not been well established [30-32]

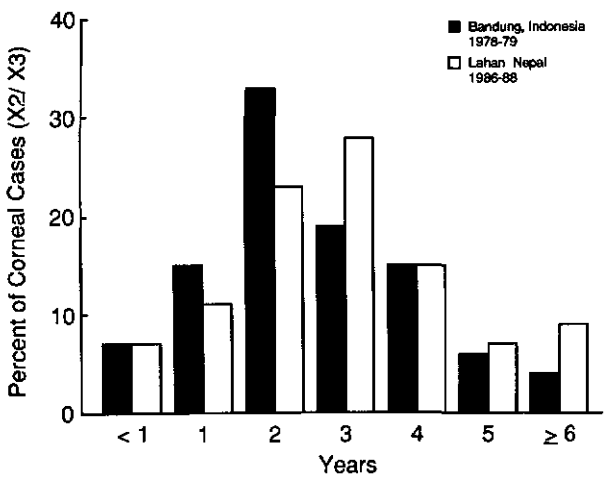

Fig. 12–2. Distribution of consecutive cases with corneal xerophthalmia (X2 or X3) presenting to the Cicendo Eye Hospital (n = 162), Bandung, Indonesia, from 1978–1979 (black bars),[7] and to the Lahan Eye Hospital (n = 295), Lahan, Nepal, from 1986–1988 [8]

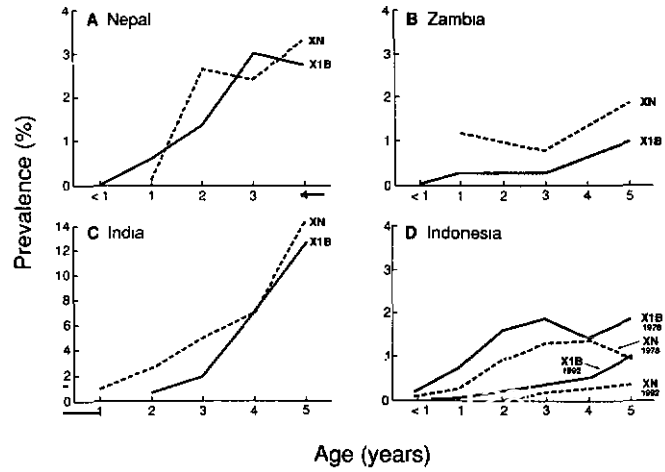

Fig. 12–3. Age distribution of mild xerophthalmia in selected countries in Asia and Africa A, Nepal,[24] B, Zambia,[106] C, India,[28] and D, Indonesia, showing data from both countrywide surveys in 1978–1979 and 1992 [7]

Although the risks of severe deficiency with associated blindness and the risk of overall mortality decline with age, vitamin A deficiency frequently extends into adolescence and early adulthood, when prevalence rates of mild xerophthalmia (XN or X1B) sometimes exceed those of preschoolers [8,20,33–36] The apparent sustained impact of vitamin A supplementation in reducing mortality into the early school-age years[37] also suggests that the health consequences of vitamin A deficiency extend into these older years On the Indian subcontinent, vitamin A deficiency has long been known to exist among adults,[38,39] especially among women of reproductive age [40–46]

Nightblindness during pregnancy and lactation is an evident consequence of the exacerbation of chronic vitamin A deficiency The condition is especially common in South Asia,[42,43,46,47] where nightblindness may occur in 15%–20% of all pregnancies, recur in subsequent pregnancies,[46] and ultimately affect up to half of all women of reproductive age residing in poor, rural areas [47] The potential health consequences to both mother and fetus, the program implications, and the relevance of moderate-to-severe maternal vitamin A deficiency to offspring are only beginning to be recognized and delineated [44,45,48–50] It is already evident that impressive gains in maternal and infant vitamin A status[51] and possibly improved survival of breastfeeding infants[52] can be obtained by supplementing mothers shortly after giving birth with a single, large dose of vitamin A (Table 14–4), although the impact on *maternal* health and survival are yet unknown

Sex

Boys have generally been found to be at higher risk of mild xerophthalmia (XN,X1B) than girls during the preschool and early school-age years,[7,14,18,26,53–57] although sex differences are less evident with respect to severe (corneal) xerophthalmia.[7] Greater male vulnerability to vitamin A deficiency is repeatedly seen in animals[58] (Chapter 6), however, cultural differences in how boys and girls are fed[11,18] and cared for may, in some populations, explain the observed variation by sex. To date, not enough attention has been paid to sex-specific causes of vitamin A deficiency for these differences in risk to have utility in designing prevention programs.

Socioeconomic Status

Vitamin A deficiency affects vulnerable groups from lower socioeconomic strata of poor countries. This relationship may be explained, in part, by the extent to which xerophthalmia and socioeconomic status (and presumably, dietary habits) covary by community. In the countrywide survey[7] and Aceh study[59] in Indonesia, families of xerophthalmic children were of lower socioeconomic status than families of age-matched, non-xerophthalmic controls from the same villages who, in turn, were worse off than all other non-xerophthalmic families in other surveyed areas (Table 12–1).[59] This suggests that villages in which xerophthalmia is present are more economically deprived than villages in which xerophthalmia is not seen.

Households with mildly xerophthalmic children have fewer possessions such as radios, watches or bicycles,[22,24,60–62] fewer draft and grazing animals[24,60] and smaller landholdings,[7,22,24,60] and poorer housing[7,22,24,59] with fewer hygienic ameni-

Table 12–1 Household Characteristics of Xerophthalmia Cases, Controls and the Remaining Aceh Study Population

Household Characteristic	*Cases (%) (N = 466)*	*Village-Matched Controls (%) (N = 466)*	*Aceh Study Households (%) (N = 15,915)*
Unprotected water source	47.5	43.8	41.1[a]
No private latrine	86.7	83.6	71.3[a]
Bamboo house walls	47.1	33.5	31.6[a]
Household head farms	57.3	55.5	53.4
Mother has < 6 yr education	94.3	86.6	80.3[a]
History of child death	12.1	9.7	7.5[a]

[a]Significant linear trend in proportions, $p < 0.001$

From Mele et al., 1991.[59]

ties[7,59–62] than families without xerophthalmic children. Parents of cases are less educated[7,14,24,32,59,60,62,63] and more likely to have had one or more children die than are control families.[7,24,59] These characteristics of poor socioeconomic status tend to be consistently associated with a 1.5 to 3.0 times higher risk (odds ratio) of xerophthalmia, although the factors by themselves cannot be used to predict the occurrence of xerophthalmia (i.e., often have predictive values of $\leq$ 12%, K.P. West et al., unpublished data, 1995).

Location

Xerophthalmia has been found to cluster within provinces or districts[7,62,64,65] (Figure 12–4), subdistricts,[63] villages,[7,29,59,66] and even households.[29] As already shown, non-xerophthalmic (i.e., clinically "normal") children living in the same vicinity as a case of XN or X1B have a lower serum retinol (Table 1–3)[67] and are likely to come from poorer families (Table 12–1)[7,59] than non-xerophthalmic children from other neighborhoods.

Clustering presumably arises from shared practices and environments. Population-based surveys in Africa and Asia have shown that children living in villages where at least one other child has xerophthalmia are at a 1.2 to 2.3 times higher risk of having xerophthalmia than children in villages where no other cases exist.

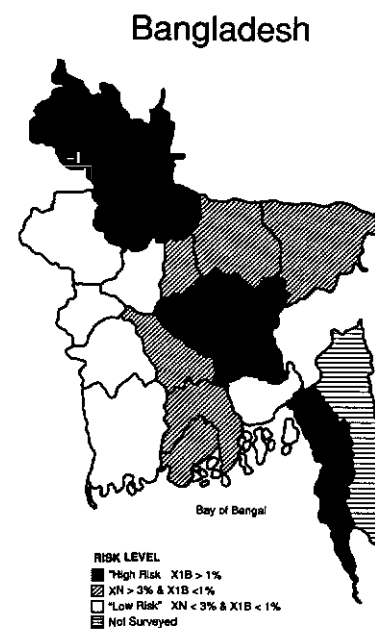

Fig. 12–4. Regional clustering of xerophthalmia (vitamin A deficiency) by severity and district in Bangladesh. (From N. Cohen et al.[65])

(Table 12–2) [29] The risk increases further within households Siblings of cases are 7–13 times more likely to have, or develop, xerophthalmia once they enter the high-risk age group than children living in households without a xerophthalmic sibling [29] These levels of clustering are stronger than have been observed for wasting or stunting [29 68 69] Risk within the household extends to the mother-child dyad, in Bangladesh, preschool-age children were 5–10 times more likely to have nightblindness if their mothers were nightblind than were children whose mothers had normal night vision [70]

Knowing the levels at which vitamin A deficiency clusters can facilitate the design, targeting, and estimation of cost-effectiveness of prevention programs [22 71] Combining region-specific prevalence rates of vitamin A deficiency with relevant population census data permits numbers of cases to be estimated[7,34 54 61 65] and the likely costs associated with achieving coverage ("efficiency") to be considered when targeting interventions In Nepal, for example, it was estimated to be 7–34 times more efficient to first prevent vitamin A deficiency in the population-dense, high-risk, low-lying plains than in sparsely populated, less-accessible communities at higher, mountainous elevations [71]

These data stress the value and efficiency of implementing prevention programs in communities of greatest need (i e , achieving high specificity) according to the available resources Where community-wide outreach is restricted, households in which (a history of) xerophthalmia exists should be targeted to screen and treat cases, supplement high-risk members with vitamin A, and provide counseling on diet or food growing More broadly, entire communities in which

Table 12–2 Crude and Age-Adjusted Village and Household Pairwise Odds Ratios for Risk of Xerophthalmia among Preschool Children[a]

	Malawi		*Zambia*		*Indonesia*		*Nepal*	
Odds Ratio	*n*	*OR*[b]	*n*	*OR*	*n*	*OR*	*n*	*OR*
CRUDE								
Village	50	1 2 (1 0–1 4)[c]	110	1 7 (0 9–3 1)	460	1 7 (1 4–2 2)	40	2 2 (1 5–3 2)
Household	2899	4 4 (2 2–8 8)	2449	7 4 (3 0–17 9)	16337	9 7 (6 6–14 2)	2909	7 7 (4 5–13 2)
AGE-ADJUSTED								
Village		1 2 (1 0–1 5)		1 7 (0 9–3 2)		1 8 (1 4–2 2)		2 3 (1 6–3 4)
Household		7 3 (3 2–16 7)		7 9 (3 5–17 8)		10 5 (7 0–15 7)		13 2 (6 0–29 0)

[a]Numbers of children < 6 years of age in each country Malawi (n = 5441) Zambia (n = 4316) Indonesia (n = 28 586), and Nepal (n = 4764)

[b]Pairwise odds ratio based on alternating logistic regression

[c]95% confidence intervals in parentheses

From Katz et al , 1993 [9]

xerophthalmic children have been identified or districts in which prevalence rates of xerophthalmia exceed minimums should be targeted for intervention

Periodicity

Seasonality is a distinguishing feature of vitamin A deficiency in parts of the world where climatic variation and attending patterns of food availability and infectious diseases are distinct [7,8,15,26,72–74] Early in the twentieth century seasonal peaks in xerophthalmia were observed to coincide with the hot, humid "diarrhea season" in Japan[75] and London,[76] scarcity of green leafy vegetables in China,[77] and the spring growth spurt in Denmark [78] The decline in apparent incidence of xerophthalmia in Danish children was attributed, in part, to a possible increase in the vitamin A content of cow's milk following the shift from stall (winter) to pasture (summer) feeding [79]

In recent years the seasonality of vitamin A deficiency has perhaps been most clearly described in the village of Ichag, West Bengal Sinha and Bang weekly tracked the incidence of mild xerophthalmia (both XN and X1B) and other diseases in a cohort of approximately 300 children for over two years before and during the conduct of a vitamin A field trial[73,74] (Fig 12–5) A pre-monsoon peak in the incidence of X1B and, to a lesser extent, XN occurred in May and June,[74] which has been observed repeatedly elsewhere [8,15,72]

The seasonal appearance and subsidence of xerophthalmia probably reflects variation in nutrient demand and intake in marginally nourished populations In South Asia, the ascending curve in March and April follows a typical growth spurt during the months after the major December rice harvest[80] that, in many poor areas, also corresponds with a period of increased caloric availability through community food-for-work programs [81] This period, however, also parallels lower

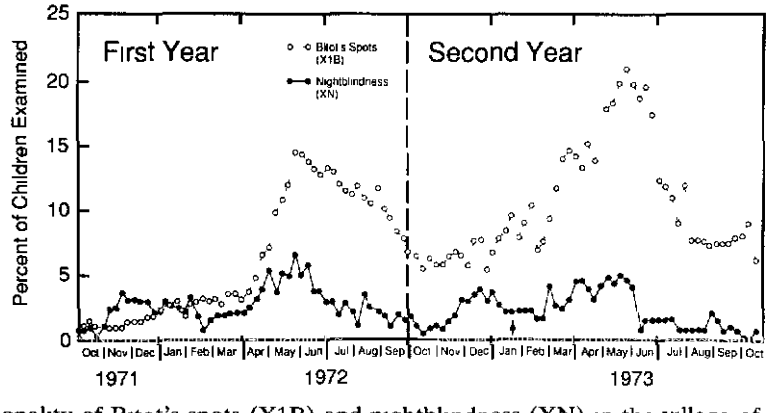

Fig. 12–5. Seasonality of Bitot's spots (X1B) and nightblindness (XN) in the village of Ichag, West Bengal, India, over a two-year period Data to the right of the arrow reflect control group only from a field trial (From D P Sinha [73])

dietary intakes of carotenoid-rich vegetables and yellow fruits among the poor,[72,82,83] coinciding with a concomitant fall in serum retinol levels.[82] An increased frequency of diarrhea and measles in May and June[28,73,74] places greater demands on vitamin A nutriture,[12] which can exacerbate moderate-to-severe deficiency.[7,13,84] Xerophthalmia usually begins to wane by mid-monsoon, following increased intakes of vitamin A during the mango season in June.[82,83] Slower growth among South Asian children during the latter half of the monsoon season reduces metabolic demand, which may further contribute to "improved" vitamin A status.[80,81]

Interventions should strive to prevent seasonal peaks in vitamin A deficiency. Large-dose vitamin A supplementation should be timed to precede the peak risk months (e.g., about March/April in India, Bangladesh and Nepal), the alternate dosing period (every six months) should come before, at least in South Asia, the major rice harvest. This "alignment" of supplementation by season effectively mutes the seasonal peaks in xerophthalmia[74] and mortality[37] (Chapter 2). Longer-term, food-based programs should place special emphasis on increasing the availability and consumption of beta-carotene-rich foods during the dry season (coupled with at least a minimum amount of preformed vitamin A year-round).

Not surprisingly, the incidence of xerophthalmia rises during drought.[72] This has been documented on the Indian subcontinent, where rates of (predominantly mild) xerophthalmia during drought can exceed 10% among preschool-age children.[72,85–87] Periodic drought and food shortage may be better predicted in the future through practical early warning systems,[85,88,89] which could permit preemptive intervention (e.g., vitamin A supplementation or enhanced availability of food sources of vitamin A).

Diet

A diet lacking the needed amounts of vitamin A, either preformed or as provitamin A carotenoids, is the basic underlying cause of vitamin A deficiency as a public health problem. Several aspects of diet in infancy and early childhood appear to be particularly critical to a child's vitamin A status and the risk of xerophthalmia and other consequences of deficiency. In particular are the adequacy of breast-feeding, and the quality of the diet offered to children during and after weaning from the breast.

Breast-Feeding

Nurslings of Russian mothers, severely malnourished from the Lenten Quadragesima fast, developed keratomalacia (cited in Blegvad, 1924[79]), suggesting that a minimally adequate maternal diet was required to protect infants from early nutritional blindness. Both Stephenson[76] and Bloch[90] noted that xerophthalmia

rarely occurred in children suckled by a healthy mother who was "capable of yielding sufficient milk."

There is substantial evidence that frequent and sustained breast-feeding protects infants and young children from severe vitamin A deficiency through the fourth year of life, under all but the harshest conditions. Children who remain breast-fed are ~65% to 90% less likely to develop xerophthalmia (i.e., odds ratios of ~0.10 to 0.35) than children of similar age who are fully weaned from the breast (Fig. 12–6).[7,10,15,24,91–93] There also appears to be a dose-responsive, protective effect related to frequency of breast-feeding. Adjusting for age, Nepalese children breast-feeding up to about ten times per day had a 68% lower risk of xerophthalmia than their non-breast-fed peers (Odds Ratio [OR] = 0.32, 95% confidence interval [CI] 0.15–0.66), children breast-feeding more frequently had an 88% lower risk (OR = 0.12, 95% CI 0.03–0.54).[24]

In Malawi, mothers of xerophthalmic children began to wean their children one month earlier, on average, than the mothers of non-xerophthalmic controls (at age three months versus four months) by giving soft porridge daily or every other day ($p \leq 0.05$).[91] This practice may have contributed to the threefold greater risk of children with xerophthalmia having been fully weaned before eighteen months (OR = 3.4, 95% CI 1.4–7.5). Premature introduction of solid foods leading to earlier cessation of breastfeeding appeared to have shortened the weaning interval ($p < 0.005$) during which cases could otherwise have benefited from breast-feeding.[91] Similar findings were reported from Ethiopia.[55] In both African studies children with xerophthalmia had been weaned from the

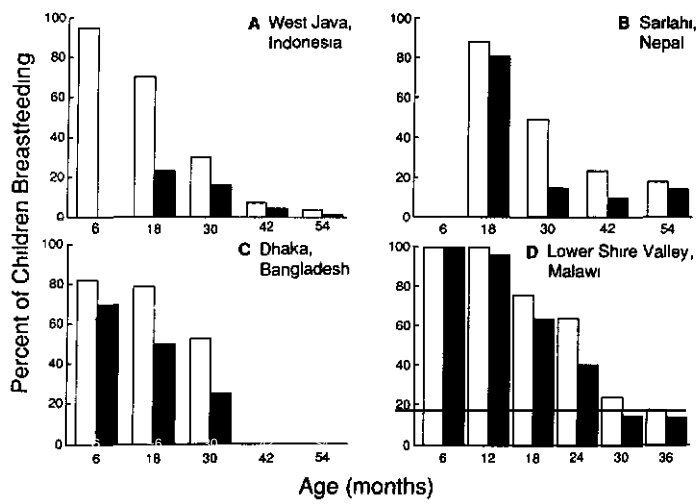

Fig. 12–6. Association between percent of children breast-feeding and mild xerophthalmia (XN,X1B), by age. Open bar, non-xerophthalmic children, black bar, cases. A, Indonesia,[7,10] B, Nepal,[24] C, Bangladesh,[93] D, Malawi.[91]

breast approximately six months earlier than non-xerophthalmic children,[55 91] suggesting that potential benefits may accrue from successful promotion of (any) extended breast-feeding

As these studies make clear, premature onset of weaning, less frequent suckling, and early cessation of breast-feeding, relative to local norms, may increase the risk of xerophthalmia in the preschool years Exactly why mothers of high- and lower-risk children breast-feed differently is not always readily apparent mothers of xerophthalmic and non-xerophthalmic children often give the same reasons for stopping breast-feeding, usually because the mother thinks it is no longer needed or because she's become pregnant [7 10]

The protection of breast-feeding against clinical xerophthalmia has not yet been demonstrated for subclinical vitamin A deficiency, but there is every reason to suspect it will since subclinical deficiency precedes severe deficiency and vitamin A supplementation of breast-feeding, nonxerophthalmic weanlings (approximately six months to thirty-five months of age) reduces their mortality[37 86 87 94–97] (Chapter 2) Maternal vitamin A deficiency,[46 98] which can result in lower milk retinol levels,[51 99] may limit the extent to which simply prolonging breast-feeding can protect a weanling child from the adverse effects of subclinical vitamin A deficiency In some situations the duration of breast-feeding may be less important than the composition of the weaning diet [59]

Weaning Diet

As breast-feeding declines, once a child begins to wean, the choice of foods becomes increasingly important During the first twelve months of weaning, Indonesian preschool, xerophthalmic children were less likely to be fed vitamin A-rich foods on a routine basis (i e , daily or every other day) than non-xerophthalmic controls (Fig 12–7) Infrequent consumption of dark green leafy vegetables (DGLV) or yellow fruits and vegetables (with provitamin A carotenoids) was associated with a fourfold to sixfold increase in the risk (odds ratio) of xerophthalmia, the effect of not consuming egg, meat (presumably including some liver), fish and milk (sources of preformed retinol) was associated with a twofold to threefold increased risk Exclusion of all of these foods from a child's diet during weaning increased the risk of xerophthalmia ~3 5 times, after adjusting for current dietary intake [59] It is likely that liver retinol stores fell during weaning, as the regular household diet was inadequate to build or maintain reserves

Childhood Diet

For many children, a weaning diet deficient in vitamin A predicts a vitamin A deficient diet through the rest of early childhood, and possibly beyond Intakes

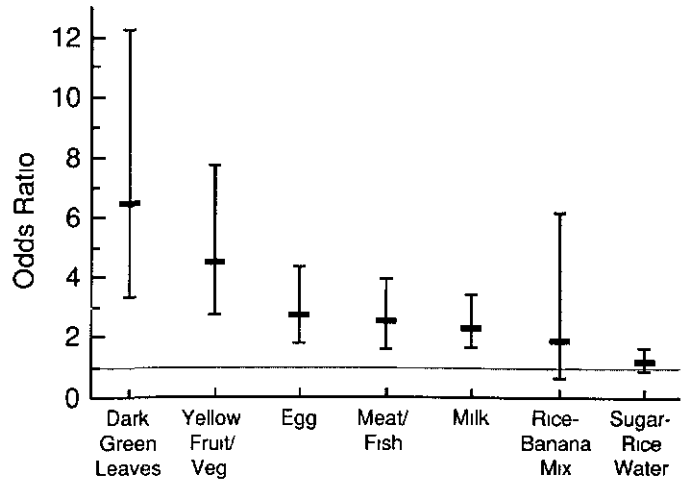

Fig. 12–7 Relative risk (case-control odds ratio) (± 95% CI) of mild xerophthalmia (vitamin A deficiency) by type of food reportedly consumed by children daily or every other day during their first twelve months of weaning (From L Mele et al [59])

of vitamin A (or provitamin A carotenoids) of children with moderate-to-severe vitamin A deficiency have regularly been found to be lower than those of children without apparent vitamin A deficiency,[7 10,32 55,59 62 63 78 90 92 100–102] yielding a remarkable degree of consistency given the diversity of the populations studied and dietary assessment techniques employed Most epidemiologic studies in recent years have relied on food-frequency questionnaires, designed to assess consumption patterns over a specified period of time (e g , past month, week, few days, or twenty-four hours) These methods have proven capable of distinguishing intake distributions of key vitamin A foods by clinical or biochemical status, thus providing a potential basis for estimating risk and for understanding, in part, the dietary basis of vitamin A deficiency and the rationale for developing food-based prevention approaches

In the Indonesian countrywide survey of 1978[7 10] and the Aceh study[59] that followed, xerophthalmic children were compared with a random sample of children[7 10] and matched controls without xerophthalmia from the same villages [58 59] In both studies the risk of xerophthalmia rose to a similar extent, in a dose-response manner, with a fall in the frequency of mango/papaya, dark green leaf and egg consumption (Table 12–3) Children eating these foods one to three times per month were at 1 3 to 2 4 times the risk of having xerophthalmia than children eating these foods at least weekly, those who rarely ate them incurred a 2 0 to 4 0 times higher risk Mango and papaya consumption were especially protective in children under three years old (age-adjusted OR ~3 0 to 9 0 for never versus weekly), though less so above this age (OR range 1 0 – 3 8) This finding coincides with a normal progression in diet from breast-milk to soft,

Table 12–3 Usual Frequency of Intake of Key Food Sources of Vitamin A by Xerophthalmia Status, Indonesia 1978 and 1983

		Frequency of Intake (%)			*Odds Ratio*		
	N	*≥ 1 wk*	*1–3/mo*	*< 1/mo*	*≥ 1/wk*	*1–3/mo*	*< 1/mo*
COUNTRYWIDE SURVEY 1978 1–5 YEARS							
Mango/papaya							
Cases (X1B)	351	59	29	12	1 00	1 34	2 07
Normals	5112	68	25	7			
Dark green leaves							
Cases (X1B)	351	68	13	19	1 00	2 41	1 92
Normals	5112	82	6	12			
Egg							
Cases (X1B)	351	24	28	49	1 00	1 84	2 44
Normals	5112	40	26	34			
ACEH STUDY, 1983 2–5 YEARS							
Mango/papaya							
Cases (XN/X1B)	367	9	69	22	1 00	1 68	2 21
Controls	364	15	68	17			
Dark green leaves							
Cases (XN/X1B)	367	16	66	18	1 00	1 72	3 85
Controls	364	28	65	8			
Egg							
Cases (XN/X1B)	367	21	57	22	1 00	1 74	3 80
Controls	364	35	55	10			

From Tarwotjo et al 1982[10] for the countrywide survey, and Mele et al 1991[59] for the Aceh Study

sweet, yellow and orange fruit [10,59] A dose-responsive, protective effect of egg consumption was present at each age from one to five years,[10 59] similar to findings elsewhere [32 63] The validity of dietary findings obtained by food frequency was supported in a substudy by comparative lower serum carotene levels (generally reflecting usual dietary intake) [10]

Other population-based studies, though varying in design and purpose, have documented similar relationships Bangladeshi children with XN were 2 5 to 5 times less likely to have consumed DGLVs, yellow fruits, eggs, milk, fish, and meat during the previous three days than children without XN, after adjusting for multiple sociodemographic and dietary factors [63] In the Philippines, the proportion of surveyed children who consumed egg, liver, and mango during the previous week fell in association with lower vitamin A status (serum retinol levels at > 20, 10–19, < 10 μg/dl) [32] In West Java, children with xerophthalmia

were less likely to consume liver [7] Distributions of vitamin A-rich food intake in Malawi were not significantly different between xerophthalmic cases and their controls,[91] although markedly lower intakes were reported among children with X1B versus normals in Ethiopia [55] In the Sudan, twenty-four recalls (conducted $\leq$ 3 times over two years) revealed significant relative risks of 1 3 to 2 3 for incident xerophthalmia and mortality respectively among children consuming food sources of vitamin A and carotenoids (< 10th versus > 90th percentiles of intake) [103 104] Detailed food weighing and compositional studies of diets of children with and without X1B in Tanzania found lower intakes of retinol, folic acid, and iron in mildly xerophthalmic children, which agreed with observed inadequate intakes of green leaves and dairy products compared with controls [102]

When planning an intervention, food preferences should be considered in addition to the size and strength of the association between vitamin A status and particular dietary components For example in Aceh, children who did not consume kangkong *(Ipomoea aquatica)* or drumstick leaves *(Moringa oleifera)* during the month before examination were at a 1 7 times higher risk of xerophthalmia (OR) than those who did, yet, 7 times as many controls had eaten kangkong in the recent past than drumstick leaves (74% versus 10% respectively) This reflects a stronger preference for kangkong among children, and indicates greater potential to enhance its intake (compared with drumstick leaves) [58]

Household Diet

Poor dietary intake of vitamin A-rich foods by children is not synonymous with lack of availability of such foods in a household In the countrywide Indonesian study, there were no differences in household consumption of DGLV, mango/papaya, and egg, even though xerophthalmic children had consumed these foods less frequently than non-xerophthalmic controls [7] Results are similar for Nepal, where households of children with and without a history of xerophthalmia have similar access to gardens and market food sources of vitamin A, however, children with a known history of previous xerophthalmia routinely consumed DGLVs (as well as dairy products) less frequently than their non-xerophthalmic controls [105]

Knowledge of these and other pertinent epidemiologic features of xerophthalmia and vitamin A deficiency at the local level can lead to more effective and efficient design, targeting and implementation of prevention strategies aimed at improving the local diet and intakes of deficient children and mothers

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13

Dietary Intervention

> a large number of foods contain substantial amounts of either vitamin A or carotenoids and many of these foods are widespread and inexpensive, even for the very poor Thus, in a logical sense, no reason exists for vitamin A deficiency to be a worldwide problem But it is a worldwide problem
>
> —James Allen Olson, 1994[1]

Vitamin A deficiency is fundamentally a nutritional disorder and thus appears to have an obvious, dietary solution increase the intake of foods rich in "vitamin A" (preformed retinyl esters or provitamin A carotenoids) to a satisfactory level among those who are deficient Finding effective dietary approaches to prevent vitamin A deficiency in most developing countries, however, is not straightforward, despite a generally sufficient supply of "vitamin A" in available foods [1-3] Where foods containing vitamin A are already available, affordable, and used by most households, the most vulnerable groups (low-income families, children) frequently do not consume adequate amounts [4-6] These populations would be expected to benefit from effective programs aimed at changing the dietary behavior (e g , eating practices) of those in need

Where vitamin A-rich foods are not locally available or affordable, one faces the greater challenge of both creating demand (for a better diet) and increasing the available, affordable supply The latter goal has been approached by encouraging food production for personal consumption and creation of local microenterprises that increase availability at the marketplace

Most attempts at changing dietary habits have concentrated on increasing the intake of *plant* sources of (provitamin A) carotene It is possible, however, that more modest increases in the consumption of vitamin A-containing foods (e g , eggs, milk, liver) may prove feasible and more effective in some situations

The form in which dietary "vitamin A" is consumed may make a greater difference than has been generally recognized In Asia and Africa, for example, provitamin A carotenoids are the major source of dietary vitamin A, with plant sources reportedly providing more than 80% of the total vitamin A intake (Fig 13–1) [7–10] These are also the regions of the world where vitamin A deficiency is most widespread and severe,[1 11 12] raising intriguing and important questions about the degree to which, and conditions under which, plant carotenoids can substitute entirely for preformed sources of vitamin A Further, inter-regional variation in dietary "vitamin A" is greatest (per capita) for availability of preformed vitamin A (retinyl esters), not for total "vitamin A activity" in the food supply (Fig 13–1) [7] While there is enormous variation in the supposed consumption of "vitamin A-rich" foods across and within geographic, demographic, and socioeconomic strata,[10 13] severe vitamin A deficiency generally does not exist as a public health problem where 40% or more of the vitamin A in the food supply is provided as preformed retinyl esters[7 14] (with the notable exception of "pockets" of xerophthalmia in Latin America[15] and the western Pacific[16–18]) This ecologic association suggests that food policies aimed at preventing vitamin A deficiency might well include ways to increase the availability and consumption of dietary sources of preformed vitamin A among high-risk groups as a potentially critical component of any food-based strategy for improving vitamin A status It does not prove, however, that this will work, given the complex and varied conditions that accompany these different consumption patterns

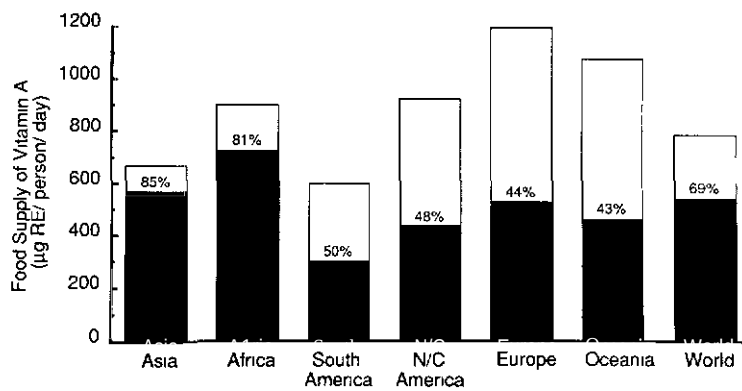

Fig. 13–1. Food supply of total vitamin A partitioned by the percentage available from provitamin A carotenoids (black segments, % indicated) and preformed vitamin A food sources (open segments), for the period 1979–1981 (From the Food and Agricultural Organization of the United Nations [7])

Food Sources of Vitamin A

Dietary approaches to controlling vitamin A deficiency require adequate understanding of (1) the content of preformed retinyl esters and provitamin A carotenoids in different foods, (2) the bioavailability of vitamin A from these foods given achievable conditions of storage and usual methods of preparation, and (3) the existing shortfall in vitamin A intake by the target (most vulnerable) populations

Preformed retinol from animal sources (e g , eggs, milk, cheese, liver, fish oils) or retinyl ester-fortified products is generally assumed to be 70% to 90% bioavailable (i e , absorbed and utilized) when consumed in usual amounts [19] Provitamin A carotenoids are less well utilized, they are obtained especially from deeply colored vegetables (e g , dark green leaves, carrots), tubers (e g , yellow sweet potatoes) and fruits (e g , mango, papaya, red palm) Although more than fifty provitamin A carotenoids have been identified, beta-carotene is the most ubiquitous and active [9 20 21] Absorption and bioconversion to retinol of provitamin A carotenoids in food is generally assumed to occur with an efficiency of 16% for beta-carotene (6 1 ratio) and 8% for other vitamin A precursors (12 1) relative to preformed retinol (Table 13–1) [7–9 19] Indian data suggest a 4 1 conversion ratio is more appropriate for beta-carotene [22] This "conversion ratio," however, is at best a gross average, and furthermore not constant The same foods prepared and consumed in exactly the same way will vary in their bioavailability of vitamin A depending on the vitamin A status of the consumer It can be expected to be lower than 6 (or 4, more efficient) in vitamin A-deficient individuals and considerably higher than 6 (less efficient) in those who are vitamin A-replete [23–26]

Although rather crude,[27 28] these conversion ratios provide a convenient and uniform basis for estimating retinol equivalency of different diets and foods

Table 13–1 Vitamin A Conversion Units

1 Retinol Equivalent (μg RE)	= 6 μg beta-carotene
	= 12 μg other provitamin A carotenoids
	= 3 33 IU vitamin A (VA) activity from retinol
	= 10 IU VA activity from beta-carotene
	= 5 IU VA activity from other provitamin A carotenoids
1 International Unit (IU)	= 0 30 μg RE
	= 0 344 μg retinyl acetate
	= 0 55 μg retinyl palmitate
	= 0 60 μg beta-carotene
	= 1 20 μg other provitamin A carotenoids
	= 0 00105 μmol retinol (SI unit)

Source FAO 1976 [175] Olson, 1987,[20] Newman, 1994 [64]

Estimates of food vitamin A content, however, are affected by the accuracy of regional, national, or local food composition tables,[29–34] which vary considerably[8 9 35] "Errors" in estimation for individual foods arise from differences in vitamin A activity associated with variations between cultivars and within species, age of the plant and degree of vegetable or fruit ripeness, seasonality, climate and soil conditions,[8 21,36 37] transport and handling, processing[21 38] and methods of cooking[21 39–42] Sampling and analytical mistakes are also important sources of error in estimation[43] older published vitamin A values are found to both underestimate[44] and overestimate[21,30] values derived by modern analytic techniques Further, modern estimates by allegedly reliable methods (compiled by the U S Department of Agriculture) of the beta-carotene content of a single, "high-vitamin A" food—the carrot—vary by as much as ~13,000 μg per 100 g raw weight,[45] a range that encompasses nearly all estimates (derived by other means) (Fig 13–2)

Profound misclassification with respect to adequacy of vitamin A intake of individuals and communities can occur when large discrepancies exist between actual and estimated values for widely consumed foods Given the variability of multiple factors involved in estimating the vitamin A content of a particular dietary item, planning an appropriate change in diet can be equally hazardous and difficult[30 44] These issues become especially troublesome when attempting to assign "risk" of vitamin A deficiency to a group, or design a food-based intervention solely on supposed dietary consumption[46]

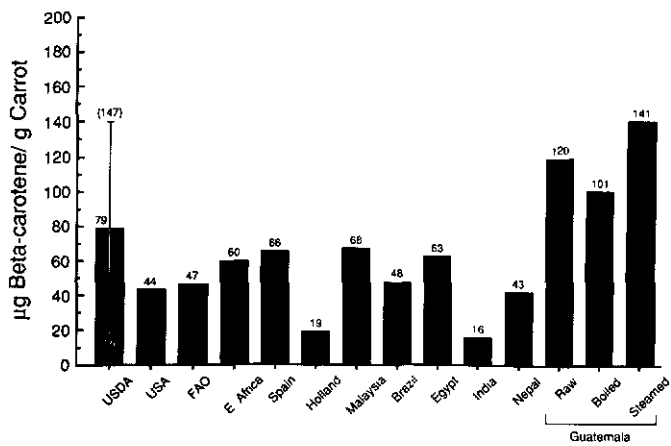

Fig 13–2. Variability in estimated beta-carotene content of the carrot based on assays in standardized laboratories USDA (U S Department of Agriculture) with range depicted from multiple, independent estimates,[45] United States and Food and Agricultural Organization (FAO),[8] East Africa,[29] Egypt,[30 178] Spain,[30 179] Malaysia,[30 180] India,[30 181] Nepal,[30 182] Guatemala[44]

Recommended Intakes

Vitamin A intake recommended by the United Nations' Food and Agricultural Organization (FAO) or nationally established recommended dietary allowances (RDAs) (usually specified as μg of retinol equivalents [RE] per day [Table 13–2]) identify levels of routine intake that are safe and will sustain health and adequate nutrient *reserves* in the vast majority (i e , mean +2 SD, or ~97%) of presumably healthy individuals in a population [7,19,20,22,47] (Chapter 8)

In addition, the FAO has estimated "basal requirements" for vitamin A needed to prevent clinical deficiency and functional impairment in the average member of a population [7] Basal requirements are likely to be the most relevant, initial goals for dietary interventions seeking to control vitamin A deficiency and its health consequences in a high-risk population An RDA of vitamin A for preschool children is typically ~350 μg–450 μg RE/day, for pregnant or lactating women it is 600 μg–950 μg RE/day (depending on the reference), corresponding "basal" levels are assumed to be roughly half these levels, or 200 μg RE and 370 μg–450 μg RE respectively It should be emphasized that an RDA (or

Table 13–2 Recommended Dietary Allowances (RDA) for Vitamin A for Preadolescent Children and Adult Women

	μg Retinol Equivalents				
	FAO		*India*	*Philippines*	*USA*
Group[a]	*Basal*[b]	*Safe*[b]	*RDA*[c]	*RDA*[d]	*RDA*[e]
CHILDREN					
< 1 yr	180	350	300	325	375
1–3 yr	200	400	400	350	400
4–6 yr	200	400	400	375	500
7–10 yr	250	400	600	400	700
WOMEN					
≥ 18 yr	270	500	600	450	800
Pregnant	370	600	950	475	800
Lactating					
1st 6 mo	450	850	950	775	1300
2nd 6 mo	450	850	950	725	1200

[a]Cut offs for some age groups vary among references This table is provided for general comparison Readers should consult original reference for exact age specifications

[b]"Basal" requirement is the amount of vitamin A needed (averaged over time) to prevent demonstrable impairment in function 'safe ' level of intake should maintain function and adequate nutrient reserves in nearly all healthy individuals From FAO 1988 [7]

[c]Indian tables are based on a beta-carotene retinol conversion ratio of 4 1 (versus 6 1 used elsewhere) From Gopalan et al , 1989 [22]

[d]From the Food and Nutrition Research Foundation 1989 [47]

[e]From the U S National Research Council 1989 [19]

equivalent) is a policy tool based on the distribution of needs and use of foods across a population, it best serves as a guideline for setting population dietary goals, interpreting the general adequacy of the diet of a population, setting nutrient concentrations for food fortification, and serving as a surrogate for the success of food-based intervention programs Persons who do not appear to meet their RDA for vitamin A, or a specified fraction thereof (e g , two-thirds), should not necessarily be construed as being vitamin A-deficient without direct, physiologic evidence of impaired status[44 48] (Chapters 2–4, 10)

Targeting and Selecting Interventions

Epidemiologic profiles of deficiency (Chapter 12) help guide the targeting and choice of food-based initiatives Initially, infants and preschool-age children[4 5 49–51] and their pregnant or lactating mothers[52–56] should be targeted, especially within poorer districts and communities,[4 5 49 57 58] based on the degree to which vitamin A deficiency clusters at each of these levels [59 60] Dietary strategies should emphasize extended breast feeding (through the second and third years of life, when possible)[4 5 61–64] and enhancing the young child's diet[4 49–51] with foods that make "age-specific sense " These should be sufficiently concentrated with preformed or provitamin A to achieve and maintain an adequate vitamin A status, and be accessible to the target population

Strategies for increasing vitamin A consumption and improving the vitamin A status of preschool-age children should emphasize increased intakes of soft yellow fruits (e g , mangos and papayas) during the first three years of life, yellow and orange vegetables and tubers and dark green leaves between the ages of one and five, and sources of preformed retinol such as egg, milk, curd, and liver (of animals or fish) from mid-infancy onward (Fig 13–3) This generic "template" has been derived from studies in numerous cultures, its specifics will depend on local circumstances

The primary goal of "dietary diversification" is to ensure a sufficient supply of plant and animal foods rich in vitamin A,[65] and to promote their adequate consumption Initiatives that would enhance production and consumption of local food sources of vitamin A are widely regarded as culturally appropriate, economically attractive, and sustainable in the long run [66] Access to a nutritious, balanced diet is also considered a basic right that underlies human development [67] These goals have been pursued through nutrition education that seeks to enhance intake of existing vitamin A-rich foods,[2 68] and encourage gardening and food preservation practices that increase and improve the quality of the vitamin A food supply Either or both approaches may be indicated, depending on local capacity and needs

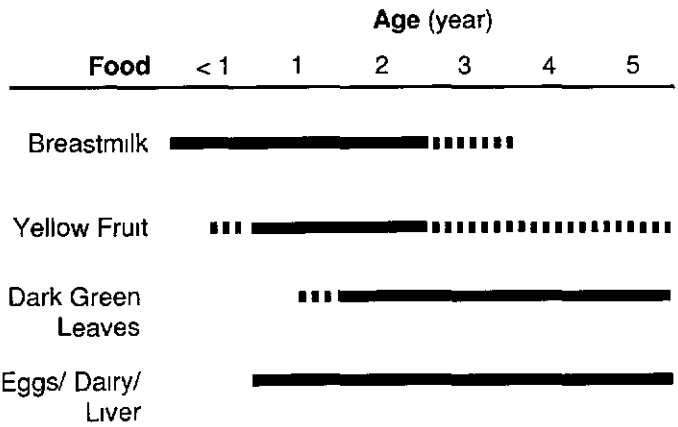

Fig. 13–3. Composite profile of age-specific protection against xerophthalmia and low serum retinol levels conferred by dietary intakes of selected types of foods Solid bar denotes ages for which epidemiologic evidence is strong Dashed bar denotes ages at which some evidence of protection exists for a food [4 5 49–51 57 62 63 69 183–188] (Adapted from K P West [189])

Improving Dietary Intake from Existing Foods

In Indonesia,[4 5] the Philippines,[50] and Nepal,[6] the availability of home gardens and the frequency with which vitamin A-rich foods were consumed by household members were similar among families with and without xerophthalmic children (XN, X1B), but xerophthalmic youngsters ate these foods less frequently than their better-nourished peers (Fig 12–3) The fact that children without xerophthalmia consume vitamin A-rich vegetables, fruits, eggs, and dairy products more frequently than do (the far less numerous) children with xerophthalmia from the same villages[4 5 49 69] attests to the possibility that children can be influenced to eat a more nutritious diet from the local fare This appears to extend to dark green leaves While dietary surveys often indicate that young children routinely consume less than 15 g of green leafy vegetables per day,[70–74] preschool-age children in Bangladesh readily consumed ~40 g (raw weight) of cooked green leaves at a single meal when they were prepared in an attractive manner [71] This amount theoretically meets the daily vitamin A needs of a young child[22 36 71] and, when consumed regularly, has been shown to reverse mild xerophthalmia [75 76] Further, mothers were receptive to the notion of feeding their children beta-carotene-rich vegetables Mothers who were counseled and participated in the appropriate preparation of vegetables (while their children underwent treatment at a diarrhea clinic) were twice as likely to be feeding vegetables to their children two months later than were other mothers from the same community.[77] Intensive and expert dietary counseling at such an individual level, however, is impractical

for population-oriented programs "Social marketing," a communications process that attempts to create widespread demand for an improved diet,[68,78,79] has begun to show promise as a population-based tool for increasing "consumer demand" for carotene-rich vegetables and vitamin A intakes of children (and their mothers)

Improving Dietary Availability and Content

In some instances, vitamin A-rich foods may not be adequately available or affordable to families who are most at risk[80] These foods can often be locally grown or (in the case of domesticated animals) raised in home, school, or other community gardens (e g , kebun in Indonesia) for personal consumption or as cash crops as part of microeconomic schemes[81-87] For such efforts to effectively raise food availability, the populace requires high-quality and inexpensive seeds or seedlings, water, organic or inorganic fertilizer, protective fencing, pest control, other extension services to enhance preservation, storage conditions, and marketing, and labor time[2,10,82] Combined, these needs represent significant outlays by poor families and communities Dietary quantity and variety can also be improved by encouraging greater awareness and consumption of indigenous ("wild") vegetables and fruits[65,88-91]

Seasonal scarcity of vegetables and fruits in the diet[74,92,93] can be alleviated to some extent by local food preservation In most tropical, rural areas this is done by drying foods in direct sunlight, often resulting in a 65% or greater loss of provitamin A activity from vegetables and fruits[41,88,91,94] These losses can be cut by 25%–50% through improved (village-based) solar[41,94-98] and shade[91] drying methods that avoid direct ultraviolet light exposure Other local processing methods are being developed that can extend shelf life, food value, and acceptability of leaf and tuberous vegetables[10,99]

Even if provitamin A-rich foods are available, they may be prepared improperly, reducing their potential nutritional benefit Carotenoids in plant food matrices are typically associated with protein complexes (carotenoproteins) and polysaccharides (such as pectin, hemicellulose, lignin) in the plant cell wall that "trap" carotenoids and inhibit their digestion and absorption[100,101] For this reason, carotene absorption from uncooked, unprocessed vegetables can be practically nil (e g , < 2% from raw, ungrated carrots)[102] Cooking, chopping, or pureeing vegetables partially breaks down these structural complexes and reduces particle size, greatly increasing carotene "presentation" to the human digestive system and resulting in improved absorption (to 25%–70%)[23,101,103-106] This can be achieved without important food carotene losses, blanching or boiling, or, better yet, steaming fresh vegetables with minimal amounts of water for several minutes typically results in losses in vitamin A activity of only 10% to 20%[21,38-40,42,107-109]

Carotenoids appear to be fairly resistant to heat[110] However, excessive cooking of vegetables can markedly reduce provitamin A carotenoid activity through

oxidation and isomerization (from all-*trans* to less biologically active *cis*- configurations) [88,101,111] Prolonged boiling for more than one hour, for example, or boiling followed by frying (a common practice in many traditional settings) doubles provitamin A losses (to 20%–45%) [21,39,40,42] These cooking losses can be partly offset by adding the cooking water or oil used in frying back into the foods to be consumed Of course, losses can also be overcome by consuming more of the vegetable [88]

Modifying traditional dietary behavior to improve nutrient availability, however, will require substantial, innovative, and persistent (targeted) consumer education [112] It is generally accepted that gardening and better food preservation and processing methods, integrated with educational efforts to change feeding practices,[68,113,114] will prove effective in improving vitamin A status of a population Unfortunately, there is scant experimental or programmatic evidence that this indeed happens [28,115,116]

Efficacy of Dietary Interventions

> There are indications that human beings in contrast to herbivorous animals, may not assimilate much of the fat-soluble A derived from plants
>
> —Olaf Blegvad, 1924[117]

> Never let sleeping dogmas lie
>
> —Bertrand Russell

Although changes in knowledge about or behavioral responses to dietary intervention, such as gardening and cooking practices and food consumption, provide a valid basis for evaluation, efficacy (improved vitamin A nutriture) is the "gold standard " It represents the degree to which vitamin A status among known recipients of the intervention changes relative to appropriate controls This information permits dietary change to be evaluated in its own right as an approach to combat vitamin A deficiency

Interventions of all sorts have generally been evaluated by the degree to which they change serum retinol levels Serum retinol, however, tends to be under homeostatic control and is relatively insensitive to variation in vitamin A (and beta-carotene) intake among individuals with adequate vitamin A status [48,118–122] However, serum retinol is a sensitive indicator of changed nutriture in vitamin A-deficient subjects and, therefore, effective for evaluating the impact of dietary interventions This is particularly true if one focuses on the degree to which the prevalence of low serum retinol levels declines (i e , a shift in the distribution of serum retinol levels to the right, as seen with fortification in Fig 15–1 and supplementation in Fig 11–3)

Circulating beta-carotene is generally assumed to reflect recent beta-carotene intake [4,5,23,123] However, there is considerable, unexplained inter- and intra-individual variation in the change in serum carotenoids in response to changes in dietary intake, reflecting gaps in our understanding of the kinetics of carotenoid absorption and metabolism [27,101,124–126] This can make serum beta-carotene a difficult and unpredictable indicator to interpret, especially in the "adequate" ranges of intake, with respect to evaluating the impact of a provitamin-A dietary intervention on vitamin A status [48,127]

Vitamin A Status Response to Foods

Evidence that the absorption of provitamin A carotenoids from vegetables and fruits is sufficient to improve vitamin A status is surprisingly weak [28,128] This may relate to variation in study designs, differences between species and cultivars of the foods tested[28] (which can influence absorption or bioconversion), food preparation and dietary habits,[21,36,39,40,42] nutritional and disease states,[129] and, perhaps consistently most important, differences in baseline vitamin A status (which can alter carotenoid absorption and conversion to vitamin A)

Individuals with Marginal-to-Adequate Status

Among individuals of marginal-to-adequate vitamin A status (e g , mean serum retinol > 25 μg/dl), increased consumption of provitamin A carotenoids from vegetables produces a variable effect on circulating beta-carotene levels and appears to have little or no impact on vitamin A status In contrast, purified beta-carotene supplements generally increase serum beta-carotene and vitamin A, at least among individuals who are marginally vitamin A-nourished at the outset) In a recent controlled trial by de Pee et al in Indonesia, lactating women with a mean serum retinol of ~0 85 μmol/liter (and Hb < 120 g/liter) in one village were assigned to receive a morning serving of cooked, local, green vegetables (containing ~3 5 mg beta-carotene, or ~585 μg RE) prepared with added fat In a nearby village, similarly nourished, lactating women were randomized in double-masked fashion to receive a morning snack wafer that was either fortified with beta-carotene (also ~3 5 mg) or not (0 1 mg) (Table 13–3) Supervised feedings were given five days per week in a manner that appeared to induce no other effects on existing meal patterns After twelve weeks, serum and breast milk retinol levels, as well as serum beta-carotene levels, rose significantly among women given the beta-carotene-fortified wafer, suggesting that the beta-carotene supplement was well-absorbed, converted to vitamin A, and transferred to breast milk However, there was no apparent response of any indicator of vitamin A status (or, indeed, even of serum beta-carotene) among women consuming cooked vegetables daily, suggesting poor absorption of beta-carotene from the cooked, leafy green and other provitamin A-rich vegetables [130,131] This wide differ-

Table 13–3 Vitamin A Status Responses of Lactating, Anemic Women before and after Consuming Test Foods Daily for Twelve Weeks, West Java, Indonesia

	Beta-Carotene-Fortified Wafer	*Control Wafer*	*Local Vegetables*
SERUM RETINOL (μmol/liter)			
Number	62	54	57
Baseline, x̄(SD)	0 84(0 31)	0 81(0 32)	0 89(0 33)
Change, x̄	+0 32*	+0 02	+0 06
(95% CI)[a]	(0 27–0 41)	(−0 07–0 09)	(−0 02–0 14)
BREAST MILK RETINOL (μmol/liter)			
Number	59	54	55
Baseline, x̄(SD)	0 88(0 59)	0 84(0 51)	0 98(0 92)
Change, x̄	+0 59**	+0 17	−0 04
(95% CI)[a]	(0 37–0 83)	(0 02–0 31)	(−0 26–0 22)
SERUM BETA-CAROTENE (μmol/liter)			
Number	56	50	53
Baseline, x̄(SD)	0 20(0 12)	0 17(0 09)	0 19(0 12)
Change, x̄	+0 91**	−0 02	0 02
(95% CI)[a]	(0 58–0 89)	(−0 04–0 00)	(0 00–0 04)

[a]95% confidence intervals interpolated from original graphs

*$p < 0.001$ between groups adjusted for age of breastfeeding child and maternal weight changes

**$p < 0.01$, between groups adjusted for above factors plus breast-milk fat changes

From dePee et al 1994 [131]

ence in response between consumption of a pure carotene supplement and consumption of vegetables was independent of the women's baseline nutritional and health status

The study by dePee et al in Indonesia is not alone in finding little impact of vegetable consumption on vitamin A nutriture under carefully controlled conditions of evaluation The response of both circulating beta-carotene and retinol was also muted among Guatemalan school children randomized to receive a daily 50 g serving of carrots for three weeks (cooked with 10 g added oil, providing ~1000 μg RE/day) Other groups received beta-carotene or vitamin A palmitate supplements (also providing 1000 μg RE) or a placebo[48] (Table 13–4) *Ascaris* or *Trichuris* infestation were common, however, baseline vitamin A status was generally adequate, with mean serum retinol at 35 μg/dl and only four children with serum retinol levels < 20 μg/dl Daily, cooked carrot intake had no measurable effect on either plasma beta-carotene or plasma retinol In contrast, plasma beta-carotene levels rose steeply among children ingesting the purified beta-carotene supplement but, unlike the results in Indonesian women, there was no discernable increase in serum retinol (Fig 13–4), probably because their vitamin A status was adequate from the outset Retinol levels did not rise

Table 13–4 Impact of Provitamin A Carotenoid Foods in Individuals with Marginal-to-Adequate Vitamin A Status

Country (Ref)	Age	Duration (Days)	Treatment Group (n_i)	Dose (μg RE)	Mean ± SD (μg/dl) Serum Beta-Carotene Baseline	Serum Beta-Carotene F/up	Serum Retinol Baseline	Serum Retinol F/up
Guatemala[48]	7–12 yr	20	Placebo (17)	0	14±12	16±5	35±11	41±12
			Carrots (17)	1000	13±10	14±5	36±11	37±11
			Beta-carotene (16)	1000	14±9	47±9*	34±11	34±11
			Vitamin A (17)	1000	14±10	13±11	35±10	37±8
Thailand[133]	Preschool[a]	14	Control (15)	0	44±16	27±9*	39±7	25±7*
		14	Ivy gourd leaves (15)	180	27±9	106±35*	25±7	49±6
Thailand[133]	Preschool[b]	14	Ivy gourd leaves (15)	198	36±12	87±22*	35±8	35±6
		14	Multi-vitamin (15)	450	87±22	57±15*	35±6	48±11*

*$p < 0.01$, follow-up versus baseline within group

[a]Control = historical control period for same subjects later crossed over to receive ivy gourd

[b]Ivy gourd = same group of children later crossed over to receive multivitamin supplements

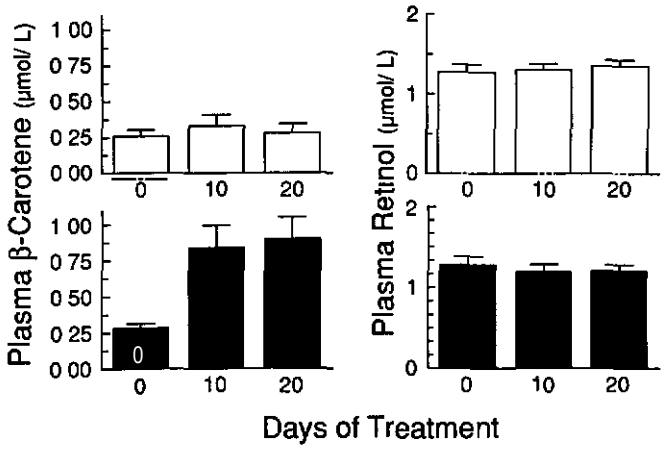

Fig. 13–4 Impact of consumption of cooked carrots (open bars) and a purified beta-carotene supplement (black bars), both with added high-fat snacks, for 20 days (1000 μg RE/day) on plasma beta-carotene and retinol levels in Guatemalan children (n_i=17) (From J Bulux et al [48])

even among those ingesting vitamin A palmitate A similar lack of serum retinol response (in the presence of a significant serum beta-carotene increment) has been repeatedly observed in well-nourished adults [120 122 126 132]

Two supervised but noncontrolled feeding studies of adequately vitamin A-nourished Thai orphans produced varied results After a two-week control period during which beta-carotene and vitamin A status declined, daily provision of ~35 g of cooked ivy gourd *(Coccina indica)* for two weeks markedly raised serum beta-carotene (from 27 μg/dl to 106 μg/dl) and serum retinol (from 25 μg/dl to 49 μg/dl) [133] The reported magnitude of the response (over so brief a period) is virtually unique The second Thai study produced a similar rise in beta-carotene after two weeks of increased ivy gourd intake (from 36 μg/dl to 87 μg/dl), but no change in serum retinol (35 μg/dl at both baseline and follow-up) This was followed by a two-week multivitamin supplementation period (without ivy gourd) in the same children serum beta-carotene fell (presumably from decreased vegetable intake, as seen elsewhere[120]) while serum retinol rose [133] Conceivably, the differences in the retinol response to dietary greens in the two Thai studies related to differences in baseline vitamin A nutriture Whereas children in the first study were of marginal status at the outset (mean serum retinol = 25 μg/dl), those in the second study had a higher, "adequate" baseline serum retinol (35 μg/dl) This may be a level of vitamin A status at which conversion of beta-carotene to retinol is far less efficient [25] The children with adequate serum retinol, however, still responded to the vitamin A (multivitamin) supplement (serum retinol rose to 48 μg/dl), which is in contrast to results reported for other vitamin A-sufficient groups

Individuals with Deficient-to-Marginal Status

Increased intake of carotenoid-rich vegetables is likely to improve vitamin A status of children with marginal-to-deficient vitamin A status (serum retinol < 25 μg/dl), the more deficient the baseline vitamin A status, the more pronounced the effect, regardless of fat intake This is in accordance with the inverse (protective) association often observed between the frequency of consumption of green vegetables and yellow fruit and the risk of xerophthalmia (moderate-to-severe vitamin A deficiency) (Chapter 12) However, as far as the available literature is concerned, the extent to which a sustained increase in vegetable (and presumably fruit) intake can improve vitamin A status appears to have its limits In India, a 50% increase in serum retinol was observed (from ~14 μg/dl to 21 μg/dl) after vitamin A-deficient children were fed drumstick *(Moringa olefeira)* or fenugreek *(Trigonella foenum)* leaves daily for two and a half months (theoretically providing a half[7] to a full[22] RDA, depending on the assumed content from food composition tables and conversion ratio [Table 13–2]) Fresh and dried (not shown) vegetables had the same beneficial effect over controls as a purified beta-carotene supplement, even *without* added fat to the meals [134] But although the benefit was sizable, neither pure beta-carotene nor the green leaf regimen raised vitamin A status to a truly adequate level (Table 13–5) A similar effect was observed among Indian children randomized to a combination of raw carrot, coriander-mint chutney, and papaya five days per week After one month, serum retinol in those receiving the added vegetables and fruit was 25 μg/dl, versus 15 μg/dl among the nonsupplemented controls ($p < 0.05$), only 5% of intervened children had serum retinol below 20 μg/dl at follow-up, compared with 91% of the controls ($p \leq 0.005$) (Table 13–4)

In Indonesia, vitamin A-deficient, preschool-age children with ascariasis (mean serum retinol < 20 μg/dl) were randomized to a placebo or midday meal of either vegetables (red sweet potato and green leaves, providing ~845 μg RE/day) or added dietary fat only (15 g–25 g), with or without being dewormed with levamisole (Fig 13–5) Beta-carotene-rich vegetables (B) given daily for three weeks raised serum retinol 5 μg/dl more than did the placebo ($p < 0.01$), adding deworming (B + D) or dietary fat (B + F) to the vegetable meal raised the serum retinol 7 μg/dl and 9 μg/dl, respectively, more than in the controls (both $p < 0.001$) The biggest change occurred among children with the lowest baseline retinol concentrations, even so, the dietary (carotenoid) intervention was unable to raise the average serum retinol level above ~25 μg/dl [135]

Two studies have reached contrasting conclusions regarding the clear but apparently limited improvement in vitamin A nutriture that results from increased carotenoid consumption among vitamin A-deficient children One study reported a rise of serum retinol into the normal range while another reported no impact at all (Table 13–5) In India, Lala and Reddy reported that ~40 g of cooked amaranth leaves *(amaranthus tricolor)* fed with 2 g–3 g oil daily for two

Table 13–5 Impact of Provitamin A Carotenoid Foods in Individuals with Marginal-to-Deficient Vitamin A Status

Country (Ref)	Age	Duration (Days)	Treatment Group (n_i)	Dose (μg RE)	Mean ± SD ($\mu g/dl$) Serum Beta-Carotene Baseline	Serum Beta-Carotene F/up	Serum Retinol Baseline	Serum Retinol F/up
India[134]	4–5 yr	75	Control (15)	0	—	—	12±11	10±11
			Beta-carotene (15)	200	—	—	14±9	22±8*
			Fenugreek leaves (15)	200	—	—	14±12	21±12*
			Drumstick leaves (15)	200	—	—	12±11	20±12*
India[176]	7–12 yr	28	Control (54)	0	—	—	—	15±19
			Carotene foods (60)	545	—	—	—	25±22*
India[103]	2–6 yr	15	Control (6)	0	—	—	25+19	23±6
			Amaranth leaves (29)	200	—	—	22±10	32±13**
Indonesia[106]	3–5 yr	74	Vegetables (32)	300	16±10	32±16***	20+8	21±9
			Salt+A (43)	306	19±18	32±18***	19±8	23±10***

$p < 0.05$ experimental versus controls at follow up

*$p < 0.10$ experimental versus control at follow up

**$p < 0.05$ follow up versus baseline within group

From Devadas et al 1978[134] Wadhwa et al 1994[176] Lala and Reddy 1970[103] and Muhilal 1977[106]

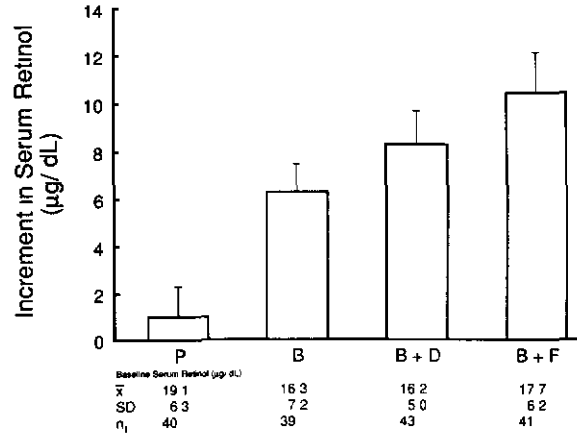

Fig. 13–5. Increment in serum retinol among Indonesian children with *Ascaris* infestation, 3 to 7 years of age, following intake for three weeks of a single, midday meal containing "placebo" (P), beta-carotene-rich vegetables and tubers (B, ~750 μg RE), with deworming with levamisole (B+D) or with added fat (B+F, ~15 g) (From F Jalal [135])

weeks to undernourished children (20% with X1A/X1B) raised mean serum retinol from 22 μg/dl to 32 μg/dl, while unsupplemented controls remained in their marginal state (Table 13–4) [103] Balance studies in four children suggested that beta-carotene absorption from amaranth had been good (~70%), perhaps explaining the exemplary serum retinol response However, absorption was worse in two children with acute lower respiratory infections (though data were not given), echoing observations six decades ago in the United States that "carotene" absorption can be halved (to ~35%) in infants with acute respiratory infections [129]

The other study, an early Indonesian trial among preschoolers with marginal-to-deficient vitamin A status (mean serum retinol ≤ 20 μg/dl), found no change in their serum retinol after two and a half months of eating cooked vegetables (amaranth, kangkong, cassava leaves, yardlong beans, or yellow sweet potato) twice a day (providing ~300 μg RE/day) Carotenoid absorption from this mix of vegetables was only 34% (perhaps insufficient for a retinol response), in line with previous estimates from mixed diets [23 104 136] In contrast, 97% of preformed vitamin A (from fortified salt) was absorbed by children in a second intervention group Their serum retinol rose, although only marginally, from 19 μg/dl to 23 μg/dl) [106] A similar rise in serum beta-carotene levels in both groups suggested other (nonintervention) dietary factors may have been at work

From a review of these studies, one is struck by the apparent inadequacy of dietary carotene to fully supply vitamin A demands There are a host of possible explanations inadequate fat, insufficient amount or duration (total dose) of vegetable intake, poor digestibility and bioavailability of vegetable carotene

(regardless of amount consumed), intestinal helminths that may hinder absorption, complicating illnesses, or other important but as yet unrecognized factors [28]

Experience with Atypical Foods

Unusually rich sources of carotene have been tested for their ability to improve vitamin A status Crude or red palm oil (RPO) *(Elaeis guineensis)* theoretically serves a dual purpose, as an oil thought to be valuable for maximizing carotene absorption, and as an excellent source of provitamin A carotenoid[137,138] that retains 70%–90% potency after normal cooking [139] Red palm oil appears comparable to sources of preformed vitamin A in improving vitamin A status and preventing clinical deficiency After two months of consuming a sweet semolina prepared with RPO (providing one RDA daily), school-age Indian children showed striking improvement in vitamin A status (serum retinol and modified relative dose response), similar to those taking a daily vitamin A supplement (also providing one RDA)[140] (Table 13–6) These findings agree with earlier studies in which RPO cured nightblindness[141] and increased serum retinol,[142] and survey data from West Africa linking routine RPO intake with elevated serum carotene and retinol levels and with reduced risk of xerophthalmia [143–145] Unfortunately, while RPO is available in many developing countries,[138,146] it is disliked In other countries, the "red" (carotene) is purposely destroyed during commercial processing, this rids the product of both its off-flavor and its vitamin A value Currently, efforts are under way to resolve the undesirable organoleptic problems with RPO without eliminating its provitamin A content [147]

An indigenous food of exceptional provitamin A content is the "sweet paste" of buriti *(Mauritia vinifera)* fruit from northeastern Brazil and the Amazon region Its therapeutic effect was tested (unfortunately without controls) in twelve

Table 13–6 Serum Retinol and DR R Ratio of Indian Children before and 60 Days after Daily Supplementation

	Red Palm Oil (N = 12)	*Vitamin A Palmitate (N = 12)*
Serum retinol (μmol/liter)		
Before	0 86 (0 14)[a]	0 74 (0 12)
After	1 89 (0 23)	1 94 (0 21)
MRDR DR R ratio[b]		
Before	0 073 (0 025)	0 095 (0 023)
After	0 023 (0 003)	0 023 (0 004)

[a]Mean (SEM)

[b]Modified relative dose-response based on the ratio of serum didehydroretinol (DR) retinol (R) ratio measured ~5 hours after a test dose of DR (cut off > 0 06 taken to reflect low liver stores of vitamin A) [177]

From Rukmini 1994 [140]

children with mild xerophthalmia (eleven with X1B, one with XN) All were given 12 g of buriti sweet daily, containing ~800 μg beta-carotene (~134 μg RE), for twenty days Xerophthalmia disappeared entirely in six and improved clinically in four others, the relative dose response (RDR) test became normal in nine of the ten children who were initially RDR+ When buriti sweet was fed to another thirty-two school-age children, the RDR test normalized in all six children who were initially RDR+ [89] These few studies should encourage further investigations into the acceptability, efficacy and practicality of utilizing indigenous food sources of vitamin A to help control vitamin A deficiency

Influence of Dietary Fat

> We believe that the high incidence of vitamin A deficiency in Ruanda-Urundi is primarily linked with the very low intake of foods of animal origin in that region
>
> —O A Roels et al 1958[105]

Carotenoids and vitamin A are fat-soluble Since dietary fat stimulates the release of pancreatic enzymes and bile salts that are important in forming mixed micelles required for hydrolysis and absorption of carotenoids from the gut [148] Therefore, a small amount of dietary fat is assumed necessary for optimal carotene absorption [20] A number of experts have, in fact, asserted that a considerable proportion of vitamin A deficiency is secondary to inadequate consumption of dietary fat, they have stressed the importance of adding sufficient fat to the diet [7 24 36 105 149] The impetus for at least some of these urgings came from the classic observation by Roels et al of seventeen school-age children with X1B in whom absorption of beta-carotene from raw, grated carrots apparently rose more than fivefold (from < 5% to ~25%) when consumed with 19 g of olive oil [105] However, even these authors believed that fat intake was less important than preformed vitamin A in maintaining adequate vitamin A status Seventy five percent of the dietary carotenoid consumed by their subjects (with a generous amount of oil) was still excreted [105]

In an equally revealing, controlled trial among preschool-age Indian children fed cooked spinach daily for a month, the addition of either 5 g or 10 g of fat (as groundnut oil) to meals increased serum retinol by 5 μg/dl to 7 μg/dl over those not receiving added fat (leading to an often-quoted belief that at least 5 g of dietary fat is necessary to facilitate provitamin A absorption[20 24 149]) The value of added oil, however, was only observed among children with better vitamin A nutriture to begin with (baseline serum retinol > 20 μg/dl, mean ~26 μg/dl) Children more deficient at baseline (≤ 20 μg/dl, mean serum retinol ~14 μg/dl) did not benefit from the added oil In those who ate their spinach meals with *no* additional fat, serum retinol increased by 10 μg/dl—virtually the same

Table 13–7 Efficacy of Adding Dietary Fat to a Daily Spinach (and Rice) Meal by Initial Vitamin A Status

Treatment/Initial Serum Retinol (μg/dl)	*Number*	*Serum Retinol (μg/dl)*		
		Baseline	*4 Weeks Later*	*Difference*
No added fat	26	20 ± 8	24 ± 6	+4
≤ 20	12	13 ± 4	23 ± 5	+10
> 20	14	27 ± 5	26 ± 6	−1
+5 g fat	22	20 ± 9	28 ± 9	+8
≤ 20	14	16 ± 4	25 ± 8	+9
> 20	8	27 ± 4	34 ± 7	+7
+10 g fat	22	21 ± 7	29 ± 6	+8
≤ 20	8	12 ± 4	26 ± 5	+13
> 20	14	25 ± 4	31 ± 5	+5

[a]Mean ± SD

From Jayarajan and Reddy [150]

level of increase experienced by deficient children who received added fat (Table 13–7) [150]

Taken together, the various studies suggest that dietary fat probably facilitates carotenoid absorption,[20,105,124,135] but lack of fat is unlikely to play a major role in producing moderate-to-severe vitamin A deficiency

Other Influencing Factors

Dietary carotenoid absorption is influenced by other factors prevalent in vitamin A-deficient populations Chronic protein restriction may be expected to reduce absorption, as seen in animals [151–157] This may also occur in moderately-to-severely malnourished children, although milder protein-energy malnutrition (determined anthropometrically) seems to exert less, if any, effect on beta-carotene absorption in children [25,127,141] Infectious diseases[103,129,158] and intestinal infestation[159] can impair both preformed vitamin A and carotenoid absorption and utilization Exogenous factors such as high dietary fiber[100] may limit carotenoid bioavailability

Vitamin A status, itself, may be the strongest determinant of carotene bioavailability There are also a host of unknown factors that influence absorption and utilization of dietary carotenoids, evidenced by variations in absorption within and between otherwise healthy individuals [28,160,161]

Effectiveness of Dietary Interventions

To be effective, dietary interventions (such as home gardening, domestic animal production, nutrition education, and social marketing) must first produce an

increase in the intake of vitamin A-rich foods by those who are vitamin A-deficient Second, the increased intake must improve their vitamin A status As already noted, the second condition cannot be taken for granted Uncertainties about the value of carotenoid-based dietary interventions under controlled conditions are magnified when considering large, "scaled-up" programs given the inefficiencies that usually accompany such expansions Although guidelines exist for evaluating the educational impact of nutrition communications programs,[162,163] there have been few controlled evaluations of the actual impact such programs have on the vitamin A status of target populations Experiences from some well-executed, small-scale programs illustrate the challenges facing larger-scale dietary interventions

In northeast Thailand, a multimedia, social marketing intervention was carefully implemented, focusing on raising awareness and increasing the availability and consumption of dark green leaves (especially the ivy gourd) by pregnant and lactating women and preschool-age children [68,112] This followed previous studies that suggested a beneficial impact of adding ivy gourd and other greens to children's diets (Table 13–4) [133] Home gardening, proper food preparation, and mother-and-child feeding practices were promoted through a comprehensive, community-based strategy Outcomes were critically evaluated two years later by comparing twelve villages in the intervention district with sixteen villages in a nearby control district Knowledge, attitudes, and reported behavior of women with respect to vitamin A-rich foods significantly improved in the intervention areas, moreover, daily vitamin A intake of pregnant and lactating women in program areas improved over that of controls (~220 μg RE, $p < 0.04$) Mothers of preschoolers reportedly grew and cooked ivy gourd at home and reported use of vegetable oil in cooking much more often than did controls (Table 13–8) [112,164] Daily intake of vitamin A by preschool-age children reportedly improved (+138 μg RE) compared with controls (+65 μg RE), presumably from increased consumption of ivy gourd and other vegetables The impact on vitamin A status, however, was disappointing Serum retinol levels, which were comparable at baseline, decreased significantly among preschoolers in the intervention group, while reportedly increasing among their controls (Table 13–8)

This important investigation highlights the complexity of factors (some known, many not) that affect real or measurable surrogates of vitamin A status As previously noted (Chapter 11), serum retinol is an uncertain surrogate for vitamin A status Yet the difference in the serum retinol response in the two groups of villagers is difficult to explain on this basis If it represents a real (and not chance) phenomenon, and assuming that no major, unrecognized changes in other aspects of health and diet differentially affected the two groups of villages, it raises the question of whether a primary (and highly successful) focus on green leaf consumption may have failed to have its intended effect This could have happened either by leading mothers to displace sources of preformed vitamin A from their children's diets in favor of less bioavailable sources of

Table 13–8 Effects of a Two-Year Social Marketing Ivy Gourd Campaign in Northeast Thailand

	Intervention	*Control*
MOTHERS OF PRESCHOOLERS (FOLLOW-UP ONLY)		
Number	109	108
Growing ivy gourd at home, %	78 2[a]	21 8
Cooking ivy gourd > 1×/week, %	54 0[a]	8 7
Cooking with oil, %	24 3[a]	2 0
PRESCHOOLER VITAMIN A INTAKE (μg RE)		
Baseline		
Number	112	113
Mean (SD)	182(245)	157(198)
Follow-up		
Number	105	104
Mean (SD)	322(784)	222(202)
PRESCHOOLER VITAMIN A STATUS (μg/dl)		
Baseline		
Number	70	70
Mean (SD)	30 1(10 0)	31 8(10 8)
% < 20 μg/dl	11 4	11 4
Follow-up		
Number	70	70
Mean	27 4(11 1)	32 6(9 0)
% < 20 μg/dl	20 0[b]	4 3

[a]Intervention versus control, $p < 0.001$ by Z-test

[b]Intervention versus control $p \leq 0.01$ by Z-test

From S Smitasiri, 1994 [112,164]

vitamin A, or through other, unrecognized changes in behavior or physiology It is widely held that a shift in dietary behavior that is perceived as beneficial is a sufficient basis for inferring improved nutritional status,[2,163] but, as the above confirms, this may not always be the case [162] In the Philippines, a weak negative correlation ($r = -0.10$, $p = 0.12$) was observed between the frequency of dark-green-leafy-vegetable (DGLV) intake and serum retinol levels in preschool-age children,[50] suggesting a similar, inverse effect These results illustrate the importance of evaluating the benefits of dietary interventions, at least in part, on their actual impact on vitamin A nutriture, and not solely on changes in knowledge, attitudes, or even feeding practices

Projects have begun to evaluate the effect of gardening on vitamin A status, in addition to changes in knowledge, attitudes, practices (KAP) and other measures of well-being [73,85,165–168] Unfortunately, none have been adequately con-

trolled A three-year gardening and nutrition education project in twenty-one subdistricts (thanas) in Rangpur, Bangladesh, emphasized raising people's awareness of nightblindness and increasing home production and consumption of DGLV by mothers and children The program may have had a modest impact on the prevalence of XN, which decreased from ~6% to ~5% in children below nine years of age ($p < 0.05$) [166]

In India, a home gardening project using volunteers was carried out in 2350 households in two drought-prone districts Each year households received vegetable seeds and seedlings from local nurseries Nutrition messages were reinforced through the media (posters, drama, demonstrations, etc) After three years, the proportion of families growing carotene-rich vegetables rose from 5% at baseline to an astounding 49%, four-fifths of whom consumed some of their produce each season Although DGLV intake by preschool-age children remained unchanged (7 g/d to 11 g/day), their intake of papaya and mango rose by 28 g, from 11 g/d to 39 g/day The prevalence of X1B among children whose families had participated for the entire length of the project was half (6%) that of children whose families had been gardening for only one year (12%) [73]

While findings from both of these project evaluations are consistent with a beneficial impact on vitamin A status, the lack of control village data makes it impossible to distinguish program effects from secular trends and other potentially confounding influences [169]

Gardening intervention was included in one of three treatment arms (public health intervention, vitamin A supplementation, MSG + A fortification, Chapter 15) in a field trial in the Philippines [170] Families in four barrios were provided with seeds and seedlings of local, beta-carotene-rich vegetables, along with extension services Although reported dietary vitamin A intake increased significantly, rates of xerophthalmia (XN, X1B, X2/X3) among children declined only marginally (from 4 9% to 3 4%, NS), and far less than in the barrios where children received vitamin A capsules (80% reduction) or vitamin A-fortified MSG (76% reduction) Serum retinol levels supported the clinical observations In light of the findings from the efficacy studies, the unclear implications of this intervention are not surprising

Home gardening programs are anticipated to confer benefits beyond making vitamin A-rich foods available One such benefit is economic Since homestead gardens are normally tended by women,[83,171] gardening might selectively enhance purchasing power of mothers and, thus, their nutritional (including vitamin A) status and that of their young children However, extensive reviews of the literature show little controlled evidence that this actually occurs [115,116,172,173] On the contrary, in one eleven-year gardening project in a Senegalese village, women ($n = 64$) operating home gardens saw their personal incomes rise, but there were no significant increases in vitamin A (or in any other nutrient or energy) intake by family members, including young children This negative nutritional

effect was attributed to the selling of produce and the use of profits for non-food purposes [174]

Summary

The overall value of a balanced diet that contains fruits and vegetables notwithstanding, it is clear that important and unresolved questions exist about the extent to which programs specifically targeted at increasing the intake of carotene-rich foods, particularly dark green leaves, will improve vitamin A status Vegetables (and presumably fruits) taken in adequate amounts appear efficacious in protecting children from severe vitamin A deficiency (low plasma retinol and xerophthalmia), this was evident from the Indian children who responded to beta-carotene diets alone [134 150] The importance of carotene-rich foods is supported by the therapeutic value of cooked green leaves in the treatment of xerophthalmia[75 76] and the repeated observation that low vegetable and fruit intakes are associated with increased risk of xerophthalmia (Table 12–3, Fig 13–3) Therefore, it seems reasonable to conclude that dietary sources of provitamin A carotenoids, prepared appropriately, can be made acceptable to children[71 77] and can improve their vitamin A status, particularly among individuals who are moderately to severely vitamin A-deficient (Table 13–5) Fat, it seems, is not a limiting factor under these circumstances

However, vegetables and fruits may be less effective in raising vitamin A status to optimal levels once subjects are no longer moderately to severely deficient (e g , xerophthalmic or with serum retinol < 20 μg/dl) A modest increase in dietary fat may help improve the value of dietary carotene among mildly deficient individuals (serum retinol 20 μg/dl–25 μg/dl) Among those of better vitamin A status (e g , serum retinol > 25 μg/dl) increased consumption of provitamin A vegetables and fruits may have little, if any, impact

Given available data, there is ample reason to suspect that a minimum amount of preformed vitamin A, at least occasionally, may be essential to achieving and maintaining satisfactory vitamin A nutriture

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14

Supplementation

Giving children a large dose of vitamin A at periodic intervals offers a direct, versatile, and relatively rapid means of improving vitamin A status and preventing associated blindness, morbidity, and mortality [1-7] Children twelve months of age and older are provided an oral dose of vitamin A (200,000 IU, 209 μmol or 60,000 μg retinol equivalents [RE]) contained in a gelatinous capsule or, as in India, an oily solution (Fig 14–1) every three to six months [1] A half dose (100,000 IU) is normally administered to infants six to eleven months of age, one-quarter of a dose (or less) may be given to infants under six months of age, particularly if they are not breast-feeding [7] Women may also be dosed with 200,000 IU within the first two months post-partum or six weeks post-partum if not breast-feeding to prevent vitamin A deficiency (Table 14–1) [3,7,8]

The nutritional aim of large-dose, periodic supplementation is to improve vitamin A status by increasing liver stores and tissue concentrations of retinol, thereby reducing the risk and severity of vitamin A deficiency and its ocular and other consequences for several months,[1] while minimizing the risk of acute hypervitaminosis A [9,10] Supplementation can be done through a network of health and other sectoral services (discussed later in this chapter)

Prophylactic vitamin A dosing does not address the underlying cause(s) of vitamin A deficiency Its use should be accompanied, whenever possible, by dietary counseling Supplementation is intended as a means of preventing vitamin A deficiency in high-risk individuals and populations until adequate and sustainable food-based programs become effective (through increased intake of foods naturally rich or fortified with vitamin A) In practice, it has generally proven difficult to withdraw periodic vitamin A supplementation due to delays in implementing effective dietary and other food-based strategies

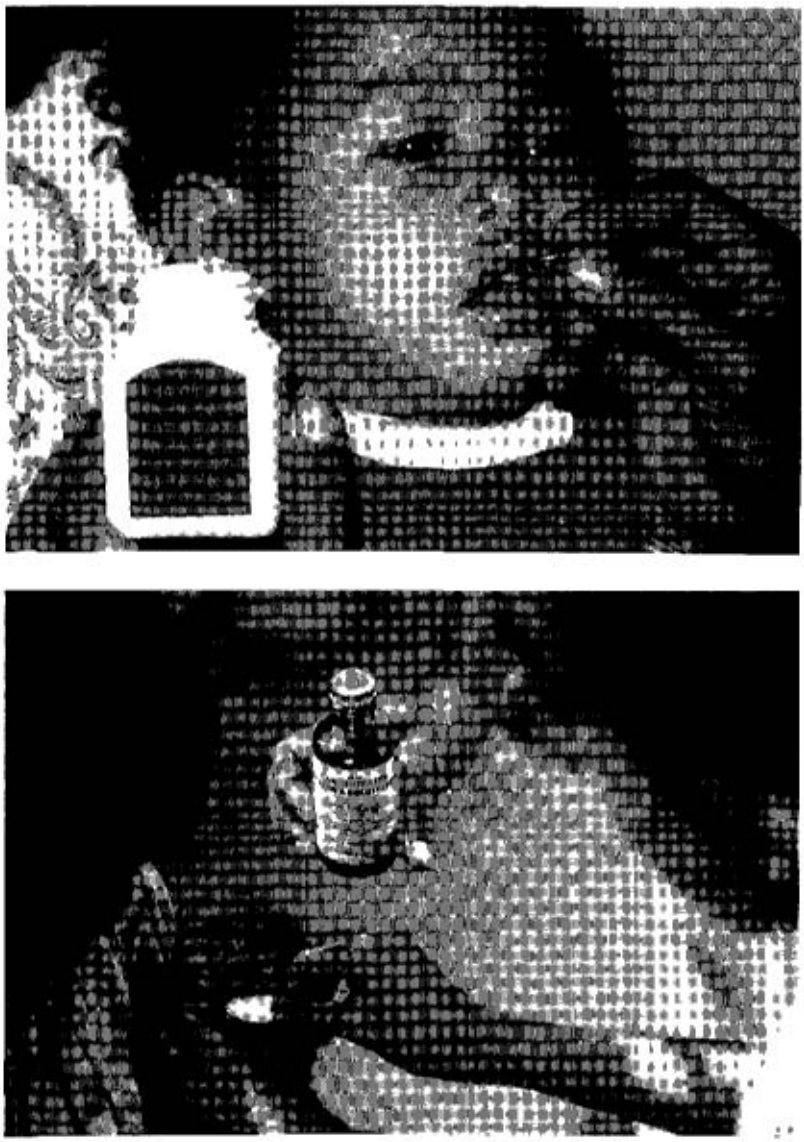

Fig. 14–1. Standard high-potency vitamin A supplements typically contain 200,000 IU (60 000 μg RE or 209 μmol/liter) per capsule (top), available from UNICEF and other sources or per 2 ml of arachis oil syrup (bottom) produced and distributed in India (Photos by A Sommer [top] and K P West [bottom])

Table 14–1 Universal Vitamin A Distribution Schedule for Preschool Children and Lactating Mothers

Children 1–6 yr	200,000 IU of vitamin A orally every 3–6 mo
Infants 6–11 mo	100,000 IU of vitamin A orally every 3–6 mo Immunization against measles provides a good opportunity to give one of these doses (see note)
Lactating mothers	200,000 IU of vitamin A orally once at delivery or during the first 8 wk post-partum if breast-feeding or first 6 wk if not breast-feeding to protect the mother and raise breast milk vitamin A levels to help protect the breast-fed infant

NOTE When infants less than six months old are not being breast-fed, supplementation with 50,000 IU of vitamin A as single dose or as divided doses of 25 000 IU, should be considered before they reach six months

Based on findings and recommendations from WHO 1988 [3] Sommer, 1994 [7] Florentino et al , 1990,[39] West et al , 1992 [44] Stoltzfus et al , 1993,[37] Humphrey et al , 1993 [24] and Humprey et al 1994 [26]

Absorption and Retention

Apparently healthy preschool-age children absorb approximately 75% of a large (200,000 IU) oral dose of vitamin A in oil and retain 50% [11–13] Although similar data are lacking in clinically ill or wasted children, utilization of a large dose is probably also lower in these children, since absorption of a small dose (3000 IU) decreases from nearly 100% in healthy children[11 14 15] to 75% during diarrhea or respiratory infection[14 15] and retention decreases from roughly 80% to 60% respectively [11 14 15] Peak (six-hour) serum retinol levels in children with diarrhea are 55%–85% that of non-ill controls following oral ingestion of doses ranging from 40,000 IU to 150,000 IU of vitamin A [16 17] Under conditions of malnutrition and infection prevailing in many developing countries, 30%–50% of a large oral dose of vitamin A can be expected to be retained [18] Despite reduced retention, high-potency vitamin A in oil is sufficiently well absorbed, even during severe diarrhea, to markedly raise serum retinol (RBP and holo-RBP) levels and achieve a therapeutic response in xerophthalmic children (Chapter 10) [19,20]

Prophylactic Efficacy

Preschool Children and Infants

An early field trial in Indonesia individually randomized nearly 2500 preschool-age children to receive 200,000 IU vitamin A or placebo over two consecutive, six-month periods Vitamin A reduced the incidence of mild eye signs (mostly X1B) during the two follow-up intervals by 87% and 91% (RR = 0 13 and 0 09 respectively) (Table 14–2) [21] In the village of Ichag in West Bengal, nightblindness was virtually eliminated and the incidence of X1B among children who had never

Table 14–2 Incidence of Mild Xerophthalmia in Indonesian Children 12–60 Months of Age at Outset of a Vitamin A Supplementation Trial

Time		Vitamin A (200,000 IU)			Control (Placebo)				
Period	*Eye Sign*	*Number*	*Cases*	*(%)*	*Number*	*Cases*	*(%)*	*RR*[a]	*95% CI*[b]
1st 6 mo	X1A/X1B	1286[c]	6	0 47	1183	43	3 63	0 13	0 05–0 30
2nd 6 mo	X1B	1197[c]	3	0 25	1072	31	2 89	0 09	0 03–0 29

[a]RR = relative risk, incidence in vitamin A/incidence in control groups

[b]95% CI = 95% confidence interval

[c]Number of children free of xerophthalmia at the outset of each time period 200 children lost to follow-up in second interval, similarly distributed by treatment group

From Tarwotjo et al , 1976 [21]

had it before was reduced by 91% (RR = 0 09) when preschool-age children were supplemented with 200,000 IU vitamin A every four months for a year, although preexisting Bitot's spots tended to persist or recur [22] Randomized trials have not measured the impact of high-potency vitamin A in controlling corneal xerophthalmia (X2/X3) However, in India, receipt of 200,000 IU every six months appeared to reduce the risk of corneal disease by 92% (Odds Ratio [OR] = 0 08, 95% confidence interval [CI] 0 02–0 35) based on the findings of a case-control study conducted among urban slums of Hyderabad,[23] this estimate assumed full and regular coverage with vitamin A in the community (i e , an estimate of efficacy)

Thus, high-potency vitamin A supplementation can be expected to reduce the incidence of both noncorneal and corneal xerophthalmia by 90% in young children Failure to prevent the remaining 10% of cases may reflect either an inability of 200,000 IU to protect individuals who are at particularly high risk (severe deficiency initially, virtually absent dietary sources, repeated infections) for the full four to six months or, alternatively, may have been due to inadequate administration, misrecording, partial delivery of the intended dose, or other reasons A loss of protection from a large dose was evident in the rising proportion of initially xerophthalmic (and, less so, non-xerophthalmic) children in Indonesia who relapsed to a deficient vitamin A status (serum retinol $< 0\ 70$ μmol/liter) after receiving one to two doses of vitamin A (within a week) totaling 200,000 IU–400,000 IU (Fig 14–2) Over 75% of initially xerophthalmic children (n = 64) were hyporetinemic at the outset Whereas serum retinol levels of these children were normal two months after dosing, nearly 40% had become deficient a year later [24] Early recurrence of vitamin A deficiency is likely to reflect, in part, particularly poor dietary intake of vitamin A combined with infectious diseases that lower vitamin A reserves [25]

Although ingestion of 200,000 IU causes side effects in a small proportion of children (discussed later in this chapter), reducing the dose shortens the interval of protection for high-risk children In Indonesia, children were randomized

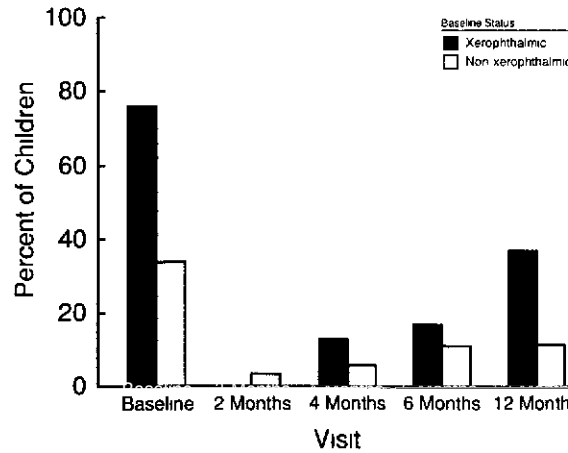

Fig. 14–2 Percent of children 3–5 years of age (with and without xerophthalmia at baseline) with serum retinol < 0 70 μmol/liter (< 20 μg/dl) at baseline and two, four, six and twelve months after being dosed with 60,000 μg RE–120,000 μg RE (200,000 IU–400,000 IU) Test for linear trend, p < 0 0001 for initially xerophthalmic and p < 0 03 for non-xerophthalmic children after baseline (From J H Humphrey, et al [24])

to a single 100,000 IU (105 μmol) or 200,000 IU (210 μmol) dosage of vitamin A and followed (Table 14–3) The larger dose was superior to 100,000 IU of vitamin A in maintaining serum retinol above 20 μg/dl for six months in children who were initially most severely vitamin A-deficient (xerophthalmic and with a positive relative dose-response [RDR+]) A smaller but significant increment in vitamin A status was evident with the larger dose in less severely deficient children (xerophthalmia *or* RDR+), whereas in normal children (no xerophthalmia, normal RDR) both dosages were equivalent (Table 14–3) [26]

These findings indicate that the recommended dose of 200,000 IU every four–six months (Table 14–1)[3 7 18 27 28] is clinically effective and probably superior to 100,000 IU,[29] even if serum levels decline with potential nonocular consequences

Mothers and Breast-Fed Infants

Vitamin A status of mothers, as well as their breast-fed infants, improves when women are supplemented with a large dose of vitamin A during the first two months post-partum In Bangladesh, women given 200,000 IU vitamin A within twenty-four hours after delivery experienced an elevation in serum retinol above control levels for at least one month (~55 μg/dl versus ~38 μg/dl, p < 0 05) Serum retinol remained ~12 μg/dl higher than among controls (p < 0 02) at three months post-partum for supplemented, nutritionally wasted mothers (body mass index [BMI] < 18), but no further effect was observed in better-nourished

Table 14–3 Effect of Different Dosages of Oral Vitamin A on Serum Retinol among Indonesian Preschoolers by Baseline Vitamin A Status

		Serum Retinol (μmol/liter)	
Baseline Status/Dosage	*Baseline*	*3 Months*	*6 Months*
NO XEROPHTHALMIA *AND* NEGATIVE RDR			
105 μmol			
mean (n)	0 63 (85)	0 59 (61)	0 95 (63)
SD	0 24	0 20	0 32
210 μmol			
mean (n)	0 64 (81)	0 64 (60)	0 96 (63)
SD	0 27	0 30	0 38
difference	**−0.01**	**−0 05**	**−0.01**
(95% CI)	(−0 09–0 07)	(−0 14–0 04)	(−0 13–0 11)
XEROPHTHALMIA *OR* POSITIVE RDR			
105 μmol			
mean (n)	0 52 (70)	0 49 (48)	0 70 (57)
SD	0 36	0 18	0 26
210 μmol			
mean (n)	0 49 (67)	0 59 (51)	0 80 (55)
SD	0 21	0 20	0 32
difference	**0.03**	**−0.10**[b]	**−0.10**
(95% CI)	(−0 07–0 13)	(−0 18–−0 02)	(−0 21–0 01)
XEROPHTHALMIA *AND* POSITIVE RDR			
105 μmol			
mean (n)	0 31 (19)	0 41 (14)	0 64 (17)
SD	0 14	0 13	0 32
210 μmol			
mean (n)	0 29 (21)	0 68 (13)	0 91 (14)
SD	0 18	0 27	0 25
difference	**0 02**	**−0 28**[b]	**−0 27**[a]
(95% CI)	(−0 08–0 12)	(−0 44–−0 10)	(−0 48–−0 06)

[a]105 μmol versus 210 μmol $p < 0.02$

[b]105 μol versus 210 μmol, $p < 0.01$

From Humphrey et al 1994 [76]

women (BMI ≥ 18) [30] Breast-milk levels remained 5 μg/dl–10 μg/dl above controls (NS) through the first six months of lactation [30] The vitamin A status of breast-feeding infants was not, unfortunately, measured

In a double-masked, randomized clinical trial (RCT) in Indonesia, postpartum changes were followed in both maternal and infant status after supplementation [31] Dosing mothers with 312 μmol (300,000 IU) vitamin A within three weeks after giving birth improved maternal vitamin A status (by serum retinol

and conjunctival impression cytology [CIC]) compared with controls through six months, breast-milk levels through eight months, and infant vitamin A status (by serum retinol and RDR) through six months post-partum (Table 14–4) [32] This was particularly impressive as the baseline status of control women was better than that of the vitamin A recipients at the outset of the study As expected, serum breast-milk levels of controls dropped precipitously during the post-partum period Vitamin A recipients had an increase followed by a more gradual decline

Safety

The toxicology of vitamin A and retinoids in humans has been reviewed in detail elsewhere [33–36] Here, we are particularly concerned with the safe use of prophylactic dosages of 50,000 to 200,000 IU vitamin A in preschool children, infants, and post-partum women and the use of smaller, more frequent doses of vitamin A by women of reproductive age in developing countries where vitamin A deficiency is prevalent [37]

Preschool Children (One–Six Years)

Early evaluations of large-dose programs suggested that up to 4% of children may experience acute, transient side effects (nausea, vomiting, headache, fever) after receiving high-potency vitamin A (200,000 IU or 300,000 IU) [22,33,37,38] The only randomized trial of sufficient size to carefully examine the effect in preschool children was conducted in the Philippines [39] Children given 200,000 IU (209 μmol) of vitamin A were four times to seven times more likely to develop headache, nausea, or vomiting after dosing than placebo recipients, those receiving 100,000 IU (105 μmol) had roughly twice the risk of these symptoms (Table 14–5) High-dose recipients were twice as likely than controls to develop one or more symptoms Most episodes (> 70%) began within twenty-four hours of dosing and resolved within twelve hours to twenty-four hours of onset [39] While the smaller (100,000 IU) dose reduced the risk of acute side effects, as we have already seen,[24,29] it also reduces the duration of protection

Older Infants (Six–Eleven Months)

At present, the World Health Organization (WHO) advises that infants six months to eleven months of age be given half a standard dose, or 100,000 IU (105 μmol), for prophylaxis [37] However, there have been no controlled trials on the incidence in this age group of acute side effects following receipt of a half dose or alternative-sized doses of vitamin A In the Filipino trial, children one year of age receiving 100,000 IU of vitamin A experienced a ~2% absolute

Table 14–4 Effect of Maternal Vitamin A Supplementation (312 μmol) Post-Partum on Mother's and Infant's Vitamin A Status

Status Indicator/ Treatment Group	*Time Post-Partum*		
	0 5 Months (Baseline)	*3 Months*	*6 Months*
MOTHER'S SERUM RETINOL (μmol/liter)			
Vitamin A			
mean (n)	1 17 (69)	1 39[a] (70)	1 23[b] (67)
SD	0 45	0 49	0 34
% < 1 05 μmol/liter	45	21	30
Placebo			
mean (n)	1 31 (71)	1 24 (69)	1 08[c] (72)
SD	0 51	0 43	0 37
% < 1 05 μmol/liter	31	33	47
MOTHER'S MILK RETINOL (μmol/liter)			
Vitamin A			
mean (n)	2 30 (70)	2 45[b] (57)	2 36 (66)
SD	1 42	1 23	1 17[b]
% < 1 05 μmol/liter	10	11[b]	9[b]
Placebo			
mean (n)	2 69 (66)	1 82 (60)	1 77 (70)
SD	1 53	1 28	0 97
% < 1 05 μmol/liter	8	32	24
MOTHER'S CIC (≥ 1 eye abnormal)			
Vitamin A[d]			
% abnormal (n)	32 (77)	23 (74)	13 (69)
Placebo			
% abnormal (n)	28 (76)	23 (74)	23 (73)
INFANT'S SERUM RETINOL			
Vitamin A			
% < 0 52 μmol/liter (n)	—	—	15 (68)
Placebo			
% < 0 52 μmol/liter (n)	—	—	36 (70)
INFANT'S RDR			
Vitamin A			
% ≥ 20% (n)	—	—	10 (67)
Placebo			
% ≥ 20% (n)	—	—	23 (64)

Note 1 05 μmol/liter = 30 μg/dl

[a]Vitamin A versus placebo, $p < 0.05$

[b]Vitamin A versus placebo, $p < 0.01$

[c]Placebo 6 months versus placebo 0 5 months, $p < 0.01$

[d]X^2 test for trend over time, $p < 0.01$

From Stoltzfus et al , 1993 [87]

Table 14–5 Incidence of Side Effects in Preschool Filipino Children within Seven Days of Receiving Vitamin A or Placebo Supplement

	200,000 IU (n = 832)			*100,000 IU (n = 803)*			*Placebo (n = 836)*		
Symptoms	*Number*	*%*	*Relative Risk*	*Number*	*%*	*Relative Risk*	*Number*	*%*	*Relative Risk*
Headache	49	5 9[a]	7 4	16	2 0	2 5	7	0 8	1 0
Nausea/vomiting	73	8 8[a]	4 0	29	3 6	1 6	18	2 2	1 0
Fever	66	7 9	1 5	49	6 1	1 2	44	5 3	1 0
Diarrhea	50	6 0	1 1	44	5 5	1 0	45	5 4	1 0
≥ 1 symptom									
Within 24 hr	143	17 2[a]	2 2	77	9 6	1 2	66	7 9	1 0
1–7 days later	47	5 6	1 2	37	4 6	1 0	38	4 5	1 0
≥ 2 symptoms									
Within 24 hr	34	4 1[a]	6 8	11	1 4	2 3	5	0 6	1 0
1–7 days later	6	0 7	1 4	8	1 0	2 0	4	0 5	1 0

NOTE 200,000 IU = 209 μmol (or 60,000 μg RE)

[a]Row (symptom) differences by chi-square test (2 *df*), $p < 0.001$

From Florentino et al., 1990 [39]

increase in the incidence of vomiting and/or nausea (5 5% versus 3 6% in controls, NS) [39] Applying these rates to late infancy (for lack of other suitable data), the enormous survival potential at this age far outweighs the mild, transient, increased risk of acute side effects

Younger Infants (Less than Six Months)

Young infants appear to tolerate a single, 50,000 IU (52 μmol) oral dose of vitamin A Indonesian newborns randomized to this dosage within forty-eight hours after birth (n = 2067) were at only 1 8 times the risk of developing a transiently "tense or bulging" fontanel within 48 hours after dosing compared with placebo controls (7 5% versus 4 1%) ($p \leq 0\ 001$) [40] Change in intracranial pressure (ICP) in half of all infants was monitored at baseline and at twenty-four hours by Doppler ultrasonography, a noninvasive device that measures blood flow within the anterior cerebral artery, yielding a "resistive index" (RI = 100 [peak systole-peak diastole]/peak systole) that reflects ICP [41,42] None of the children developed a resistive index indicative of elevated ICP Indeed, the ICP of infants with a bulging fontanel was normal in both groups Fontanelles were all clinically normal within seventy-two hours Affected infants showed no increased irritability, lethargy or vomiting on repeated clinical examination over a forty-eight-hour period [40] These findings suggest that transient bulging of the anterior fontanel in the small percentage of newborns who received vitamin A (and in those who did not) is benign and, in the absence of any detectable rise in intracranial pressure, likely to be clinically insignificant [43]

In Nepal, infants under one month of age participating in an RCT (n = 223) showed no increase over placebo controls in the risk of bulging fontanel, vomiting, irritability or other morbid symptoms twenty-four hours after receiving 52 μmol (50,000 IU) of vitamin A Among infants one month to six months of age (n = 2617), receipt of 105 μmol (100,000 IU) of vitamin A was associated with a (nonsignificant) 1 6% absolute increase in both forceful vomiting (95% CI 0 2% – 3 0%) and fever (95% CI 0 7% –3 9%), and a 0 5% excess risk of bulging fontanel (95% CI −0 1% –1 1%) [44]

The incidence of side effects may increase when infants receive repeated large doses, as in the Expanded Programme for Immunization (EPI) [45] Field trials (RCTs) in Bangladesh provided infants with 50,000 IU[46,47] or 25,000 IU[48] of vitamin A at each of the program's three diphtheria-pertussis-tetanus (DPT) visits (that occurred at ~6, 11 and 16–17 weeks of age) In all these studies, there was a sizable increase in the number of infants who developed a bulging anterior fontanel following vitamin A supplementation compared with controls (Table 14–6), 10% –15% versus 1% –3% [46–48] In all three trials, the incidence of bulging fontanel increased with each successive visit, suggesting a cumulative response to repeated vitamin A dosing over the ten-week interval While posing little apparent health risk, high rates of bulging fontanel may be negatively

Table 14–6 Infants Developing a Bulging Anterior Fontanel during Randomized Vitamin A Supplementation–EPI[a] Trials in Bangladesh

Study Author (Ref)	*Vitamin A*	*Control*
de Francisco et al [46]		
Dosage per visit, IU	50,000	0
Number infants	97	94
Bulge, number infants (%)	11(11 5)	1(1 1)*
Mahalanabis et al [47]		
Dosage per visit IU	50,000	0
Number infants	40	40
Bulge, number infants (%)	6(15 0)	1(2 5)
Baqui et al [48]		
Dosage per visit IU	25,000	0
Number infants	86	81
Bulge, number infants (%)	9(10 5)	2(2 5)**

[a]Infants received stated dose of vitamin A or placebo at each of three EPI (Expanded Programme for Immunization) visits at ~6 ~11, and ~14 weeks of age (varies slightly by study)

*$p < 0.10$
**$p < 0.01$

perceived by mothers, which could adversely affect compliance with a supplementation or, worse, with an EPI program This would argue for giving a smaller prophylactic dose of vitamin A (e g , ≤ 50,000 IU) only once in early infancy, preferably soon after birth, or, alternatively, only two doses at longer intervals (e g , 25,000 IU at six weeks and again at sixteen weeks)

Infants appear to tolerate a large dose of vitamin A (up to 50,000 IU) with, at worst, a low incidence of mild, transient side effects While it is, therefore, unlikely they will suffer any subsequent defects, there are little data on chronic risks In Nepal, a 27% increase in four-month mortality was observed among infants one month to three months of age who received a 105 μmol (100,000 IU) dose of vitamin A [49] Although not statistically significant (95% CI −11% to 89%), the direction and size of the effect, coupled with a (nonsignificant) trend of increased mortality in recipients of better nutritional status (by arm circumference), suggest that this dosage may be excessive for infants under four months of age Further, in Indonesia half this amount (52 μmol) given at birth[40] was safe and highly effective in *reducing* infant mortality [50]

Investigations are under way to carefully search for any potential subtle developmental changes in the third year of life that may be associated with bulging fontanels induced by vitamin A supplementation (50,000 IU), or occurring spontaneously, immediately after birth (J Humphrey, personal communication, 1995)

Maternal and Fetal Effects

There have been no reports of maternal intolerance to a large oral dose of vitamin A given soon after childbirth Because "excessive" maternal intake of vitamin A *early* in pregnancy has the *potential* for being teratogenic,[8 34 51 52] it is prudent to ensure that targeted women are only dosed post-partum with a large dose of vitamin A, prior to the return of ovulatory menses and risk of conception Such risk is virtually absent in women who are dosed within one month following childbirth, in breast-feeding populations, fewer than 1% of women conceive before the third month post-partum [53-60] Therefore, the potential risk of teratogenicity is exceedingly low for maternal, high-potency vitamin A supplementation within two months of giving birth These restrictions do not apply to women of childbearing age at risk of going blind from xerophthalmia (Chapter 10)

Effectiveness

One reasonably high goal for high-potency vitamin A effectiveness would be a 90% reduction (i e , estimated efficacy) in the incidence of xerophthalmia in preschool-age children Program impact would, on average, be expected to be lower since reduction depends on both efficacy and program performance, such as the adequacy of target-group definition and routine levels of coverage achieved However, community-based programs for dosing preschool-age children have received insufficient (controlled) evaluation

The Aceh study assessed program impact using a randomized design, 229 of 450 villages were assigned to the government's semiannual, universal vitamin A capsule distribution (UNIVAC) program, which achieved 78% coverage of preschoolers at both rounds [61] After one year, vitamin A supplementation reduced the prevalence of new cases of XN by 70% and X1B by 80% (RR = 0 30 and 0 20 respectively),[62] a level of impact that indicated there was an unusual lack of bias in coverage (expected reduction 90% x 78% = 70%) Nearly identical reductions in new, prevalent cases of XN (73%), X1B (66%), and in the two conditions combined (78%) were observed among preschoolers in Sarlahi, Nepal, following sixteen months (four rounds) of high-potency vitamin A supplementation compared with controls [63]

During each of the five years of vitamin A distribution in the urban slums of Hyderabad, India, the relative risk of corneal xerophthalmia (X2/X3), based on reported admissions to hospitals serving program and nonprogram areas, was RR = 0 59, 0 44, 0 12, 0 16, and 0 17 respectively,[23] suggesting that a cumulative effect may have occurred during the first three years Thereafter, the impact leveled off, possibly due to imperfections in program coverage (estimated to have been ~87%) [23]

As a rule, vitamin A supplementation programs can be expected to have little impact on xerophthalmia if coverage is below 25% Reasonable control of xerophthalmia can be expected when capsule coverage exceeds 65%, and may approach the limits of efficacy once 85% or more of children are reached [6,10] But without concerted effort to dose the most disadvantaged children and communities, effectiveness will suffer [64] A survey-based evaluation in Bangladesh suggested that the national vitamin A program had reduced the prevalence of corneal xerophthalmia by only ~53% in high-coverage areas (> 75% capsule receipt in the previous six months), the estimated reduction in XN was only 37%–47%, and in X1B, less than 25% [65] This modest impact of a program in the presence of apparently adequate coverage was probably due to low coverage of highest-risk children [2,65]

Of course, effectiveness can also be evaluated with respect to other outcomes (such as impact on mortality reduction) that can be expected with vitamin A prophylaxis, for which estimates linked to coverage have been made [66,67] In addition, Beaton et al [5] have estimated the probabilities of achieving levels of mortality reduction based on results of mortality studies (Chapter 2)

Implementation

Vitamin A capsules containing 200,000 IU as retinyl palmitate (and 40 IU vitamin E) in oil are available to most countries from UNICEF through the Essential Drugs Programme of WHO They can also be purchased directly, provided by various organizations, or, as in India, manufactured locally [68] In 1993 alone, UNICEF supplied over 125 million high-potency capsules to sixty-three countries under routine and emergency conditions (J Csete, UNICEF, personal communication, 1994) at a delivered cost of approximately US$0 02 per capsule India distributes in-country another 50 million doses as an oily syrup each year (Fig 14–1) Shelf life of large-dose vitamin A supplements generally appears to be excellent Manufacturer tests for vitamin A content of capsules stored in closed containers at 23°C for up to thirty-one months have shown 97% retention [3] Capsules retained 98% of their original retinol activity after two months of sea and overland transport in Africa [69] Periodic analysis of capsules during large community trials suggests that 85%–90% of vitamin A in supplements is retained after twelve months to eighteen months' storage under field conditions [70]

High-potency vitamin A supplementation can be targeted by location, age, and season according to local epidemiologic features (Chapter 12) Vitamin A supplementation programs normally target (initially) children six months to seventy-two months of age, concentrating in areas where xerophthalmia (or vitamin A deficiency) constitutes a public health problem [7,10] and where child mortality rates are high Where seasonality is distinct (Chapter 12), steps should be taken to ensure distribution is timed to precede the high-risk season

The supplemental doses can be delivered to children through different mechanisms, chosen to achieve different levels of coverage of those at risk (program sensitivity) The lowest coverage and cost for an impact is obtained by targeting children with xerophthalmia, measles, and other clinical illnesses or malnutrition presenting to health workers, clinics and hospitals (Chapters 7, 10) Wider coverage of children in high-risk areas can be achieved through clinics or community-based outreach services, but these generally omit the remote, high-risk underserved areas The third approach is the distribution to all children in high-risk areas (universal distribution) [16,10] Regardless of the approach taken, effective supplementation programs clearly identify their target groups, set standards of performance, and achieve high vitamin A coverage of those targeted through an organization that is adequately staffed, trained, supervised, and supplied for the chosen level of activity [10] Periodic careful evaluation of both *process* (are the capsules reaching warehouses and clinics, and being distributed?) and *outcome* (did xerophthalmia rates at least decline?) are essential to ensuring continued program rigor and effectiveness

Treatment (Medical or Therapeutic Approach)

Adequate provisions for effective treatment of vitamin A-deficient children should be the goal of any national vitamin A prophylaxis strategy [1,10] Health workers staffing health facilities where vitamin A deficiency exists should be trained in the diagnosis and treatment of xerophthalmia and other precipitating conditions, such as measles Administration of high-potency vitamin A for "treatment" represents a subset of targeted delivery,[16] it focuses on preventing blindness, severe morbidity, and death related to vitamin A deficiency by supplementing children who are obviously deficient, ill, or wasted (it has high specificity) Incremental costs relate to training existing staff, maintaining records, and ensuring a continuous and adequate supply of vitamin A supplements to health facilities [1,10] Even with program elements in place, however, effectiveness is naturally limited by health care utilization patterns

Targeted Distribution

This approach offers the greatest flexibility and cost effectiveness (hence potential for sustainable vitamin A supplementation) to reach more than those few children who present to health care facilities It takes full advantage of existing contacts between health providers (and other sectoral workers) and the community to get vitamin A to children in high-risk areas The approach requires substantial planning and coordination across sectors—from ministerial through local levels—to set goals, to implement, and to evaluate progress A variety of health services can be included sickness and growth monitoring clinics,[71,72] antenatal and postnatal services, and community-based home-visiting, family-planning,

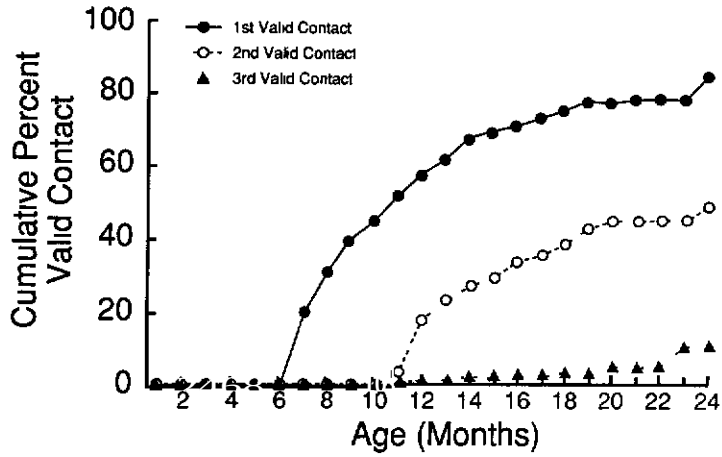

Fig. 14–3. Cumulative percent of Ghanaian children having a "valid" immunization contact, that is, at which a high-potency vitamin A supplement could be given (i e , ≥ 6 months of age for a first contact or subsequent contacts at least four months apart, 55% of children had an initial valid contact during the latter half of infancy [6–11 months], 48% of children had a second valid contact by 24 months of age) (From D C Arhin et al [76])

birth-attending, and immunization[45] services Access can be further increased through schools and their outreach capabilities, agricultural extensions, and community services of local and international nongovernmental organizations In refugee settings, vitamin A should be made available to children during registration, food distribution, and immunization [4] All of these scenarios represent "windows of opportunity" for prophylaxis [72]

If one relies primarily on a passive system of targeted distribution, providing supplements only to children attending health facilities, then low and sporadic health clinic utilization will lead to poor coverage [173-75] Greater coverage requires outreach and active community-based programs In the Bolgatanga-Frafra District of Ghana, 55% of surveyed, preschool-age children between six months and eleven months of age received at least one EPI contact during which a vitamin A capsule could be administered, this had increased to 84% by 24 months of age (Fig 14–3) [76] The age and level of coverage varies widely from country to country The spacing of immunization (and other) contacts becomes important for evaluating the probability of dosing children two or more times with vitamin A In the Ghanaian study, 48% of the children had received two properly spaced immunizations (≥ 4 months apart) before twenty-four months of age, representing an of opportunity to reach half of all children twice with vitamin A, albeit irregularly, between six months and 23 months of age [76] As overlapping program options are explored, home-based documentation of capsule receipt may become important to ensuring appropriate spacing of vitamin A supplements

Universal Distribution

"Universal" distribution involves community-wide delivery of vitamin A to target-age children on a regular basis in high-risk areas Although WHO recommends three-month to six-month intervals between doses, most programs are organized as semiannual distribution campaigns Existing health auxiliary or other, local volunteer staff are mobilized to administer the supplement, usually on a house-to-house basis

Factors that predict adequate coverage remain poorly defined, although local empowerment appears to play an important role in program performance In Indonesia, children were more likely to be dosed with vitamin A if their mothers were aware of the potential benefits of capsule distribution and the consequences of xerophthalmia [77,78] Health volunteers who routinely offered health education to the community also achieved higher rates of capsule coverage [77] Involvement of local women's groups raised attendance, and vitamin A capsule coverage, at the monthly "pos yandu" clinics in Eastern Indonesia [78] In Aceh, where distribu-

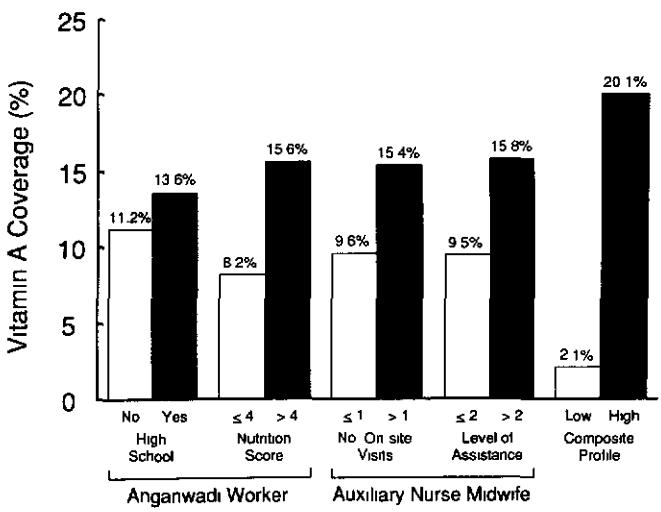

Fig. 14-4. Determinants of vitamin A supplement coverage achieved by community health workers ("anganwadi" workers) in the Integrated Child Development Services, Gujarat, India Nutrition score was based on seven questions (maximum score = 7) related to child feeding and other practices Number of visits refers to the times an auxiliary nurse-midwife (ANM) visited the anganwadi worker in the previous three months Level of assistance was based on activity of the ANM in helping an anganwadi (maximum score = 9) The composite profile takes the coverage of workers who had a combination of no high school education, a nutrition score ≥ 4, ≥ 1 ANM visit, and an assistance score of ≤ 2 by the ANM, and compares this with workers who had the complement of these scores All differences, $p < 0.0001$, except for high school comparison (NS) (From S Gujral et al [81])

tors were predominantly male (86%), capsule coverage was higher in smaller, more rural villages than in urban areas Volunteers representing the local "status quo ' (farmers or nonfarmers with little education) were better distributors than those who were "upwardly mobile" (higher education, wealthier and more urbanized) [79] In an evaluation of India's Integrated Child Development Scheme,[80] vitamin A supplement coverage (one of a number of services) was twice as high when female community health workers ("anganwadi" workers) understood basic nutrition or received frequent visits or encouragement from supervising auxiliary nurse-midwives Combining these factors led to a twelvefold increase in coverage (20%, versus 2% in their absence) (Fig 14–4), although overall delivery rates remained low and unexplained [81]

The relative strengths and weaknesses of universal distribution have been reviewed in detail [1 2 6 10 62 82–85] Difficulties most often encountered include recurrent depletion of central stores, transport delays and breakdowns in local procurement that lead to peripheral capsule shortages, poor supervision and motivation of local workers (partly related to a lack of incentives), and inadequate local coordination and record-keeping Children may be incorrectly targeted (i e , outside the designated age group) or receive the wrong dose [86] Mothers may become noncompliant if negative perceptions are not addressed in a timely manner [1] Finally, policy questions exist about how and on what criteria to phase out and eventually halt community-based vitamin A supplementation [86] Answers will emerge when, and if, alternatives (such as food-based dietary strategies or fortification) are developed and shown by evaluation to ensure adequate, more sustainable improvement in the vitamin A status of target children in high-risk areas (Chapter 13)

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15

Fortification of Dietary Items with Vitamin A

There are two kinds of developing countries those that have begun to fortify and those that have not yet begun to fortify their food supply

Food fortification involves adding nutrients to foods in order to maintain or improve the quality of the diet of a targeted group or population [1,2] Fortification has long been an accepted strategy for improving vitamin A nutriture in industrialized countries [3,4] The epidemic of xerophthalmia in Denmark during World War I that paralleled the substitution of margarine (lacking "fat-soluble A") for butter in the Danish food supply foretold the clear need to fortify such products [5] Margarine is now among those food items most frequently fortified with vitamin A in the world [4] Universal iodization of salt to prevent iodine deficiency disorders (IDD) is a rapidly approaching achievement [6] Today, the motivation to fortify foods or condiments with vitamin A in developing countries continues to be the prevention of vitamin A deficiency and its health consequences (xerophthalmia, other morbidity, mortality) Although "targeting high-risk groups" is a guiding principle, broader segments of a population (not always specifically targeted) may gain nutritional and health benefits through fortification as well [7-9]

Food fortification (or nutrification) offers a direct, effective and potentially sustainable way to correct vitamin A deficiency [2] Criteria for food fortification have been discussed extensively elsewhere [4,7,9-13] The aim is to add vitamin A to a regular dietary constituent (food or condiment) of the targeted population at a specified level (e g , half the RDA) that will correct an existing dietary deficiency without posing significant risks of overdosing those who habitually consume the largest quantity of the fortified product This means that, ideally, the amount of carrier food consumed by the population would be within a relatively narrow

range, so that the selected fortified level can be both effective (even for low food-carrier consumers) and safe (for high consumers)

As with any fortificant, the added vitamin A should not appreciably alter the dietary carrier's appearance, color, texture or organoleptic properties (taste, smell) Further, potency should remain high under usual conditions of processing, transport, storage and cooking As a minimum criterion, the product should retain potency for six months to cover time between production and consumption, and whatever loss that ensues should occur at a predictable rate [2,13] In most circumstances, fortification should be a relatively centralized process, this limits capital outlay and manpower requirements and simplifies maintenance of quality control

Once a fortified product is developed and ready for the market, international standards for maintaining quality assurance and labeling are provided by the Codex Alimentarius of the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) [14]

Food fortification is often called "passive" supplementation because it offers an opportunity to improve dietary (micronutrient) quality without necessarily changing existing food habits The concept is straightforward However, successful implementation of vitamin A fortification has proved challenging Thorough product development and testing has been required, along with persistent explanation and advocacy within both the public and private sectors to gain support, alleviate anxieties and misconceptions, and ultimately ensure success One need only be reminded of the long history of salt iodization, a simple technology that first met all the requirements of fortification and has effectively controlled IDD in North America and Europe for the past six decades [15] Yet thirty years after successful pilot projects were launched in South and Central America, salt iodization has still to be effectively instituted to control IDD in South Asia[15–17] and Africa[15,18]—although it is now a stated international goal [6]

Vitamin A Fortification in Developing Countries

Experiences in developing countries with vitamin A fortification highlight the nutritional benefits to be gained, as well as the challenges faced, by this promising strategy Foods that have been fortified with vitamin A or imported as fortified products in developing countries[4,13] include sugar [7] monosodium glutamate (MSG, a popular flavor enhancer),[19,20] wheat,[21–23] rice[24–26] and other grain products,[27] tea,[28,29] dairy foods (especially dried skim milk),[30–34] margarine,[4,35] edible oils,[36,37] formula foods, and specialty items However, to date, only vitamin A-fortified MSG (in Southeast Asia) and sugar (in Central America) stand out as products that have undergone extensive testing, have been distributed widely in-country, and have been evaluated for their public health impact

Monosodium Glutamate In Indonesia and the Philippines

Two countries, Indonesia and the Philippines, have pioneered the use of MSG for vitamin A fortification MSG+A Their experiences provide valuable insight into the process, from identifying a suitable vehicle, or carrier, for the nutrient to testing and attempting to scale-up production

Vehicle Selection

In the late 1970s, national surveys in Indonesia investigated potential dietary vehicles for vitamin A fortification MSG was observed to be consumed at least weekly by three-fourths of the children one year to five years of age (including 70% of children with X1B), the proportion rose to over 80% for children two years and older In contrast, only 50%–55% of children consumed other potentially fortifiable staples such as wheat or sugar [38] An economic analysis suggested that MSG would be less expensive to fortify than other products, since less vitamin A would be wasted on the nontarget population Further, in Indonesia MSG is centrally processed by only two companies Per capita intake was reportedly ~0 6 g/day (standard deviation [SD] ±0 7 g) Preschool children were found to consume, on average, 0 2 g to 0 3 g/day (SD ±0 3 g) [20 39] Although the coefficient of variation for intake was ~100%, variability in consumption across socioeconomic and demographic strata was smaller for MSG than for other potential vehicles, reducing the risk of excessive vitamin A intake by the largest consumers [38] Further, it was feasible to segregate a product "stream" of MSG for fortification the 50% of production packaged in the smallest containers (1 5 g) that penetrated the target markets (poorer consumers) In a scaled-up program this would cut the recurrent manufacturing costs in half by restricting vitamin A addition to MSG sold in the poorer markets Also, by spreading the cost over all MSG consumed, the marginal cost of fortified MSG purchased by poor communities would be halved This strategy also permits a higher concentration of vitamin A, boosting the supplementary intake of those in greatest need without running the risk of overdosing larger consumers, who were far less likely to need the added vitamin A

The Philippines pioneered the use of MSG for vitamin A fortification even before Indonesia [19 40–42] A single manufacturer,[19] coupled with nearly full-market penetration (~94% of Cebuano children one year to sixteen years of age reportedly consumed MSG each week), made MSG an attractive candidate for fortification Families normally purchased two small sachets per day for a per capita intake of ~0 7 g/day,[40 41] similar to Indonesia, although preschool-child intakes were not specified Breads, biscuits, corn grits, and table salt were also nearly universally consumed by children,[41] however these foods were either not centrally processed or difficult to fortify with vitamin A

MSG has been fortified by first coating dry vitamin A palmitate beadlets (Type 250 CWS [cold water soluble], Roche) with a whitener and flow agent to make a premix, this is then metered into crystalline MSG at a ratio of 2 98 and mechanically mixed to ensure homogeneous dispersal of the product [43] Strict quality control standards have been established for MSG+A [44] Laboratory tests have shown excellent vitamin A retention (~75%) at high temperature (37°C) for six months[4] and a half-life of over two years under dark, dry conditions,[43 45] although potency can rapidly deteriorate with continuous exposure to light and moisture [43]

Both countries deemed MSG to be safe based on toxicological[46] and epidemiologic[47] evidence, its inclusion on the generally-recognized-as-safe (GRAS) list of the U S Food and Drug Administration,[48] and its widespread consumption [38 41] Fortification levels that would provide an approximate RDA for Filipino children (2050 μg RE/g)[41] and half an RDA for Indonesian preschool children (810 μg RE/g)[20] were chosen, based on dietary intake data for MSG and dietary vitamin A and the nutritional goals of each country

Prophylactic Efficacy of MSG+A

Preliminary trials suggested that consumption of both vitamin A-fortified MSG and sugar could significantly improve vitamin A status of vulnerable groups In Cebu, ensured intake of MSG+A (provided free, directly to families as two 2 2 g sachets daily, for nearly two years) raised serum retinol levels of Filipino children from a mean of 21 μg/dl to 29 μg/dl, compared with no change or a modest decline in serum retinol among children of other barrios who were either dosed semiannually with vitamin A or whose families received a package of public health interventions (PHI) (Table 15–1) [40] Regular intake of MSG+A decreased the proportion of deficient (< 20 μg/dl) children relative to the PHI group, whose vitamin A status appeared to change little The largest reduction in the prevalence of xerophthalmia also occurred among MSG+A recipients

Table 15–1 Impact of Different Vitamin A Interventions, Cebu, the Philippines

		Serum Retinol (μg/dl)			
	Number of	*Baseline*		*Follow-up*	
Intervention	*Children*	*Mean*	*Sd*	*Mean*	*SD*
MSG-A	387	21	13	29[c]	14
Vitamin A supplements[a]	343	20	12	20	14
PHI[b]	391	19	12	16	12

[a]Six monthly 200,000 IU (60,000 μg RE) vitamin A distribution

[b]Public health intervention (PHI) package including gardening, nutrition and health education, and sanitation inputs

[c]MSG+A versus PHI, $p < 0.01$

From Solon et al , 1979 [40]

(declining from 4.2% to 1.0%), though capsule recipients were not far behind (3.1% to 0.6%). There was little change in xerophthalmia rates among those exposed to PHI/gardening intervention (4.9% at baseline versus 3.4% at follow-up).[40-42] Although the study was not randomized and the number of barrios was small (n = 12 barrios, 4 per group), the effect may be considered an estimate of the potential "efficacy" of vitamin A-fortified MSG consumption (directed to families who routinely consume this product).

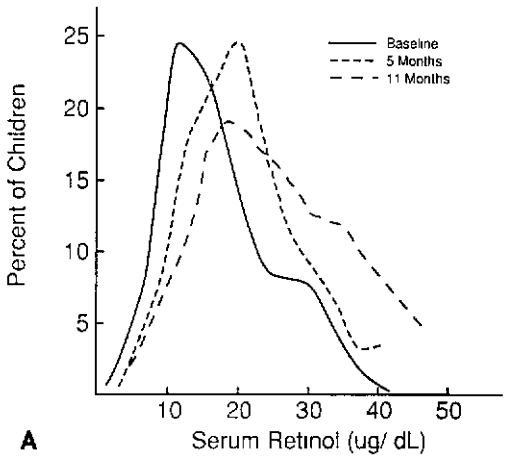
The positive impact of MSG+A on serum and breast-milk retinol in Indonesia, under actual program conditions (providing an estimate of effectiveness, discussed below), supports the benefits of MSG+A reported from the Philippines.[20,49] The apparent lack of effect of the PHI/gardening intervention in Cebu remains unexplained, though it seems likely that it either failed to change behavior or account for intermediary determinants of vitamin A status (e.g., measles and diarrheal rates, which were not reported). In addition, the degree to which vegetable gardening raises vitamin A status of children remains questionable (Chapter 13).

Effectiveness of MSG+A

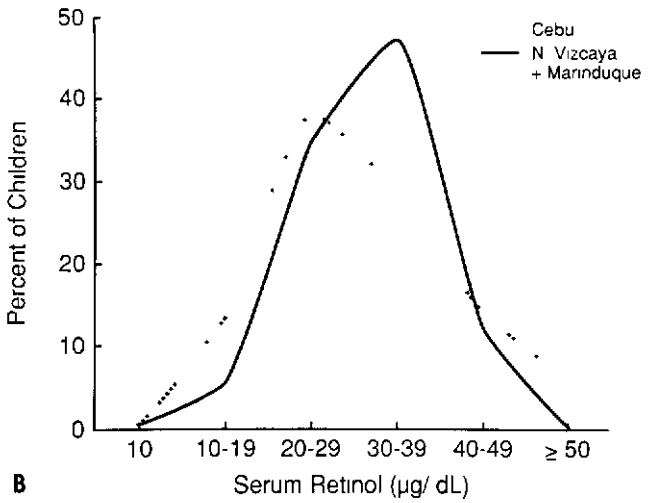
"Effectiveness" represents the impact of an intervention under usual conditions; it is the net effect of efficacy (biologic impact under conditions of full coverage and compliance) modulated by the inefficiencies (interrupted supplies, hostile environments, noncompliance, etc., that reduce coverage) of program operation. MSG+A has proven effective in raising the vitamin A status of a target population when delivered through usual marketing channels, resulting in a consistent shift in serum retinol levels to the right (reducing the proportion of the population most deficient). As expected, the impact increases with the duration of fortification, at least through the first year or more of operation (Fig. 15–1).

In Indonesia, MSG+A was distributed and sold without advertising or promotion through small village shops in forty-eight subvillages; shops in forty-four nearby subvillages (serving as controls) continued to receive unfortified MSG. Children living in program villages experienced incremental increases in serum retinol at six months and, again, 11 months after beginning fortification (Table 15–2, Fig. 15–1, Panel A),[20] while breast-milk retinol of lactating mothers rose relative to controls (Fig. 15–2).[49] These improvements in biochemical indices of vitamin A status had a demonstrable health impact: a reduction in the prevalence

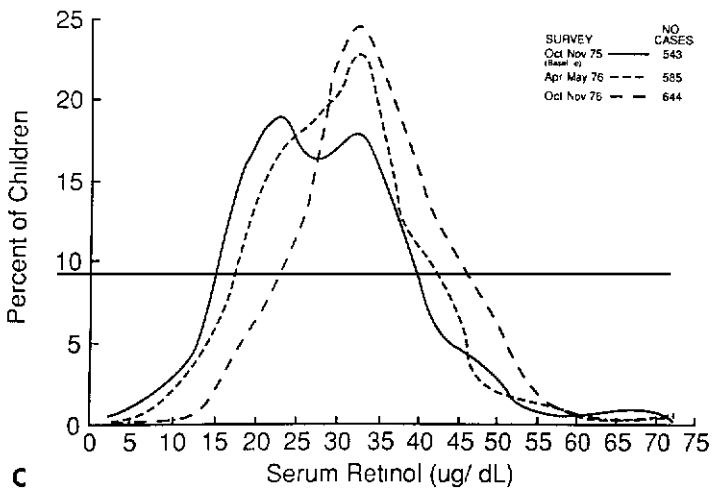
Fig. 15–1 Changes over time in serum retinol distribution in children following vitamin A fortification. Panel A: West Java, Indonesia. Baseline, five and eleven months after introduction of MSG+A through normal markets.[20] Panel B, Cebu (control) and Northern Vizcaya and Marinduque (intervention provinces) after almost two years of MSG+A through markets.[40] Panel C, Guatemala. Baseline, six and twelve months after introduction of vitamin A-fortified sugar as a national program.[7]


Baseline
5 Months
11 Months
Percent of Children
25
20
15
10
5
10
20
30
40
50
Serum Retinol (ug/ dL)

A


Cebu
N Vizcaya
+ Marinduque
Percent of Children
50
40
30
20
10
0
10
10-19
20-29
30-39
40-49
≥ 50
Serum Retinol (µg/ dL)

B


SURVEY
NO CASES
Oct Nov 75 543
Apr May 76 585
Oct Nov 76 644
Percent of Children
25
20
15
10
5
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Serum Retinol (ug/ dL)

C

Table 15–2 Serum Retinol of Children before and during MSG + A Marketing, Bogor, Indonesia

	MSG + A Program			*MSG Controls*		
	N	$\bar{x}$	*SD*	*N*	$\bar{x}$	*SD*
Baseline	205	0 67	(0 33)	240	0 78	(0 35)
5 mo	258	0 78	(0 32)*	289	0 71	(0 30)**
11 mo	217	0 92	(0 33)*	290	0 72	(0 33)**

*$p < 0.001$ (increase) compared with baseline in program area.

**$p < 0.05$ (decrease) compared with baseline in control area

From Muhilal et al., 1988[20]

of X1B (by 85% after eleven months), a ~10 g/liter rise in hemoglobin (Chapter 5), improved linear growth (Chapter 6), and a 46% reduction in preschool-child mortality (Chapter 2) The breadth of these changes strikingly illustrates the potential health benefits to a high-risk population of vitamin A fortification of commonly consumed dietary items

In the Philippines, a scaled-up, pilot fortification program in two provinces (Nueva Vizcaya and Marinduque) followed the successful preliminary demonstration of efficacy,[40] cost-effectiveness,[41] and cost-benefit[8] projections for MSG+A noted above Vitamin A-fortified MSG was sold in 2 g sachets in the test provinces The province of Cebu, receiving nonfortified MSG, served as the control At baseline, serum retinol levels of preschool-age children in the combined program provinces (n = 1156) were virtually identical to those of Cebuano children (n = 591) (not shown)[42] After twenty months of selling MSG+A the mean increase in serum retinol was modest [28 μg/dl to 31 μg/dl ($p < 0.01$) and 31 μg/dl to 32 μg/dl in the two test provinces, versus 29 μg/dl to 31 μg/dl in Cebu][42] However, children in the test provinces (n = 1291) showed a distinct

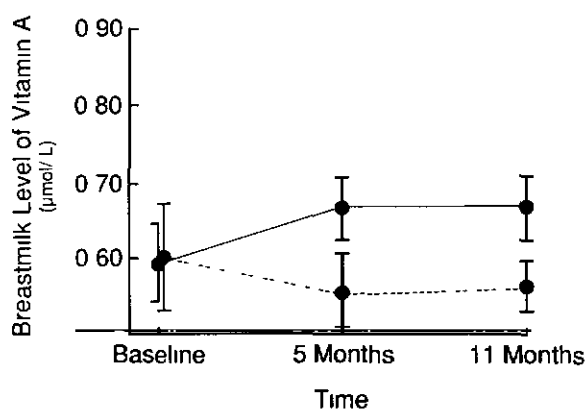


Fig. 15–2. Mean (±2 SD) breastmilk retinol (vitamin A) levels of women in MSG+A villages (solid line) and control villages (broken line) at baseline, five and eleven months after introduction of MSG+A, West Java, Indonesia (From Muhilal et al[20])

shift to the right of their serum retinol distribution compared with children in the control province (n = 608) (Fig 15–1, Panel B) The proportion of children most deficient in vitamin A was reduced, and as a consequence, xerophthalmia rates declined (from ~3% to 1%) At least three factors explain the absence of an even greater impact (1) mean serum vitamin A at the outset was already near the normal range (~30 μg/dl), (2) the technology for packaging MSG+A caused problems because the fine premix powder interfered with complete sealing of the sachets against humidity, resulting in clumping of MSG, loss of vitamin A potency, and lower area sales,[19] and (3) the added cost of fortification was passed on to the consumer in the form of reduced MSG content per sachet,[19] prompting consumers to switch to larger, nonfortified packets

Cost Considerations

Initial cost projections from the Philippines were ~US$0 11 per capita (during the first few years) for MSG+A production and distribution (including set-up costs), decreasing to under $0 10 over a five-year period [8] Indonesia learning from the lessons in the Philippines, recognized that while the full cost must eventually be borne by the consumer to insulate consumption from the uncertainties of the health budget, it needed to be done in a less obvious and more gradual manner One plan was to spread the marginal cost of vitamin A fortification (~24% of the fortified amount) over the cost of all MSG produced and packaged in the country Since only half would be fortified (small sachets consumed by the targeted poor markets), the marginal increase in purchase price would be ~12% to all MSG consumers The government considered a partial subsidy of two-thirds of the cost during the first year, phasing it out over three years The consumer would, therefore, face an annual 3%–4% increase in the price of (all) MSG, against a background inflation rate of ~10%, for three years, after which the cost of fortification would be self-sustaining

After nearly two decades of research and development demonstrating clear nutritional and health benefits from MSG fortification with vitamin A, production has not yet gone full scale in either Indonesia or the Philippines This has been largely due to problems with physico-chemical instability of the MSG+A as fortified in the pilot trials,[19] serving to emphasize that fortification technology (for large-scale programs) should be based on a fully tested and proven "industry standard " Although a whiter, free-flowing vitamin A palmitate premix has been recently developed and laboratory tested,[43] in-country testing remains to be completed These technical difficulties have increased resistance on the part of major MSG manufacturers to collaborate[19 42] (S Wilbur, personal communication, 1994), stalling efforts in both countries and resulting in *zero* effectiveness This, despite a willingness on the part of the Indonesian government to adopt regulatory legislation mandating fortification of MSG as part of its national strategy for preventing vitamin A deficiency [50]

Sugar Guatemala and Other Areas of Latin America

Vehicle Selection

In the early 1970s, white, refined sugar was identified as a potentially fortifiable food carrier for vitamin A in a number of Central and South American countries Tests in Chile[51] and Guatemala[7,52,53] found vitamin A-fortified sugar (sugar+A) to be bioavailable,[54] organoleptically acceptable, and stable under ambient, humid conditions (90% retention after six–eight months) and after cooking (85%–99% retention in a wide range of products) Ongoing tests (in the 1990s) continue to show 50% to 85% vitamin A potency after nine months [55] In Guatemala, from where most data are available, white sugar was found to be nearly universally consumed (85%–90% of all sugar consumption was white sugar, the remainder was brown, unprocessed *panela,* mostly used in remote, rural areas) Sugar was also centrally manufactured, by a small number of refineries, and distributed nationwide through established market channels [7]

Fortification of sugar with vitamin A is straightforward A premix is prepared by combining vitamin A palmitate (CWS 250) with refined sugar (in a ratio of 22 76), followed by adding a stabilizer/vegetable oil solution (2 parts per 100) during continuous mixing [7] In Guatemala, the premix is distributed to seventeen sugar refineries and mechanically added to the sugar at a ratio of 1 1000 prior to drying the sugar, providing a calculated vitamin A concentration of 15 μg RE/g [53,55,56] This level was chosen on the basis of extensive dietary data showing average preschool-child intakes of sugar to be 33 g/day (range 10 g–50 g) in rural areas The selected level of concentration provides ~100% of a child's RDA (~400 μg RE) (Table 13–2) through fortified sugar, after allowing for programmatic losses [7] Similar goals have been set for Honduras, Costa Rica, and Panama [7,55]

Efficacy of Sugar+A

Early studies among children and adults in Chile[51] and school-age children in Brazil[57,58] indicated a positive impact of fortified sugar on vitamin A status Four remote Andean villages in Chile (per capita sugar intake ~40 g/day) were supplied with vitamin A-fortified sugar (38 μg RE/g) as their sole source of sugar, through local shops After three months the percentage of children (< 18 yr, n = 160) with serum retinol < 20 μg/dl declined from 76% to 29% Fortified sugar was then discontinued, three months later the prevalence of deficient serum retinol (< 20 μg/dl) had risen to 51% Adults showed a similar pattern [51] The two controlled, Brazilian studies were conducted among non-xerophthalmic nursery school children (eight months to eighty-nine months) with extremely low and highly variable serum retinol levels (baseline means of 7 μg/dl–12 μg/dl, SDs ranging from 7 μg/dl–50 μg/dl) Six months later the proportion with deficient

values appeared to have declined[57,58] although the variability was too great to reliably interpret

In an El Salvadoran clinical trial, pregnant women given ~30 g of fortified sugar per day (at ~11 μg RE/g) from the last trimester through the first four months post-partum (n_i = 25) had their serum retinol levels raised by ~10 μg/dl over controls ($p < 0.01$) [59] The increased vitamin A intake appeared to attenuate the post-partum decline in breast-milk retinol observed among the controls Infants breast-fed by mothers who received the fortified sugar experienced a gradual increase in serum retinol relative to controls for at least the first four months of life (+0 6 μg/dl at birth, +1 1 μg/dl at two months, +2 4 μg/dl at four months), although the differences were not statistically significant [59]

These preliminary studies provided the basis for instituting vitamin A fortification of white sugar, particularly in Guatemala [7,54,60]

Effectiveness of Sugar+A

Because sugar fortification was mandated nationwide, the Guatemalan evaluation lacks concomitant controls But semiannual nutritional surveillance during the first two years of start-up provided strong evidence that the program was achieving an impact on vitamin A status, as evidenced by the shift in serum retinol levels toward greater adequacy (Fig 15–1, Panel C) [7,54,60] Increases in serum retinol were observed in wasted and nonwasted children, indicating that fortified sugar was reaching even the most disadvantaged children [7] Sugar fortification also appeared to improve the mobilization and utilization of body iron (Chapter 5), and may have enhanced seasonally adjusted linear growth as evidenced by increased height-for-age [7] Breast-milk retinol levels rose from a mean of ~25 μg/dl to a mean of ~40 μg/dl during the first two years,[7,54] which was estimated to provide a dietary increment to the breast-fed infant of ~90 μg RE/day (~60% increase to infants consuming 600 ml of breast-milk daily) [7]

Subsequently, sugar fortification in Guatemala was suspended for nearly eight years due to civil conflict, lack of enforcement of the fortification law,[55] a glut in the international sugar market (reducing market prices and profits), and a rise in the Swiss franc on the international currency exchange (raising the price of vitamin A) Failure of the program during this period was a direct result of its mandated financing scheme Sugar manufacturers were required by law to absorb all of the cost of fortifying sugar with vitamin A [7,60] When changes in world markets combined to render sugar fortification too expensive, the program was stopped—providing a vivid example of why consumers ultimately must bear the cost of fortification if it is to be sustained Fortification also ceased in Costa Rica, El Salvador and Panama, although it continued to some extent in Honduras [55]

Sugar fortification was restarted in Guatemala in 1988,[53] and more recently in El Salvador, partly motivated by discoveries relating increased vitamin A

nutriture to improved child survival [55] Renewal in Guatemala offered a second opportunity to evaluate program performance and, at the same time, assess the devastating impact of its suspension for much of the previous decade A new "baseline" survey reported hyporetinemia (< 20 μg/dl) in 26% of preschool children and an abnormal relative dose response (RDR+) in 33%, serum retinol levels that were *lower* than they had been before the first fortification program began in 1975 [7 61] Six months after restarting fortification, the percentages of vitamin A-deficient children by serum retinol and RDR+ had declined to 10% and 14% respectively (Fig 15–3)[53] (when population coverage was 89%) [62] Although other factors that might account for improved vitamin A status cannot be ruled out, the findings are consistent with renewed impact

Since it was restarted in 1988, program performance has fluctuated Formal estimates of the proportion of sugar fortified for domestic consumption is 100% for Guatemala, 40% for El Salvador and 30% for Honduras [55] However, a household survey in periurban Guatemala City in 1990 (n = 91) showed low and erratic levels of vitamin A in the sugar consumed mean (SD) vitamin A content was 5 5 μg/g, or one-third the intended amount The median content was 3 3 μg/g (range, 0–30 μg/g), in line with quality control checks that found 94% of all samples containing *some* vitamin A (up from 0% in 1987[1]) but only 30% with acceptable levels (12 μg/g–18 μg/g) [55 62] Even at this low concentration, however, fortified sugar was providing ~25% of a young child's daily vitamin A intake (79 μg RE/day) [63]

Cost Considerations

Recently the Guatemalan sugar fortification law was revised to exempt sugar intended for industrial purposes (i e , non-human consumption), relax the "ac-

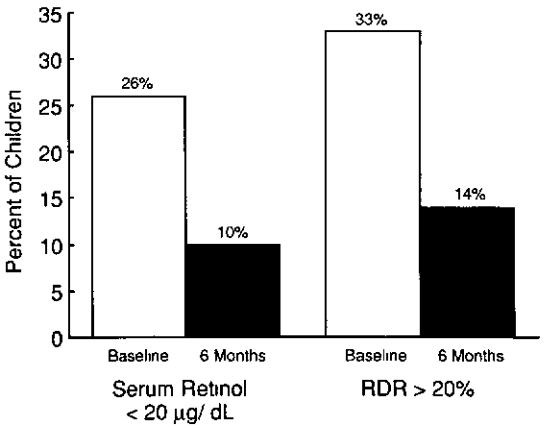


Fig. 15–3. Proportion of children with serum retinol < 20 μg/dl and a positive relative dose-response (RDR > 20%) at baseline and six months after the restart of sugar fortification with vitamin A in Guatemala, 1988–1989 (From O Pineda [53])

ceptable range" to 10 μg–20 μg RE/g (making it easier for producers to comply with standards), and provide tax incentives for fortifying imported sugar However, producers must still shoulder the costs of fortifying sugar with vitamin A [55] Originally, the estimated production cost per recipient in the population (1976 dollars) was $0 07 per capita, representing 1 1% of the retail price of sugar [7] Today, sugar+A is estimated to cost $0 29 per recipient (1993 dollars), still representing only 1 6% of sugar's retail price [55] Given Guatemala's earlier experience, however, it is not clear whether the above legislative changes will ensure a sustainable, effective program

Alternative Vehicles for Vitamin A Fortification

Beyond MSG and sugar, which have received most of the attention, other vehicles may offer opportunities to control vitamin A deficiency These include rice, wheat, dairy products, edible oils, and margarine, and other foods such as tea and (perhaps) certain types of salt The list has largely been limited by lack of imagination—many of the choice carriers need to be identified through exploration of locally available options, keeping an open mind about all dietary staples consumed by the target population

Rice

As a staple grain consumed by half of the world's population, rice could be an attractive vehicle in some settings Its first and most immediate limitation is that the rural poor often produce their own rice, or procure it from thousands of local millers, precluding carefully supervised addition of vitamin A fortificant The technology for adding vitamin A to rice exists, however One promising technique, advanced in Thailand two decades ago,[26 64] involves preparing a premix of nutrient-dense, simulated-rice granules, extruded either from pasta or rice flour, that are metered into the bulk grain (e g , 1–2 parts per 100) [4 24 26] Vitamin A content of premix granules synthesized from rice flour, with added antioxidants, appears stable under tropical conditions of storage (half-life of two to three months) and cooking (~55%–96% retention) [24] Bioavailability,[25] based on RDR results in recipient Brazilian children, is comparable to that of preformed vitamin A on an equimolar basis [25] Sensory panel testing favored the fortified over the nonfortified rice [25]

Vitamin A fortification of rice may be effective and practical where centrally processed or distributed rice targets the poor (such as government food-for-work programs or subsidized ration outlets), and in refugee feeding or dry-ration distribution Otherwise, feasibility, cost, and quality control constraints pose major challenges

Wheat Flour

With consumption of wheat increasing in many developing countries (in response to such factors as constraints in the supply of rice and the development of affordable, commercial wheat products), wheat fortification with vitamin A may provide a growing opportunity to improve vitamin A intake of the poor The technology for fortifying wheat flour with dry vitamin A palmitate (250 SD) exists [4 21 23 27 65] Vitamin A retention in low-extraction wheat flour exceeds 95% for up to a year under temperatures of ≥ 40 C°,[13 23 66] although high moisture content may increase losses markedly [22] Approximately 70% of vitamin A activity remained after baking traditional bread products with fortified flour [23 67 68] Organoleptic properties of fortified wheat flour appear unchanged from those of the unfortified product [13]

Many tropical countries import wheat, either through commercial channels or as food aid, the latter route tends to self-target the poor and, therefore, presumably the most vitamin A-deficient[69] Imported wheat is often milled at a limited number of private (e g , Philippines, Indonesia) or government (e g , Sri Lanka) mills prior to national distribution, making centralized fortification feasible Wheat (and other grain) flours exported from the United States under Public Law 480 are already fortified with 2200 IU–2645 IU vitamin A per 100 g,[70] but their consumption and impact on vitamin A status in high-risk populations are unknown

During the late 1960s, a concerted effort was launched to fortify wheat flour with vitamin A (240 μg RE per 100 g) in major, government-owned bakeries in Bombay and other Indian cities,[21] however, its economic success and nutritional impact are unknown [4 13 23]

Locally grown and milled wheat suffers many of the same practical problems as locally grown and milled rice Two decades ago, Vaghefi and Delgosha proposed that vitamin A be added to baker's yeast tablets rather than mixed into the wheat flour The tablets were produced in Iran's only yeast plant, thus serving the centralized and quality control needs of vitamin A fortification Although yeast may be a poor matrix to standardize for such a purpose, tests showed ~70% vitamin A retention in Persian breads baked by this method [68] The idea has not yet been seriously pursued

Milk Products

Vitamin A naturally occurs in milk[71] but is lost when the butterfat is removed,[4] making it necessary to reintroduce fat-soluble vitamin A (and D) to nonfat dry (skim) milk (DSM) [72] This is especially important when it is intended as food aid for developing countries,[32 33] particularly in disaster relief situations[73 74] where children are at high risk of blinding xerophthalmia [75 76] While high-protein refeeding of severely malnourished children (without apparent vitamin A deficiency)

may improve hepatic mobilization of vitamin A from the liver,[77–79] it may also precipitate vitamin A deficiency[78] and even xerophthalmia[77,80] in vitamin A-depleted children Feeding non-vitamin A-fortified milk products (e g , sweetened, condensed milk) to infants has been repeatedly implicated as the underlying cause of widespread deficiency and keratomalacia [30,81] Years ago, infants given isonitrogenous, isocaloric DSM (plus fat) formulas for three months, with (5,000 IU/100 g) or without vitamin A, showed comparable growth However, those not receiving vitamin A suffered a marked reduction in serum retinol (declining from 25 μg/dl at baseline to 16 μg/dl at follow-up, versus infants consuming vitamin A-fortified formulas, who maintained levels of ~30 μg/dl [p <0 012]) [31]

Since 1959, WHO/FAO/UNICEF have recommended that DSM intended for use in malnourished populations be fortified with 5000 IU vitamin A per 100 g [32,82] All DSM shipped from the United States under Public Law No 480 (Title II, Food for Peace Program) has been fortified with vitamin A since 1965,[33] initially at levels of only 2200 IU–4400 IU per 100 g,[73,83] these were raised in recent years to 5000 IU–7000 IU per 100 g [70,84] Other donor countries have been slower in meeting the recommendations [33,73]

Edible Oils

In some countries, processed oils and fats are increasingly penetrating lower socioeconomic markets and, therefore, may prove suitable as carriers of vitamin A An outstanding recent example is a popular margarine in the Philippines that has been highly hydrogenated (and, therefore, does not require refrigeration) In Cebu, the margarine was found to be consumed weekly by ~40% of children under six years,[41] usually spread on cooked rice A double-masked, placebo-controlled trial was jointly implemented by the government and the local manufacturer Two tablespoons (~27 g) of a fortified version of the margarine (providing 431 μg RE per two tablespoons) was consumed daily by one group of preschool-age children three years to six years old (n = 296) and a similar amount of the standard, nonfortified product by controls (n = 285) After six months, the mean serum retinol level of children who ate the fortified margarine increased by 3 μg/dl (from 26 μg/dl to 29 μg/dl), while values among controls declined by 2 μg/dl (from 27 μg/dl to 25 μg/dl) (final difference between groups, p < 0 001) [35] The prevalence of hyporetinemia (< 20 μg/dl) in the intervened group decreased from 26% at baseline to 10% at follow-up, but remained unchanged (27%) among controls (Fig 15–4) Xerophthalmia also disappeared among children receiving fortified margarine, but developed in ~2% of controls.[35] The product earned a limited endorsement by the Department of Health, two months later, product sales rose by ~20% over the previous year (F Solon, personal communication, 1994) This example indicates that fortification, when appropriately done, can be profitable to the private sector while benefiting the population at risk

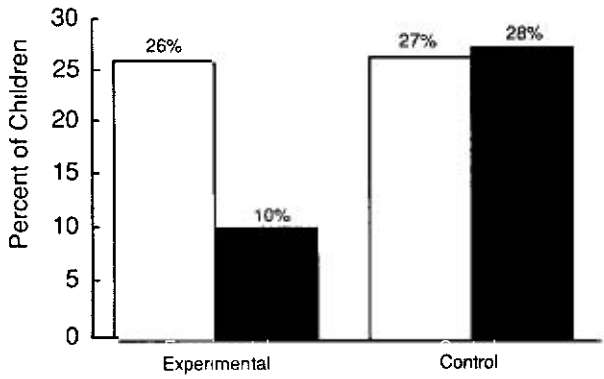


Fig. 15–4. Proportion of preschool-age children with serum retinol levels < 20 μg/dl at baseline (open bars) and after six months (black bars) of providing vitamin A-fortified margarine (experimental) or nonfortified margarine (control) to households in the Philippines (From F S Solon et al [35])

In Brazil, consumption of centrally processed soy bean oil is rising rapidly among lower socioeconomic groups Vitamin A-fortified soy oil is undergoing testing at a level of 200 IU (60 μg RE) per g,[13,36,37] which could provide an FAO safe level of intake[85] (300 μg–450 μg RE) to children consuming 5 g–10 g, or two teaspoons, per day Stability has been found to be excellent for up to six months (~98%), although it has varied thereafter depending on conditions of storage (e g , ~40% retention after eighteen months) Repeated use of the same oil in cooking also reduces potency by ~50% [13,37] Trials documenting impact on vitamin A status have not yet been done

Other Foods

Tea

An effective technology for fortifying tea (dust) with vitamin A (~125 IU/g–250 IU/g or 375 IU–750 IU per brewed, 150 ml cup)[4] was established long ago in India[29] and Pakistan [28] Stability (using powdered or emulsified vitamin A palmitate) was excellent after one hour of continuous boiling [4] Though tea fortification continues to offer a potentially effective option, little further work has been devoted to this approach, largely because the youngest children do not consume adequate quantities, if any, of tea It's conceivable that national supplementation via this route might have a beneficial effect by raising the levels among women of childbearing age

Salt

Given its ubiquitous use, its controlled processing, and its proven efficacy as a carrier for iodine, salt has been repeatedly suggested as a potential vehicle for

Table 15–3 Impact of Consuming Brick Salt Fortified with Vitamin A (440 IU/g) on Serum Retinol, West Java, Indonesia, 1982

	Baseline				*Six-Month Follow-Up*			
Serum Retinol	*Vitamin A-Fortified*		*Control*		*Vitamin A-Fortified*		*Control*	
(μg/dl)	*n*	%	*n*	%	*n*	%	*n*	%
< 10	33	17 2	25	13 5	3	1 6	21	11 3
10–19	103	53 6	97	52 4	72	37 5	103	55 7
≥ 20	56	29 2	63	34 1	117	60 9	61	33 0
Total	192	100 0	185	100 0	192	100 0	185	100 0

Baseline $X_2 = 1\,57$ $p = 0\,46$

Six months $X_2 = 36\,49$ $p < 0\,001$

From Hussaini 1982 [86]

vitamin A fortification [38,41,86–88] A field trial in West Java, Indonesia, evaluated the effect of fortified brick salt (440 IU/g) on vitamin A status of preschool-age children (one year to six years) living on rubber plantations Consumption of ~4 5 g of salt (~1 teaspoon providing 2000 IU of vitamin A per day) (n = 192) daily for six months markedly raised vitamin A status over placebo recipients (750 IU capsule × 1) (n = 185) In children who ate the fortified salt, the percentage with serum retinol < 10 μg/dl declined from 13 5% at baseline to 1 6% at follow-up, the percentage with serum retinol between 10 μg/dl–19 μg/dl decreased from 54% to 38% The vitamin A status of placebo controls remained essentially unchanged (Table 15–3) [86] Although salt as a vehicle is encouraging, the hygroscopic (water-absorbent) qualities and impurities (that accelerate the destruction of vitamin A) of more commonly consumed, coarse salt have limited its potential usefulness [13,88]

Biscuits

For years, biscuits and crackers have been investigated as potentially fortifiable vehicles for vitamin A [41] A recent randomized trial among lactating women in Indonesia (Table 13–3) demonstrated the value of feeding wafers fortified with beta-carotene Daily consumption of these wafers (providing 3 5 mg or ~585 μg RE/day) resulted in a significant rise in serum beta-carotene and serum and breast-milk retinol compared with controls [89]

Summary

Despite the apparent success of vitamin A fortification of a wide assortment of dietary items in wealthier market economies like the United States, it has not yet met with similar acceptance and implementation in developing countries

Food fortification with vitamin A clearly can be effective, it should be aggressively pursued as a potential intervention strategy But with the exception of the national sugar fortification program in Guatemala and (more variably) its neighboring countries,[7 53–55 61 90] most projects in developing nations have consisted of small demonstration and feasibility studies This appears to reflect, in part, the limited choices of viable vehicles, but also a lack of imagination Vigilance is needed to identify and exploit all potential vehicles, particularly since they are likely to increase with time More aggressive pursuit of fortification may yet yield broader applicability and impact

It should be clear that implementing a national fortification program is a major undertaking that requires sound scientific rationale, industrial capacity, training, advocacy,[91] adequate legislative support,[14 92] economic viability, community acceptance and long-term sustainability, monitoring and quality control

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