Annu. Rev. Nutr. 1989. 9:63–86

VITAMIN A AND INFECTION: Public Health Implications

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CONTENTS

INTRODUCTION 63
EFFECT OF VITAMIN A ON INFECTION 64
Vitamin A Deficiency 64
Vitamin A Supplementation 66
EFFECT OF INFECTION ON VITAMIN A STATUS 67
Systemic Infection 67
Role of Ocular Infection in Xerophthalmia 70
EFFECT OF VITAMIN A ON MORTALITY 71
Vitamin A Deficiency 71
Vitamin A Supplementation 72
VITAMIN A AND MECHANISMS OF HOST RESISTANCE 75
Epithelial Integrity 75
Lymphoid Tissue 76
Specific Immunity 77
Nonspecific Mechanisms 79
CONCLUSION 79

INTRODUCTION

Animal models of vitamin A (VA) deficiency have revealed pervasive, multisystem involvement, that in many ways mirrored classic descriptions of human VA deficiency described around the turn of this century. Clinical and, later, epidemiologic attention initially focused on the dramatic ocular effects of VA deficiency (xerophthalmia) and its magnitude, now estimated to affect five million and blind 250,000 children in Asia alone each year (135, 159).

0199-9885/89/0715-0063$02.00

The more insidious manifestations of VA deficiency such as decelerated growth, metabolic alterations, and increased infection have long been addressed in the laboratory, where their combined impact frequently leads to death of experimental animals. Despite the anticipated subtlety of nonocular consequences of VA deficiency and difficulty in identifying them within the complex milieu of child health and disease in developing countries, recent data indicate that VA plays an important role in resistance to infection and its deficiency significantly increases a child's risk of systemic morbidity and mortality (as animal data have long suggested). Although mechanisms involved remain largely unknown, this review examines many of the important observations to date on this particular nutrition-infection interaction (121) with emphasis on its public health consequences for child health.

EFFECT OF VITAMIN A ON INFECTION

Vitamin A Deficiency

ANIMALS Progressive VA depletion in animals initiates a set of widespread systemic responses including deceleration in growth, loss of appetite, epithelial metaplasia, and reduced secretory functions (2, 170), as well as impairment of specific and nonspecific components of the immune response (95, 165). Infection is often an early complication (49, 161) and leads to substantial mortality prior to the development of xerophthalmia (147). Vitamin A–deficient animals raised under germ-free conditions develop many of the classic keratinizing lesions of VA deficiency but continue to survive. In contrast, equally VA-deficient littermates raised in a conventional environment acquire infection and die prematurely (13, 14). Broad-spectrum antibiotic treatment of VA-deficient animals can markedly extend survival (2). These and other studies (95) strongly suggest that vitamin A plays a pivotal role in maintaining resistance to infection and survival that should have its correlate in humans.

CHILDREN Vitamin A status is assessed clinically by examining the eyes for signs of xerophthalmia, which exhibit a gradient of severity. These signs include nightblindness (XN) (usually detected by history), conjunctival xerosis in the presence of overlying cellular and bacterial debris (Bitot's spots, or X1B), corneal xerosis (X2), and corneal ulceration, or keratomalacia (X3) (134). In children, uncomplicated XN and X1B represent mild xerophthalmia that rarely progresses to corneal disease. Until recently, these mild eye signs were held to confer little inherent health risk per se and served more as markers for the likelihood of nutritional blindness in the community (58). While appropriately classified as mild eye disease, XN and X1B appear to reflect advanced, systemic VA deficiency with consequent physiologic

abnormalities and increased health risk, as animal models would predict. This distinction was recognized in clinical cases by Ramalingaswami more than 40 years ago (111).

Prospective studies that use VA status to estimate the relative risk of developing infection in free-living populations provide insight into the magnitude and temporal sequence of the VA deficiency–infection interaction. Four longitudinal studies in Asia have investigated the relationship between preexisting VA deficiency and incidence of respiratory and diarrheal infections among children. In West Java, Indonesia, approximately 4600 preschool children were examined every 3 months over an 18-month period (139). At each round, mild xerophthalmia (XN or X1B) was diagnosed according to standard, clinical criteria (134); respiratory infection was defined by the presence of clinically significant cough, rhonchi, or rales leading to a diagnosis of bronchitis or pneumonia; and diarrhea was defined by a history of four or more loose, watery stools during any 24-hour period during the previous month. Only children who were free of apparent infection (by exam or history) at an interval-initiating examination round and seen at the next follow-up were included in the analysis (average n per round = 3135). Children with mild xerophthalmia at any two consecutive examinations were 1.8 and 2.7 times more likely to develop respiratory and diarrheal infections, respectively, during the interval than were children with clinically normal eyes ($p < 0.001$).

A second prospective study in south-central India examined more than 1500 preschool children every 6 months over an 18-month period for xerophthalmia while maintaining weekly morbidity surveillance by trained field staff (86). Details of the clinical examination and definitions of disease were not reported. Data were analyzed similarly to the Indonesian study. Children with mild xerophthalmia at the outset of a 6-month interval were twice as likely to develop respiratory infection than were nonxerophthalmic children ($p = 0.06$). There was no relationship between preexisting xerophthalmia and incidence of diarrhea.

Two further studies of limited sample size were carried out in India and Thailand. Over one year, 310 preschool children living in a West Bengal village (Ichag) were examined monthly for xerophthalmia by a pediatrician and underwent weekly morbidity history surveillance by trained health assistants (125). Person-time analyses revealed no association between xerophthalmia and the occurrence of upper respiratory infection (nasal discharge, sneezing, cough), which affected children approximately 70% of the time throughout the year. This high, continuous burden of disease may have overwhelmed any association that may have existed with VA deficiency. The fourth study assessed risk of morbidity by history over a 3-month period among 146 Thai children 1 to 8 years of age, stratified by serum retinol lev-

els at baseline (17). Respiratory disease was defined by a history of breathing problems, cough, or runny nose accompanied by fever at any time during the interim. Diarrhea was defined as in the Indonesian study (139) over the same interval. The reported incidence of respiratory infection rose in a dose-response fashion from 11 to 26 to 39% with decrements in serum retinol from adequacy (>0.70 μmol/L) to deficiency (<0.35 μmol/L) ($p < 0.01$). In both studies, no change in diarrhea incidence was observed by VA status.

These epidemiologic studies suggest that preexisting VA deficiency (causing mild xerophthalmia or biochemical deficiency) may increase a child's risk of developing respiratory infection, defined either clinically (86, 139) or by history (17). Only one study attempted to differentiate lower from upper respiratory infection (139), and in none were pathogenic organisms isolated. The data suggesting that VA deficiency causes diarrhea are less consistent, possibly reflecting an actual weaker association or imprecise recall methods used to ascertain diarrhea, especially in terms of etiologic agent, stool volume, consistency, frequency, and duration.

Vitamin A Supplementation

Early, clinic-based observations among severely malnourished and frequently xerophthalmic children reported positive therapeutic effects of orally administered VA on general health and growth (16) and on reducing the frequency of diarrheal stools (107, 111, 153). These uncontrolled, clinical reports left claims of efficacy in doubt.

A small, randomized (but unmasked) trial among 166 preschool children in several Thai villages showed a 45% lower reported incidence of respiratory infection (13%) during the 2–4 months following oral receipt of 60,000 μg Retinol Equivalents (RE) of VA compared with controls (23%, $p < 0.1$). No change was observed in diarrheal rates (17). A randomized, double-masked trial in West Bengal (following a year of surveillance, noted above) provided supplements of either 60,000 μg RE or a placebo orally every 4 months to the 310 preschool children in Ichag village (126). No difference between groups was observed in diarrheal or upper respiratory infection rates by weekly reporting (125). A recent, in-hospital trial in Bangladesh failed to show any advantage for mass dose VA when combined with standard treatment among children presenting with acute, dehydrating diarrhea (B. Hening, personal communication). These studies leave essentially unresolved (due to sample size constraints or by design) questions concerning the prophylactic efficacy of VA in reducing the occurrence, duration, or severity of respiratory infections or diarrhea among children in the community who may be subclinically deficient in VA.

Two recent, randomized, double-masked clinical trials in select population

groups in developed countries have tested specific hypotheses about the role of VA supplementation in physiologic doses in preventing morbidity. In one trial among 147 presumably well-nourished Australian preschool children with a history of recurrent respiratory problems (by parental diary), there was a reported 19% decrease in the frequency of respiratory illness with a daily dietary supplement of 450 μg RE (1 US Recommended Dietary Allowance) over an 11-month period (108). The entire effect was explained by 25% reduction in children with a previous history of lower respiratory infection ($p < 0.05$).

A second trial in the United States evaluated the efficacy of intramuscular injection of VA (600 μg RE every other day for 28 days) in reducing bronchopulmonary dysplasia (BPD) in 40 preterm, very low birth weight infants (124), a group shown to be at high risk of VA deficiency (123). Treatment resulted in a nearly 50% reduction in the incidence of BPD compared with controls (45 vs 85%, respectively, $p < 0.01$) and a 60% reduction in airway infection (21 vs 55%, $p < 0.03$). These pulmonary effects may have been mediated in part by enhanced epithelial repair and restoration of mucociliary function in the airway following injury.

EFFECT OF INFECTION ON VITAMIN A STATUS

Systemic Infection

Beyond the influences on anorexia and reduced dietary intake, infection adversely influences VA status by interfering with VA absorption, utilization, and excretion.

METABOLIC EFFECTS Enteric infections of bacterial, viral (94), parasitic (6, 71, 72, 128), or unspecified (80, 114, 128) etiology significantly impair absorption of orally administered doses of VA [reviewed in (168)]. Reports on parasitic disease–induced malabsorption of VA are not always in agreement (115, 145), possibly because of species differences and variation in the load of infestation. Malabsorption of both preformed vitamin A (75, 80, 122) and provitamin A carotenoids (51, 65, 122) occurs during systemic, febrile illnesses, including those involving the respiratory tract (51, 65, 128). Absorption improves following appropriate treatment (4, 5). Poor absorption during infection may be, in part, due to intraluminal abnormalities (e.g. bacterial overgrowth, osmotic imbalances) affecting micelle formation and altered integrity and function of the intestinal wall (118).

While infection clearly impairs vitamin A absorption, physiologically adequate amounts of oral VA can still be absorbed (88, 114, 115, 133), even among malnourished children with severe diarrhea and respiratory disease, and the efficacy of treating corneal xerophthalmia with large, oral doses of

VA has been established (133). From the public health perspective, these data imply that oral VA supplementation can be efficacious in preventing VA deficiency and xerophthalmia in communities where infectious disease and malnutrition are endemic, although the duration of protection may be shortened.

The circulating pool of VA decreases markedly during severe, systemic infections such as pneumonia, bronchitis, septicemia, and rheumatic or scarlet fever (4, 29, 30, 56, 78, 115, 122), as well as during experimentally induced hyperthermia (30, 84); the decrement in serum retinol parallels the clinical severity of infection (30) and degree of fever (56). Following the acute, febrile phase, serum VA level spontaneously increases (4, 78), although it may take weeks to return to normal (115, 122). Adequate VA intake during recovery can minimize the adverse effects of infection on serum retinol (and beta-carotene) levels (122). These acute-phase circulatory changes during systemic stress may reflect accelerated peripheral usage, depressed hepatic mobilization, and/or abnormal excretion (67, 112) of VA rather than exhaustion of liver stores (145). Protracted infection, however, may lead to increased utilization and total body losses over time, as suggested by autopsy data showing that individuals who die of lower respiratory (54) and other severe infections (110) have markedly depressed hepatic retinol levels compared with those who die accidentally.

EPIDEMIOLOGIC ASSOCIATION Clinic- and population-based, cross-sectional studies have reported positive associations between VA deficiency and prior infection. In Asia, the Middle East, and Africa, children with mild xerophthalmia (XN or X1B) were more likely to have had antecedent respiratory infection (135, 160), diarrhea (18, 146), and worm infestation (105, 135) during the 1–4 weeks previous to examination compared with nonxerophthalmic children (controls). Xerophthalmic children were more likely to have reported chronic diarrhea (19, 38) and respiratory infection (38) over the previous one or more years than nonxerophthalmic children. Not all studies have demonstrated an association with each disease symptom, and one survey in the Philippines failed to show any link between mild xerophthalmia and these common morbidities (132), although a positive association was observed between xerophthalmia and both tuberculosis and pertussis. Urinary tract infection is more frequent in children with xerophthalmia (16, 21, 36). The proportion of Bangladeshi children with bacterial infection ($>10^5$ bacterial count per ml of freshly voided urine) increased directly with severity of xerophthalmia (20). Urinary tract infection may represent more an effect than a cause of VA deficiency, since bacteria may colonize and invade keratinized epithelium of the urinary tract and bladder as occurs on the conjunctival surface of the eye.

Variation in the observed infection–vitamin A relationship may reflect different patterns of infection and nutritional status across populations as well as inherent difficulties in establishing temporal sequence from cross-sectional studies. Recent cohort studies have specifically investigated the influence of preexisting infection on VA status. In the previously described longitudinal study of 4600 preschoolers in West Java, children were examined by a physician every 3 months. The risk of having developed xerophthalmia 3 months later among children with diarrhea or respiratory disease at a previous examination was twice as high as it was for those without these infections ($p < 0.01$) (143).

In Brazil a unique opportunity arose to assess the influence of chicken pox (varicella) on liver reserves of VA, as estimated by the relative dose response (RDR) test (25). Vitamin A status of 93 children attending a day-care program was assessed by RDR, using oral doses of 60,000 μg RE, and reassessed 1, 4, and 6 months later. An outbreak of chicken pox 3 months after dosing affected 36 children. At baseline ~40% of those children who later became infected and those who remained infection-free tested RDR-positive, reflecting the initial prevalence of inadequate VA status (1, 69). All children were expectedly RDR-negative at 1 month postdosing. At 4 months (1 month postepidemic), however, 10% of infected vs none of the noninfected children were positive. At 6 months, the proportions were 74 and 10%, respectively, demonstrating accelerated depletion of hepatic reserves of VA following such infection.

Usually, severe VA deficiency leading to corneal destruction is immediately preceded by systemic infection. Thus, onset of corneal xerophthalmia among children presenting to hospitals and clinics is frequently precipitated by concurrent lower respiratory tract disease (135), intestinal parasites, and diarrhea (102). Protein energy malnutrition often complicates the clinical picture, however, as a predisposing risk factor for infection, and if sufficiently severe, for corneal xerophthalmia (135).

The devastating effect of measles infection on VA status deserves special mention. Measles remains a widespread, life-threatening disease for children in many developing countries; it depletes VA levels (135) and interferes with growth (59). In-hospital measles case fatality rates reach 20–25%, and community-based mortality ranges from 1–15% [reviewed in (60)]. In marginally nourished children, serum VA levels may already be depressed prior to measles onset (113), drop precipitously during the acute phase of illness (11, 135, 163), and fail to recover to their preinfection levels for weeks (55, 83, 113, 135). In Africa, where measles appears to be particularly severe (90, 91, 119), 1–4% of hospitalized measles patients develop concurrent corneal disease caused by underlying, severe VA deficiency (46, 60). Typically, 25–75% of children presenting to hospitals with corneal xerophthalmia report

having had measles during the previous 1–3 months (135). In one study, measles raised the risk of developing corneal xerophthalmia 13 times (135).

A strong predictor for corneal disease, measles exerts an equivocal influence on mild xerophthalmia with either a weak (160) or no association (19, 132, 135) reported. These equivocal results are likely due to the contrasting courses of severe and mild xerophthalmia, the former usually precipitated by severe infection (in this case by measles) in ill, malnourished children who are already VA deficient, and the latter, which represents a more chronic, insidious progression of deficiency.

Initial nutritional (including VA) status, dietary intake during infection and recovery, and the occurrence of secondary infection all influence a child's risk of postmeasles VA deficiency and corneal disease. While the decompensating effect of measles has been widely recognized, deterioration in VA status can be expected during most severe, febrile illnesses among children with only marginally sufficient VA status at the outset (4, 25, 56).

Role of Ocular Infection in Xerophthalmia

EFFECT ON MILD XEROPHTHALMIA There is no evidence that local pathogenic invasion plays an important role in the etiology of mild xerophthalmia. Bitot's spots, which overlie xerotic patches on the conjunctiva, are formed in part by saprophytic bacilli (68, 79, 148). The xerosis bacilli colonize keratin debris formed from the xerotic process but do not invade the superficial epithelium (79). With VA therapy bacilli disappear, concomitant with resolution of the Bitot's spot. This improvement appears to be more a response to a change in the ocular surface than to any direct bactericidal effect.

EFFECT ON SEVERE XEROPHTHALMIA The only real evidence for an initiating role for local infection in xerophthalmic corneal ulceration comes from laboratory studies that demonstrate that the corneas of VA-deficient animals are more susceptible to melting following abrasion (12, 96) and to infection from pathogens such as pseudomonas (34) or herpes simplex virus (96), locally applied to an injured corneal surface or injected intrastromally. There is little evidence in humans, however, that local infection plays an important initiating role. Although potentially pathogenic bacteria were recovered from a large percentage of children with xerophthalmic ulceration, there were no nonxerophthalmic controls for comparison (162). In hospital-based studies in Indonesia, pathogenic bacteria were recovered from xerotic but nonulcerated corneas as commonly as from ulcerated ones (135), and corneal dissolution was observed to occur readily in the absence of significant bacteria or inflammation (138). In a randomized trial, frequent antibiotic therapy failed to influence the course of xerophthalmic corneal healing (135), and among

children presenting with less-than-total corneal destruction, VA alone reversed the process, resulting in maintenance of still-normal cornea and scarring of the ulcerated areas (135, 140).

EFFECT OF VITAMIN A ON MORTALITY

Vitamin A Deficiency

MILD Acute lower respiratory infections (23) and diarrheal diseases (131) are the two leading causes of early childhood mortality in developing countries and account for more than 5 million child deaths each year. Emerging evidence that mildly xerophthalmic children are more likely to develop these infections leads to a reasonable suspicion that these children may also be at higher risk of dying. Although cause-specific mortality data are not available, indirect evidence suggests this risk may be real.

The same longitudinal study of Indonesian preschoolers in West Java that reported a higher incidence of respiratory and diarrheal morbidity (139) also found a four-fold increased risk of mortality among children with XN and X1B relative to their nonxerophthalmic peers (142). A dose-response trend was evident at each age: mortality rates rose directly with the severity of xerophthalmia [XN < X1B < (XN + X1B)]. The effect was consistent across ranges of nutritional status (weight for height) and, at least within this mildly wasted population, the effect appeared to be stronger on mortality than was low weight for height (A. Sommer, unpublished observations). Mild xerophthalmia explained approximately 16% of preschool child mortality in this population.

SEVERE Corneal ulceration and keratomalacia represent severe VA deficiency and are typically accompanied by serum retinol levels below 0.35 μmol/L (normal lower limit: 0.70 μmol/L). Corneal xerophthalmia is a medical emergency for two reasons: irreversible blindness is imminent in the affected eye(s) and the risk of death is extremely high, usually from concurrent, severe systemic infection and malnutrition. Immediate VA therapy (with appropriate antibiotics and other nutritional support) generally halts corneal destruction (134) and likely improves the child's chance for survival.

Early hospital-based studies in Europe reported case fatality rates among children with keratomalacia of 50–100% (52, 149, 150). The immediate cause of death was frequently bronchopneumonia. Although susceptible to influences of selection bias, these rates may still represent the untreated case fatality experience of nutritional blindness in many rural, underserved areas of developing countries today.

Even with treatment, death rates following corneal xerophthalmia are excessive: As recently as 25 to 30 years ago, in-hospital mortality among

children with combined kwashiorkor and keratomalacia was 80–95% (83, 120). Recently, in-hospital mortality was 29% among Tanzanian children admitted with corneal ulceration due to VA deficiency (46).

A high risk of mortality follows severely VA-deficient children after recovery and discharge from the hospital. Approximately 20% of severely xerophthalmic children in India died 3–17 months following discharge from health centers (85, 164). In Indonesia 13% of admitted patients died within 14 months of discharge (136); in an earlier report, 43% died during a 6-year period following treatment (156). Data on cause of death are scant, but the high mortality probably represents the continued health burden from chronic infection and malnutrition as well as the influences of an entirely new set of risks associated with being visually impaired in an unchanged, stressful home environment.

The high mortality of severe xerophthalmia is inextricably related to concurrent moderate to severe protein-energy malnutrition, which profoundly reduces resistance to infection (27, 106, 121). Mortality rates among severely malnourished nonxerophthalmic children have ranged from 11% (137) to 25–35% (35, 47, 64). Among 161 Indonesian children admitted to an eye hospital for corneal xerophthalmia, 3% died within a week of presentation, all of whom were severely malnourished (135). During the ensuing 14 months, mortality was almost entirely a function of protein-energy status: 25% of those severely malnourished died vs 4% of those not severely malnourished. Thus, mortality of treated children with corneal xerophthalmia may be expected to be closely related to the severity of coexisting protein-energy malnutrition.

Vitamin A Supplementation

COMMUNITY-BASED STUDIES Two field trials, both in Indonesia, have addressed the question of whether VA supplementation to the community at large can reduce childhood mortality in regions where deficiency is endemic. In a randomized, controlled trial in 450 villages in northern Sumatra (~25,000 preschool children), semiannual VA capsule (60,000 μg RE/ UNICEF) distribution reduced preschool child mortality by 34% over the course of a year (141). Cumulative mortality rates in VA program and control villages diverged with each capsule distribution cycle (~2 and 8 months following baseline examination, Figure 1). Several aspects of this large trial are noteworthy:

1. During a baseline survey, children with xerophthalmia (2%) in program and control villages were treated with VA and excluded from analysis, which suggests the reduction in mortality occurred primarily among children with mild but clinically inapparent VA deficiency.

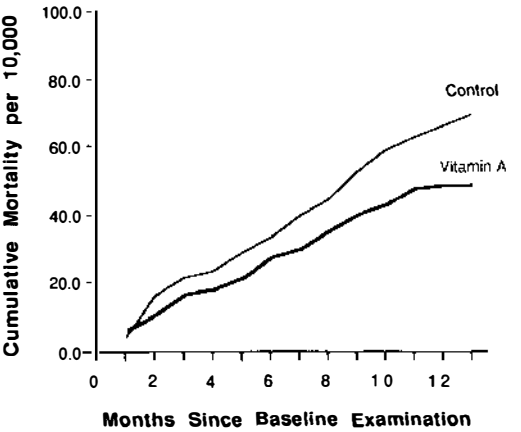

Figure 1 Cumulative 12-month mortality rates among children 1–5 years of age, sexes combined, in vitamin A supplementation and control villages, Aceh Province, northern Sumatra, Indonesia. Vitamin A capsule distribution in program villages occurred approximately in the second and eighth months after baseline examination. Adapted from (141).

2. The basic analysis was performed on an intent-to-treat basis (i.e. by village allocation), which ignored a reported 7–22% lack of coverage within the distribution program area. Subsequent analyses indicated that the impact may have been greater had all program village children received their allocated supplements (155).
3. The reduction in mortality was largest among boys, consistent with their higher risk of xerophthalmia at baseline (41) and significant growth response to supplementation (167).
4. That the trial was not double-masked (by intent) has raised legitimate concerns (37, 48, 77, 144) and signals the need for replicative mortality studies to be carried out with placebo controls, where possible, to allow for masking of treatments (152).

A second field trial among more than 11,000 children in 10 communities tested the effectiveness of VA-fortified monosodium glutamate (a widely consumed flavor enhancer), distributed unobtrusively through normal marketing channels, in improving child health (92, 93). The study was single-masked but not randomized by virtue of the type of intervention. Over a one-year period, VA status among the fortified recipient population improved, with xerophthalmia decreasing from 1–0.3% and retinol levels in serum and breastmilk rising in comparison with those of control villages. In addition, hemoglobin levels increased, linear growth accelerated, and mortality decreased 31% among children in the fortified versus control villages. The

cumulative mortality in the supplemented group diverged linearly from that of the control group throughout the year of follow-up (Figure 2).

These two Indonesian trials, in populations 1200 miles apart, with different levels of childhood mortality, and employing different VA interventions and study designs, offer the strongest evidence to date that in areas where xerophthalmia is endemic, VA supplementation may improve preschool child health and survival beyond its known effect in controlling nutritional blindness. The reduced mortality among VA-supplemented children was presumably mediated by enhanced resistance to predominantly respiratory and/or diarrheal infection, although neither study was designed to evaluate the impact of supplementation on interim morbidity. Results from these two field trials plus the original observational study in West Java (142) suggest that approximately one fourth of the 600,000 child deaths that occur annually in Indonesia (158), or 150,000 deaths, are preventable by improving VA status in preschoolers. Attention has now focused on whether such an effect can be expected in countries where the constellation of competing risk factors that limit child health and survival are different from those in Indonesia.

HOSPITAL-BASED STUDIES There are limited clinical trial data on the impact of VA supplementation in reducing in-hospital child mortality from infectious causes. A small, randomized trial (n = 180) in Tanzania reported a nearly 50% reduction in mortality from a control rate of 13% among children hospitalized with severe measles who received two consecutive daily oral doses of 60,000 μg RE VA (p = 0.13) (11). Virtually all the reduction

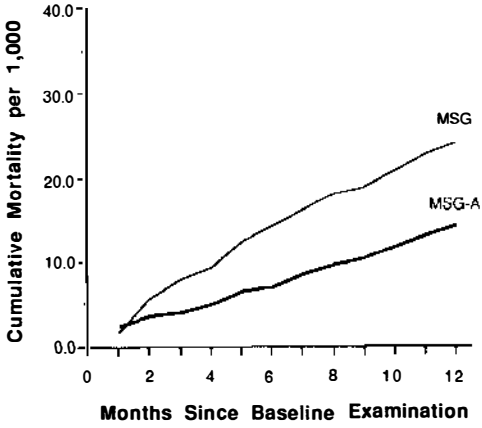

Figure 2 Cumulative 12-month mortality rates among children 1–5 years of age, sexes combined, in villages receiving commercially marketed vitamin A–fortified (MSG-A) and unfortified (MSG) monosodium glutamate, West Java, Indonesia. Adapted from (93).

occurred among children under two years of age ($p < 0.05$). The results agree with those from a clinical trial among 600 hospitalized measles patients in London nearly 60 years ago (44). Half of the children were alternately allocated to receive 300 Carr-Price units of VA (approximately 20,000 IU) plus 2000 IU vitamin D as cod liver oil each day for 1–3 weeks. All children otherwise received the normal hospital diet. Mortality among the vitamin A and D treatment group (3.7%) was less than half that of the control group (8.7%, $p < 0.01$).

VITAMIN A AND MECHANISMS OF HOST RESISTANCE

Efforts to explain the cellular dynamics mediating this apparent link between VA deficiency and infection have long been under way (49, 89). Unlike our understanding of the role of VA in the visual cycle (166), however, mechanisms underlying the somatic functions of vitamin A, including its involvement in the immune response and nonspecific elements of disease resistance, are only beginning to be revealed. This active area of basic research has been reviewed extensively (28, 39, 95, 165, 171, 176). Broadly, VA appears to modify epithelial integrity and function, lymphoid mass, specific immunity (i.e. cell-mediated and humoral), and nonspecific mechanisms of host resistance.

Epithelial Integrity

Vitamin A is required for growth and differentiation of rapidly renewing tissues of the body. Nowhere has this requirement been more evident than in the epithelial linings of the respiratory, gastrointestinal, and genitourinary tracts (and, of course, the conjunctival and corneal epithelium). This involvement confers on VA a critical role in maintaining the integrity of the epithelia and presumably the barrier function of epithelia in resisting pathogen invasion (32, 66).

Experimental VA depletion causes squamous metaplasia of nasal (7, 9, 53), tracheolarnygeal, and bronchial (2, 82, 151, 170, 175) epithelium with loss of mucous-secreting goblet and ciliary cells. Occurrence of these lesions generally coincides with the weight-plateau stage of VA deficiency. The histologic picture is complex; keratinizing metaplasia develop in foci adjacent to areas of minimal morphologic change (82), and rapid and slow epithelial cell proliferation occurs in both areas, respectively (82, 175). Keratinized foci in the tracheobronchial epithelium may provide both a nidus for pathogen growth and colonization as well as a direct port of entry for microbial penetration (121). Generalized squamous metaplasia in the respiratory tract appears to increase susceptibility to infection (10), but infection also acceler-

ates keratotic changes in vitamin A–deficient epithelium at an intensity not seen in VA-replete tissue (7, 9), a histologic enactment of the VA-infection synergism. Loss of the mucociliary layer normally protecting the epithelial surface would appear to have multiple consequences for host pulmonary defenses, including impaired pathogen escalation from the conducting airways, reduced secretion of mucosal antibodies (especially secretory IgA immunoglobulin), and enhanced viral and bacterial adherence to injured mucosum.

Studies of human respiratory tract changes in VA deficiency are scant. Vitamin A–deficient children who died of bronchopneumonia exhibited at autopsy marked squamous metaplasia throughout the larynx, trachea, and bronchi (15, 153). Diffuse infiltration of the respiratory epithelium was evident at autopsy even in areas with minimal metaplasia (5, 157).

Vitamin A deficiency also induces epidermoid metaplasia in salivary glands (submaxillary, parotid, sublingual) and their ducts (2, 43, 100, 161, 170), which undergo loss of cellularity (174) and secretory function (3) and display a propensity for subsequent infection as deficiency progresses (49, 161, 170). The gastrointestinal tract does not keratinize (171), but impaired goblet cell differentiation (117) and mucosal cell proliferation (173) in the small intestine have been observed early in VA deficiency. These and other alterations in gut-associated lymphoid tissue and local immune function (73, 74) may serve to weaken local defenses against enteric invasion.

Extensive epithelial keratinization along the urinary tract and in the pelvis of the kidney has been reported in experimental VA deficiency (161, 170) and in autopsied children who died of infectious causes (15, 153, 169). Normally sterile, the urinary tract in VA-deficient, malnourished children appears to be at increased susceptibility to bacterial colonization and infection (21, 36).

Lymphoid Tissue

Vitamin A deficiency adversely affects lymphatic tissue, although the degree to which this effect occurs varies across species and has often been confounded in studies by protein-energy malnutrition. The thymus and bone marrow (or bursa of Fabricius in chickens) are the primary anatomic sites for precursor lymphoid cell differentiation into the T and B lymphocytes, respectively. Early autopsy case studies reported atrophy of the thymus in severely wasted, VA-deficient infants and children (15, 169, 170). Vitamin A–deficient and protein-energy malnourished animals similarly exhibit marked atrophy of the thymus (33, 62, 170), and the joint effects appear to be additive (62). In the absence of inanition, however, the influence of early VA deficiency on thymic weight in animals is less clear. The influence ranges from a weight-adjusted reduction of more than 30% (174) with dramatic losses in cellularity (8, 24, 174) to little (26) or no change in size (97, 130) compared

with pair-fed controls. Where thymic atrophy has been induced by VA deficiency, viral infection by inoculation appears to exacerbate the loss of lymphocytes from the cortex (8).

The bursal weight of the chick consistently decreases relative to body weight (33, 103) and involutes prematurely (7) after complete or partial (33) withdrawal of VA from the diet. The process is aggravated by superimposed infection (7, 8). Whether similar atrophic changes occur in the "bursa equivalent" of mammals is not known, although VA appears to be functionally important for myeloid and lymphoid cell differentiation in bone marrow (61).

Early VA deficiency has been reported to induce both a moderate reduction (10–15%) in splenic weight (98, 174) with marked loss of cellularity (174) as well as no change in mass (97, 99, 130, 154) or cellularity (130) in relation to body weight. The size of the spleen also appears to be subject to nutritional-species interaction, disproportionately decreasing in rats (62) but enlarging in mice (130) when VA deficiency and protein-energy malnutrition coexist. Splenic hyperplasia has been described at autopsy in a VA-deficient child (157). Surprisingly, altered splenic mass appears to have little bearing on functional changes of immune-competent cells during VA deficiency (see below).

Regional lymph node mass generally appears to increase in VA deficiency; the increase is attributed to accumulation of cellular debris and altered lymphocyte and macrophage trafficking patterns. In mice, the summed weight of regional lymph nodes was similar to that of controls early in deficiency (prior to the weight-plateau stage) but enlarged once weight loss had begun (130). The bronchial (170), cervical (96, 98, 99), and mesenteric (99) lymph nodes enlarge during VA deficiency and further increase with superimposed infection. Nodal enlargement has been reported from an early autopsy report in a VA-deficient, undernourished child (157). In contrast, the numbers of Peyer's patches enveloping the gut wall are decreased in VA-deficient guinea pigs (73).

Specific Immunity

HUMORAL Diminished antibody responses to challenges by a wide variety of antigens (e.g. red blood cell, toxoid, bacterial) occur in VA-deficient animals (26, 50, 62, 70, 104, 109, 127, 129, 130) frequently in the presence of normal (26, 127) or elevated (50, 129) circulating total immunoglobulin (Ig) levels.

Recent work in mice suggests that early VA deficiency may compromise the humoral response via a defect in antigen-specific B cell clonal expansion in the presence of normal total Ig secretion, isotype switching, and antibody secretion rates per cell (129). T helper cell–stimulated antibody synthesis appears to be implicated, since T cell–dependent serum IgG responses are

affected earlier in VA deficiency and more dramatically than the relatively T cell–independent IgM response to antigen. Thus, VA deficiency may act on humoral immunity, in part, by impairing T helper cell differentiation or function (129, 130).

Data are equivocal on the efficacy of VA supplementation in enhancing humoral immunity in animals; they show both increased (104) and no (40) effects although the vitamin clearly displays adjuvanticity (31, 39, 42, 45). One clinical trial has been carried out to date among children in Bangladesh. Moderately to severely malnourished children, randomly assigned to receive 60,000 μg RE VA orally, showed no differences in antitetanus toxoid antibody titers following immunization compared with equally malnourished controls (22). More work is needed to assess the influence of VA on vaccine efficacy among VA-deficient populations.

CELL-MEDIATED Experimental VA deficiency impairs cell-mediated immunity; the impairment is most consistently shown by depressed splenocyte blast transformation to T cell mitogens (particularly concanavalin A) prior to onset of the weight-loss stage (24, 76, 97–99). Intercurrent (viral) infection does not alter the differential response from pair-fed controls (98). The splenocyte transformation response quickly reappears following low-level VA supplementation (76).

Conversely, neither early (24, 97) nor late VA deficiency accompanied by inanition (62) appears to affect relative blast transformation in the thymus (26, 97). Lymphocytes residing in peripheral secondary lymph nodes exhibit an amplified mitogenic response only as deficiency progresses into the late weight-plateau/early weight-loss stages (98, 99). In one small study among mildly malnourished Indian children, those with Bitot's spots exhibited depressed circulation of T cells compared with nonxerophthalmic children (113). Region-specific variation in lymphocyte distribution or activation may reflect changes in mitogen recognition by T cells that are mediated by VA deficiency–induced alterations in cell membrane glycoprotein synthesis and receptor function (172). The extent to which these T cell alterations occur in VA-deficient children and their significance in host resistance remain unknown.

Impaired delayed-type hypersensitivity (DTH) has been reported in vivo in mildly VA-deficient mice (130). Vitamin A–deficient Indian children exhibited a depressed DTH response to Bacillus Calmette-Guérin immunization (57), while VA supplementation had no effect on the DTH reaction to skin-test antigens in malnourished Bangladeshi children (22). In some populations, protein-energy malnutrition and VA deficiency may compete to first limit cell-mediated immune function.

Nonspecific Mechanisms

Vitamin A appears to influence an array of non-antigen-driven mechanisms that are critical to the mounting of an adequate host response to infection, including phagocytosis (63, 101, 116), peripheral blood lymphocyte trapping and localization (81, 154), natural killer (NK) cell lysis (98), maintenance of leukocyte lysozyme activity (87), and mucosal barrier resistance to microbial penetration (32, 66).

CONCLUSION

Available animal data suggest that a "vicious cycle," that of VA deficiency increasing risk of infection, which further aggravates VA deficiency, may be operative in high-risk human populations. Laboratory experiments show VA-deficient animals have reduced resistance to infection, but researchers have only begun to elucidate the immunologic and histologic mechanisms that may explain this effect. Infection appears to accelerate pathologic changes initiated by VA deficiency. Clinical and epidemiologic data suggest these interactions exist in human populations as well, most apparent for respiratory infection. Effects on specific etiologic agents are unknown. Further data are needed to establish the reliability of this hypothesis. Similarly, conditioning factors related to the host (prior nutritional and morbidity status), environment (dietary, other exposures), and agents (pathogen-specific) and their relative importance in this cycle await further clarification.

As in animals, the severity of the synergism between VA deficiency and infection in humans may be responsible for excessive childhood mortality in many developing regions of the world. In one country (Indonesia), endemic VA deficiency may account for 150,000 avoidable preschool child deaths each year. Further data on the impact of improved VA status in reducing infant and preschool child mortality under various conditions of risk are needed to substantiate and provide further quantification of this impact across different cultures.

The dominant direction of the VA deficiency–infection interaction in the community cannot be easily teased apart but has implications for public health policy in resource-limited developing countries. One direction implies that allocating resources specifically toward increasing VA intake (via nutrition education, improved horticulture, etc.) in a marginally deficient population could decrease childhood infection (frequency, intensity, or duration) and its morbid sequelae, including mortality, beyond just controlling nutritional blindness. The opposing direction implies that allocating resources toward interrupting the transmission of infectious disease through environmental improvement and immunization would reduce infectious morbidity and re-

lated mortality and would prevent more widespread VA deficiency and its complications. Resource allocation is never an all-or-none process, but recognizing the existence of such interaction between vitamin A deficiency and infection and understanding the relative importance of each path may help keep child health and survival strategies in developing countries as valid, targeted, and effective as possible.

ACKNOWLEDGMENTS

This paper was prepared under Cooperative Agreement No. DAN-0045 between the International Center for Epidemiologic and Preventive Ophthalmology and the Office of Nutrition, US Agency for International Development (USAID), Washington, DC. Support from Task Force Sight and Life (Roche) of Basle, Switzerland is gratefully acknowledged. This paper is based in part on a USAID state-of-the-art paper by G. R. Howard, K. P. West Jr., and A. Sommer entitled "Vitamin A and Infection: Current Concepts," 1987.

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