Bellagio Meeting on Vitamin A Deficiency & Childhood Mortality

Proceedings of—

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Organized by Helen Keller International
Sponsored by the Charles A. Dana Foundation
Chaired by Professor Abraham Horwitz
Convened by Professor Alfred Sommer

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In February of 1992, Helen Keller International convened a meeting of concerned scientists, health officials, and policy makers to examine the role of vitamin A status on the health of children in developing countries. We challenged this group to reach clear and appropriate conclusions, where warranted, and to consider the policy implications which may arise in order to guide program managers and decision makers around the globe.

For Helen Keller International, their conclusions affirm the results of our own efforts over the years to explore the importance of vitamin A in child sight, health, and survival. Starting in 1972, we assisted the government of Indonesia to assess the degree to which xerophthalmia, the blinding consequence of severe vitamin A deficiency, was a public health problem and to evaluate interventions for its control and prevention. More importantly, this research documented that inadequate vitamin A status, even mild levels of deficiency, heightened child mortality. Subsequently, Helen Keller International and our partners conducted a community-based trial which verified the positive impact of improved vitamin A status on childhood health and mortality. Our results spurred other researchers to undertake similar trials in other countries. Today, we are working to ensure that the techniques we pioneered for the control of nutritional blindness are adopted by every health agency, worldwide, for both the reduction of childhood mortality and the needless loss of sight.

The following proceedings summarize the technical data presented and critically reviewed at the meeting. They capture the breadth of the group’s discussion in fully exploring the physiologic and epidemiologic roles of vitamin A in health and its impact on childhood mortality. Immediately following the meeting, the group’s conclusions and the underlying scientific rationale were presented as the Bellagio Brief — Vitamin A Deficiency and Childhood Mortality. Reproduced as Chapter 1 in these proceedings, the Bellagio Brief has been published in a number of journals and has been translated into French and Spanish.

For the many prospective readers around the world, we hope these proceedings serve as a state-of-the-art review of vitamin A deficiency and its impact on childhood mortality and as an introduction to the body of knowledge existing today on vitamin A. But most of all, we hope that these proceedings serve as a guide to further inquiries and research.
We thank the Charles A. Dana Foundation for their direct support of the meeting and its publications. We extend our appreciation to the Rockefeller Foundation for providing conference facilities and support. We give our heartfelt thanks to Professor Abraham Horwitz for his guidance and contributions as meeting chair. We again thank all the meeting participants for the immense time and energy they devoted to the task set before them. And finally, we offer a special thanks to Dr. Alfred Sommer, who served as the principal investigator for the research efforts in Indonesia many years ago, for his ongoing friendship and support.

John M. Palmer
Executive Director
BELLAGIO BRIEF –
VITAMIN A DEFICIENCY
& CHILDHOOD MORTALITY

The 1986 UNICEF report, "State of the World’s Children," drew attention to emerging observations suggesting that improving vitamin A status might have great potential for reducing childhood mortality. The 1990 report of the Commission on Health Research for Development noted gathering confirmation of the potential impact of vitamin A deficiency on child health and survival, and declared that "if these findings are confirmed, the strategic implications would be astounding."

In light of recent calls by the "World Summit for Children" and the "Bellagio Declaration" for worldwide control or elimination of vitamin A deficiency and the recent spate of published scientific data, a meeting of concerned scientists, health officials, and policy makers was convened on February 3-7, 1992, at the Rockefeller Study and Conference Center in Bellagio, Italy. The purpose was to examine the role of vitamin A status on the health of children in developing countries; to reach clear and appropriate conclusions where warranted; and to consider policy implications arising from these conclusions that might guide program managers and decision makers.

The group considered published data concerning the biochemistry and molecular biology of vitamin A, its role in cellular differentiation and immunity, animal models and clinical case reports of vitamin A deficiency, the epidemiology of vitamin A deficiency in human populations and the environment in which it arises, prospective observational field investigations, hospital treatment studies, and controlled, community-based prophylaxis trials.

The meeting was supported by the Charles A. Dana Foundation, organized by Helen Keller International, and chaired by Professor Abraham Horwitz.

CONCLUSIONS

- Vitamin A is essential for normal health and survival.
- Vitamin A deficiency increases mortality among children 6 months through 6 years of age; improving the vitamin A status of deficient children dramatically increases their chance of survival.
- Vitamin A deficiency increases the severity, complications, and risk of death from measles. Improving vitamin A status before the onset of measles...
**BELLAGIO MEETING ON VITAMIN A DEFICIENCY & CHILDHOOD MORTALITY**

(prophylaxis), or after measles occurs (treatment), markedly reduces the severity of complications and associated mortality.

- Vitamin A deficiency increases childhood morbidity, particularly the severity of infectious episodes (e.g., diarrhea, pneumonia). Improvement of vitamin A status reduces the severity of infectious episodes.

- Vitamin A is essential for normal vision and ocular function. Deficiency results in night blindness and other manifestations of xerophthalmia, including corneal destruction (keratomalacia) and blindness.

- Increased morbidity and mortality occur at levels of vitamin A deficiency less severe and chronic than required for night blindness and xerophthalmia. Therefore, the definition of vitamin A deficiency for public health purposes must be revised and made more sensitive to milder degrees of deficiency.

- Tens of millions of the world’s children are vitamin A deficient; one million or more needlessly die or go blind every year.

- Improving the vitamin A status of deficient children and treating all cases of measles with vitamin A, even in populations in which xerophthalmia is rare, can substantially reduce childhood disease and mortality.

- Increasing the vitamin A intake of deficient children through diet or supplementation is an important component of a comprehensive child survival strategy.

**SCIENTIFIC RATIONALE**

The evidence that vitamin A deficiency increases childhood morbidity and mortality and that this can be prevented by improving vitamin A status is overwhelming.

Since 1913 when vitamin A was discovered, progressive depletion of vitamin A in animals has been shown to result in histologic and functional abnormalities in cells throughout the body, alterations in immune function, wasting, severe infection, death, and in those animals that survive, blindness. The more severe the vitamin A deficiency, the more common, and severe, are the consequences.

For over 60 years physicians have reported the same histologic changes, increased rates of infection, and greater severity of measles in children who were vitamin A deficient—all conditions which could be cured or prevented with vitamin A. In each of three hospital-based, randomized, controlled trials on the treatment of measles (one in London in 1930, two in the past decade in Africa), children who received vitamin A died at less than half the rate of children who received routine therapy. The African measles mortality trials and a recent African trial on measles morbidity also examined the incidence and severity of measles complications. All found that measles complications, in both incidence and severity, were reduced in children who received
vitamin A. Other community-based observational studies and intervention trials, particularly a just-completed morbidity trial in Ghana, indicate that better vitamin A status reduces the risk of other severe infections as well, particularly diarrhea and pneumonia.

In 1983 a community-based observational study examined 4,000 preschool-age Indonesian children seven times over an 18-month period. Other factors being equal, a close, statistically significant dose-response relationship existed between the severity of vitamin A deficiency and the risk of infectious episodes and death. That the presence and severity of vitamin A deficiency had a direct relationship with mortality suggested the possibility that improving the vitamin A status of children in communities where vitamin A deficiency was prevalent might reduce childhood (age 6 months to 6 years) mortality.

Over the past decade a series of controlled, community-based prophylaxis trials have been conducted in order to verify this potential reduction in mortality. In each trial, mortality among children randomly assigned to receive supplemental vitamin A was compared with that of their concurrent controls. The results of six such trials (two each in Indonesia, India, and Nepal) have been published. In all six trials, the vitamin A group experienced lower mortality. Pooling the data of the six trials (approximately 100,000 children and 1,000 deaths) by using meta-analytic methods indicated that, on average, vitamin A supplementation programs can reduce childhood mortality by 34%. The size of the impact observed in each of the six trials was consistent with the 34% overall reduction (heterogeneity, p ≥ 0.32). The probability that the vitamin A programs reduced childhood mortality was highly significant (p < 10⁻⁹). The consistency of these findings is particularly persuasive given variations in the underlying mortality and other health indices of the study populations and the differences in the design and conduct of the trials.

Cause-specific mortality was examined in three of the community-based prophylaxis trials. In all three, dramatic reductions in deaths were associated with diarrhea (the major cause of death in children over 5 months of age) and measles.

These same trials, and at least two other trials directed at the impact on xerophthalmia, confirm that improvement of vitamin A status in deficient children will prevent even the most severe forms of xerophthalmia, including keratomalacia and blindness.

Modern molecular biology has begun to unravel the mechanisms by which vitamin A exerts its powerful, pervasive effects. It is now known that vitamin A directly affects the expression of at least 300 different genes — a number that is likely to grow — which in turn affect cellular differentiation, the integrity of epithelial structures, and immunologic function. While the precise mechanisms by which vitamin A manifests its impact

* Subsequently, the meta-analysis was restricted to children ≥ 6 months of age at the time of intervention. The adjusted analysis also resulted in 34% reduction in childhood mortality. See chapter 5: Meta-analysis of Published Community Trials.
have yet to be delineated, the biological plausibility of those effects is well established.

The consistency of animal models with the clinical reports, observational studies, community-based prevention, and hospital treatment trials (and their biologic plausibility), all support the generalizability of the clinical findings. Given the weight of existing evidence, additional trials that withhold vitamin A from deficient children 6 months of age and older would appear to be unwarranted.

The reduction in child mortality achieved by prophylactic community-wide improvement of vitamin A status and vitamin A treatment of measles cases is comparable to the impact of the most effective of the other child survival strategies. Methods for improving vitamin A status include periodic distribution of large-dose capsules appropriate for age, fortification of readily consumed dietary staples, and increased intake of vitamin A-rich foods, alone or in combination. The cost of a large dose of vitamin A from UNICEF delivered to any country is only 2-4¢ US. In one intervention study in Nepal with high underlying mortality, the cost per death averted was only $11 US. The costs associated with improving vitamin A status will be minimized when such programs are integrated, as appropriate, with other child survival strategies, with attempts to control other relevant micronutrient deficiencies, and with existing community health activities.

Much of the mortality reduction found in both community and hospital-based trials occurred in children with only marginal pre-existing deficiency. In most trials, study children were without xerophthalmia, as those children with ocular disease were excluded at baseline. In others, xerophthalmia was rarely, if ever, encountered in the population. Increased morbidity and mortality occurs at levels of vitamin A deficiency less severe and chronic than required for night blindness and xerophthalmia. Thus, it is imperative that programs to improve vitamin A status be targeted towards populations which may be marginally or mildly deficient.
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GLOBAL & POLITICAL CLIMATE IN WHICH CONSEQUENCES OF VITAMIN A DEFICIENCY & ITS PREVENTION ARE BEING RECOGNIZED

Abraham Horwitz

It is important to place this meeting in Bellagio in a historical perspective and to be cognizant of some of the events that have preceded the now rapidly accelerating global movement to assess and prevent vitamin A deficiency in the Third World. Knowing what has come before will help us to understand the current directions and help us to shape future actions. A global survey of the problem by Oomen and colleagues\(^1\) and considerable early work in India on the effectiveness of large-dose vitamin A distribution\(^2\) in the 1960’s drew attention to both the extent of xerophthalmia and the possibilities of its control in the community. However, the momentum of the past two decades can largely be traced to Item 9 of Resolution V of the World Food Conference in 1974 that, within the context of broader nutritional goals, stated “Governments should... establish a world-wide control programme aimed at substantially reducing deficiencies of vitamin A [and other micronutrients] ...as quickly as possible.”\(^3\)

The Conference was followed by a meeting in Jakarta sponsored by the World Health Organization and the US Agency for International Development to review epidemiologic data on xerophthalmia and vitamin A deficiency. The findings were published in 1976 as the first WHO technical series report on this problem.\(^4\) The International Vitamin A Consultative Group (IVACG) was established soon thereafter as a technical resource and to provide a forum to enhance communication and networking on vitamin A deficiency and its control. A second technical report from a second meeting in Jakarta in 1980 updated the global situation to that point in time.\(^5\) However, it was only after this report was published that the importance of vitamin A deficiency for child survival surfaced,\(^6\) marking a new era of critical research and increased commitment for its prevention.

Several resolutions, declarations, and other pronouncements have stimulated a rising tide of political momentum and financial support to prevent vitamin A deficiency (Table 2.1). These have largely been in response to: (a) a convincing body of epidemiologic, clinical, and laboratory evidence on the etiology and consequences of vitamin A deficiency, and (b) the emergence of cost-effective technologies with which to mount strategies for its control.

In May 1984, the World Health Assembly issued Resolution WHA 37.18, which urged members to give high priority to the prevention of vitamin A deficiency and its blinding consequences and requested the World Health Organization to support such
efforts. A "Ten-Year Programme" was prepared for advocacy, surveys where needed, program formulation and initiation, and training with a $25 million budget allocated for the first five years. This Action Programme, which envisioned "a time when vitamin A deficiency would rank among nutritional scourges of the past," was approved during the 11th Session of the Subcommittee on Nutrition of the UN Administrative Committee on Coordination (ACC/SCN) in February 1985. That year an SCN interagency meeting with the WHO and Food and Agricultural Organization (FAO) was held to develop operational objectives; examine management, organizational, and training needs at the international and country levels; develop suitable educational materials; and begin creating public awareness. WHO was recognized as the lead agency and IVACG as a main resource for scientific and technical matters. Although underfunded, the Plan appears to have stimulated a great deal of further research and has fostered commitment among member governments and agencies to confront this problem.

In the mid-eighties, multilateral agencies began to issue policy-related statements in direct response to new research findings. Examination of a draft report from the first large community trial on vitamin A and child mortality in Aceh, Indonesia, led the ACC/SCN to conclude in 1986 that the 34% reduction in preschool child mortality that
had been observed in the treatment versus control villages was probably attributable to
the vitamin A supplementation. The SCN further judged that it was reasonable to
expect reductions in child mortality of this size from periodic vitamin A distribution
programs carried out under similar conditions.10

In May 1987, findings of a nearly 50% reduction in case fatality from a measles-
vitamin A treatment trial in Tanzania,11 combined with data up to that time, led to a joint
WHO and UNICEF recommendation that children with severe measles be treated with
vitamin A in areas of endemic vitamin A deficiency or where measles case fatality
typically exceeds 1%.12 These findings have been further strengthened by more recent
research in South Africa.13,14 Considering vitamin A deficiency as a “significant yet quiet
killer of children,” an independent Commission on Health Research for Development
set up in 1987 embraced the vitamin A child survival hypothesis by indicating that if the
recent findings were confirmed “the strategic implications would be astounding.”15

In November 1989, following a review of available data from field studies, IVACG
noted that evidence was accumulating to ascribe a role to vitamin A in reducing child
mortality, cautioning that the impact will likely vary with the severity of deficiency and
other ecological factors.16 Emphasis was placed on improving diets and raising vitamin
A status where intakes are low.

The confidence expressed in. nori; policy-oriented, global calls to action has intensi-
fied over the past four years as vitamin A deficiency control has found its place on
“Health for All by the Year 2000” agendas. The “Bellagio Declaration on Overcoming
Hunger in the 1990’s” in November 1989 called for the eradication of iodine and vitamin
A deficiencies as one of its four major recommendations.17

From the historic World Summit for Children, held at the United Nations in
September 1990 and attended by heads of state and senior policy makers from 151
countries, came the “World Declaration on the Survival, Protection, and Development
of Children” with an attending “Plan of Action.”18 The Declaration recognizes the loss of
an estimated 40,000 children each day from malnutrition and disease and commits its
cosignatories to work for optimal growth and development of children through the
eradication of hunger, malnutrition, and famine. Commitments are specified as mea-
surable targets in the Plan of Action: e.g., to reduce rates of death by one-third (or to < 70
per 1,000) and malnutrition by one-half among under-fives. The goals are further
articulated in an Appendix to the Plan, where the commitment of the world community
extends to the “virtual elimination of vitamin A deficiency and its consequences,
including blindness.”18

The need to “keep the promise” made at the World Summit was the main theme at
a Policy Conference on “Ending Hidden Hunger” in Montreal in October 1991.19 More
than 300 representatives from 55 governments and 50 cooperating agencies committed
themselves to intensive combat against micronutrient malnutrition: vitamin A, iron,
and iodine deficiencies. This effort was viewed not only as a health issue but as an
important new goal for national and international development, an ambitious but
achievable target if there is adequate social and political commitment at all levels, and as
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a "cutting edge" of a new war on malnutrition. Operational targets were set with a calendar of activities covering management, assessment, interventions, monitoring, and evaluation. At that meeting, Dr. Alfred Sommer concluded in his keynote address that "control of vitamin A deficiency should, for most countries, be an affordable and achievable goal. The bottom line is that significant numbers of children will benefit from improved vitamin A. The time to act is now."

These declarations and meetings have served multiple purposes, including as an advocacy role in pointing out the extent and consequences of the problem in widely understood terms, as a consensus on ideals and attainable or sustainable goals within targeted periods of time, and as promoters of international cooperation and resource mobilization. The political decisions in the process of being made must be based on firm, scientific evidence or they may not reach their agreed-upon objectives; thus, the reason for meeting today in Bellagio in 1992 has been to provide the scientific credence needed to move forward toward our global goals in the 1990's.
REFERENCES


It is important that the implications of vitamin A deficiency and adequacy on child health and survival be understood within the context of current demographic trends, other causes of child morbidity and mortality, constraints on primary health care services and other resources, socio-economic conditions in developing countries, and the rapidly changing political environment in which aid is apportioned. These conditions, in turn, serve to underscore the appropriateness of vitamin A deficiency prevention and its potential cost-effectiveness as a component of a child survival strategy.

Demographic Trends and the “Health Transition”

Unacceptably high risk of infant and child mortality in developing countries has driven the global effort to improve child survival, both as a moral mandate and as a component of “real development.” Infant mortality has steadily decreased over the past 30 years in most countries, the decline being strongly and positively associated with levels of maternal literacy among many other factors. While large absolute reductions in infant mortality have occurred where rates were highest in the 1960’s (e.g., > 100 per 1,000), infant mortality clearly remains excessive (e.g., > 60 per 1,000 live births) in 65 countries and 1-4 year mortality typically ranges between 10-30 deaths per 1,000 children, leaving the reduction in infant and child mortality an urgent priority.

Crude birth rates have also dropped relative to the 1960’s, but not at the same pace as the decline in infant mortality, leaving many countries in the rapid population expansion phase of the “demographic transition,” with consequent high growth rates. However, rates of natural increase will continue to produce a dramatic change in the shape of population pyramids over the next 30 to 40 years, including a marked increase in not only the numbers of adults in the reproductive years but also in the elderly. These trends, coupled with continued increases in life expectancy, reductions in infectious disease mortality and fertility decline, will produce what has been called a “health transition,” characterized by a larger, aging population with a steady amplification of the public health importance of chronic, non-communicable diseases. In anticipation of this trend, it is probable that some resources will be redirected from child health and survival to preventing diseases related to aging, adding further to the urgency to establish effective child survival strategies at low costs.
Health Resource Limitations

Economic constraints, current aid priorities, and sectoral competition severely limit the health care delivery options for developing countries. Debt servicing consumes 4-5% of the gross national product in low and medium income developing countries, compared to 1-1.5% for health. Until debt servicing is carried out under more forgiving financial terms, it appears that a massive debt burden will prevent many countries from advancing their economies, investing in future productivity, providing adequate social services, and protecting future generations through improved nutrition, health care, and education. Development aid will not be sufficient in amount or proportion to resolve this situation. Currently only about one-third of all official international development aid reaches those two-thirds of developing countries that are most in need. Worse still, less than 15% of development assistance is directed at social sectors — health, water, and education.

Most developing countries spend less than 5% of their national budget on health; often up to 80% or more of what is allocated is spent on tertiary care facilities in urban centers. Most of the remaining 20% that is reportedly spent on public health and prevention supports payroll, leaving minute fractions of many national budgets for supporting actual service delivery. Child survival must operate within these economic constraints while competing with other current and emerging public health priorities (e.g., care for AIDS or care for the aging). National and international bodies must have confidence that each adopted child survival technology be effective and operable within existing and projected resource constraints.

Major Causes of Preschool Child Morbidity and Mortality

Approximately 14 million children under the age of five years die each year in the developing world (Figure 3.1). Diarrheal diseases account for 4 million or 28% of all deaths. Preschool children in developing countries have, on average, 2 to 5 episodes of diarrhea — accounting for 10 to 25 days of illness — each year. Although persistent diarrhea accounts for only 3-11% of all episodes, it accounts for ~50% of all diarrheal days and 30-40% of all diarrhea-related deaths. Presently, oral rehydration saves an estimated 1 million child deaths each year, owing to an estimated 38% usage rate. However, at least another 2.5 million deaths or 62% of the remaining 4 million could be averted with timely and adequate oral rehydration therapy. Nutritional factors (e.g., protein-energy malnutrition and vitamin A deficiency) can also modify the severity of diarrhea (and therefore the risk of dying), but the possibilities of implementing nutritional therapy and dietary management in the routine treatment of diarrhea are still debated.

Acute respiratory infections (ARI), including those related to measles, are responsible for 4.2 million deaths (~30%). It is estimated that children are afflicted by ARI ~24% of all days of the year. The annual incidence of pneumonia can be 10 to 20 per 100 children in low to medium income developing countries.
Measles claims 1.5 million deaths per year, accounting for 11% of all under five deaths. The vast majority of these deaths occur in the first two years of life, when case fatality rates (CFRs) may range between 4% and 8% compared to 1% to 2% among 3- to 5-year-old children. The course of measles is often complicated by secondary infection which dramatically increases the risk of death (e.g., case fatality rates of over 10% when accompanied by diarrhea of 7 days duration or longer). Measles and its associated mortality can be prevented by vaccination. While measles immunization coverage worldwide has reached 70% to 80% and is climbing in many countries, consistent and adequate coverage is not yet at hand. Again, the state of nutrition — especially with respect to protein-energy and vitamin A stores — can greatly influence recovery from primary infection and complications, including the risk of developing corneal xerophthalmia or dying. In this regard, vitamin A supplementation has clearly been shown to prevent or reverse measles-related corneal destruction and reduce case fatality rates by ~50%. Routine vitamin A therapy for children with measles is recommended by the WHO and UNICEF wherever case fatality rates exceed 1% or where vitamin A deficiency is designated as a public health problem.9

Neonatal tetanus and malaria each claim nearly 1 million deaths (6% to 7% of all deaths) while other, mostly infectious causes are responsible for the remaining 4.2 million deaths. Africa is only beginning to realize the threat the AIDS pandemic poses to child survival. AIDS will claim the lives of hundreds of thousands of children and monopolize the attention of health systems and donors in the years ahead.

A Paradigm for Understanding Cause of Death

The chain of causative events leading to the death of a child after illness is complex but can be partitioned into proximal, underlying, basic, and root causes. Proximal causes include those major illnesses discussed above but also include flaws and inadequacies in the delivery of care. Thus, proximal causes are technically defined as causes for which sound, discrete solutions can be offered; e.g., specific therapies or preventive measures such as vaccines. These solutions often compete with each other for scarce resources and mainly require passive participation on the part of parents and patients.

Underlying causes are broader in definition and their consequences cut across disease boundaries (for example: low birth weight, malnutrition, breast feeding or lack thereof, inadequate child spacing, and poor hygiene). Solutions to these causes are imbedded in the behavioral change of individuals; e.g., exclusive breast feeding for 4 to 6 months after birth, timely introduction of appropriate weaning foods, family planning, personal hygiene, growth monitoring, etc. These solutions are not as easy to define and achieve as those for proximal causes, since they require greater understanding of cultural values, more comprehensive health worker training, and active participant behavior.

Basic causes are defined and handled at societal and community levels. They relate to gender bias, information access, self-esteem, crowding, pollution, etc. Interventions involve broad-based community action, socio-cultural change, and political commitment (in contrast to the individual, often technical efforts to resolve underlying causes). Although the solutions to address basic causes tend to be "out-of-reach" for many individual professionals and agencies, it is important to recognize that most health and development efforts do have an impact on these causes in some fashion.

Root causes include poverty, political inequity, war, environmental degradation and vulnerability, and violations in human rights and in the international economic order. The poorest developing countries are particularly vulnerable to the consequences of such root causes. Solutions require organized class, national/regional, and international action such as democratization, debt forgiveness, broad access to credit, jobs, peace, and other global actions.

While most biomedical research is aimed at discovering and applying solutions to the proximal causes of child mortality, it is increasingly recognized that underlying factors and even basic causes of ill health must be addressed if even "successful" solutions are to be effective. All would agree thatremedying root causes would be most desirable, but proximal and underlying interventions are at once affordable and immediately "doable" with important benefits even in the short term.
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BIOCHEMISTRY OF VITAMIN A

Frank Chytil

Food Sources of Vitamin A

Broadly, "vitamin A" refers to preformed natural retinoids (compounds structurally related to retinol) and precursor carotenoids that possess the biological activity of retinol. Preformed vitamin A is found in animal products (e.g., egg yolk, liver, dairy products, and breast milk), largely in the form of retinyl esters (dominated by retinyl palmitate). Carotenoids, of which ß-carotene is the dominant vitamin A-active form, are found in plants such as dark green leaves, yellow and reddish fruits, vegetables (including a wide variety of traditional plants), and red palm oil. In developing countries, approximately 85% of dietary vitamin A intake originates as carotenoids, compared to an estimated 50% in developed countries.

Digestion, Absorption, and Transport

Retinyl esters, carotenoids, and lipids are released from foods during their digestion in the stomach. Lipids and other constituents entering the small bowel stimulate cholecystokinin release from the intestinal mucosa that, in turn, stimulates the gall bladder to release bile into the lumen. Bile salts stimulate the release of pancreatic enzymes and emulsify the vitamin A-containing lipid globules to form mixed micelles that incorporate and deliver retinyl esters and carotenoids to the brush border of the intestinal epithelium. At the brush border, retinyl esters are hydrolyzed to form retinol, which is believed to be efficiently (i.e., 70-90%) absorbed into enterocytes (intestinal cells) by facilitated diffusion. Within the mucosa, retinol appears to be escorted by a cellular retinol-binding protein called CRBP II prior to it becoming esterified and incorporated into the chylomicra. Vitamin A is then carried into the lymphatic circulation and the general circulation, bound to the chylomicra. These chylomicra are further pro-
cessed in plasma to form remnants which are cleared primarily in the liver by parenchymal cells (hepatocytes). Approximately 50-80% of the total vitamin A in the body is stored in hepatic lipocytes ("stellate" cells).5-6

The passive absorption of carotenoids from the intestinal lumen is more dependent on bile salt formation and is less efficient (i.e., < 50%) than retinol.5-6 Absorption of carotenoids decreases further when dietary fat is lacking.7-9 This may be of etiologic importance in the development of xerophthalmia within cultures in which most vitamin A is consumed as carotenoids but the dietary fat intake of children is extremely low. The efficiency of carotenoid absorption also tends to decrease with increased carotenoid intake, possibly reflecting incomplete digestion of vegetable matter. Within the enterocyte it is presumed that approximately 16% of absorbed β-carotene (and even less of other carotenoids) is enzymatically cleaved to retinaldehyde, reduced to retinol, and esterified to retinyl ester. Once esterified, retinol derived from β-carotene is bound and transported by the chylomicra to the liver as occurs with preformed vitamin A.10

The vitamin A molecule, whether in transit from storage depots or moving between and within cells, is largely bound to carrier proteins. Retinol is released from the liver into circulation, complexed with retinol-binding protein (holo-RBP when bound to vitamin A) and transthyretin (previously prealbumin). There is evidence that the delivery of vitamin A to peripheral tissue may be mediated by surface receptors for RBP on target cells.16 An interphotoreceptor (interstitial) retinol-binding protein has also been isolated within the eye.11 Within target cells retinol, retinoic acid, and retinal are shuttled to their metabolic sites in the cytosol or to the nuclear membrane by specific, cellular retinoid-binding proteins (CRBP, CRABP, CRALBP, respectively).5,12

It should also be noted that there appears to be extensive recycling of retinol from the liver to peripheral target tissues and back again.5,13 Tracer studies in animals suggest that retinol may be recycled 7 to 9 times before being irreversibly metabolized by tissues14 and that only ~20% of RBP-bound retinol in circulation at any one time originated from the liver.5-6 Vitamin A is also conserved through an entero-hepatic cycle whereby retinoid-glucuronides (components of bile salts) can be reabsorbed from the intestine, recirculated to the liver, and reutilized as a retinol source.13 These conservation mechanisms could be important for extending vitamin A nutriture and related functions during periods of low vitamin A intake.

Function

The most completely understood function of vitamin A at the molecular level, to date, is the role of retinyl aldehyde (retinal) in forming visual pigment in the photoreceptor cells (rods) of the retina of the eye. In this process all-trans retinal isomerizes (changes shape) to 11-cis retinal which complexes with the membrane glycoprotein, opsin, located in the rod outer segments to form rhodopsin ("visual purple"). On exposure to light, the 11-cis
retinal molecule isomerizes back to the straight all-trans form which releases retinal from the opsin and changes the charge potential on the protein molecule. This action initiates an electrochemical impulse that travels along the optic nerve to the brain to create a visual image. Thus, when vitamin A is deficient, the rhodopsin level decreases in the eye and night blindness ensues.

The second, far-reaching, systemic role of vitamin A relates to its regulatory influence on cell differentiation, affecting such diverse functions as spermiogenesis, egg hatching, implantation, organogenesis, pregnancy maintenance, post-natal organ maturation, and immunity.15 The most apparent of these is the central role of vitamin A in initiating and maintaining differentiation of epithelia, which undergo dramatic morphologic change in a vitamin A-deficient state16-17 (although vitamin A has an influence on myeloid cells as well).16 These dramatic epithelial alterations can be broadly classified as metaplasia and keratinization (e.g., as occurs in the trachea and urinary bladder)17 or atrophy without keratinization19 (e.g., as occurs in the testicular seminiferous tubules),15-18 suggesting that more than one mechanism of action exists for different forms of vitamin A. For example, retinol supports spermiogenesis, whereas its oxidative intermediary, retinoic acid, does not.18 However, retinoic acid widely regulates epithelial differentiation, which appears to occur largely via the direct interaction between nuclear retinoic acid receptors and the DNA molecule.15,19 The functional significance of retinyl aldehyde outside of its role in the eye, if any, is less clear at the present time.

Vitamin A deficiency-induced tissue change presents a consistent sequence and is time-dependent across species, including the human species. Thus, the earliest observed morphologic alterations occur in the salivary glands, followed by lesions in the respiratory tracts and genito-urinary tracts, and, lastly, in the eyes and para-ocular glands.17 In advanced vitamin A deficiency, secondary changes can be observed, such as weight loss, anemia, cessation of bone growth, degenerative muscular lesions, and lymphoid hypoplasia. When animals are repleted with vitamin A (in special retinoate recycling experiments), tissue recovery is rapid and is also reproducibly time-dependent.20 Ocular and para-ocular lesions exhibit improvement within 1 day, microscopic lesions in the oral cavity recover within 2 days, and respiratory and genito-urinary epithelia recover within 3 to 5 days.20,21

Summary

The biochemical and pathophysiological consequences of vitamin A deficiency are not limited to the eye, in which the effects of deficiency generally manifest only after moderate to severe depletion. An altered vitamin A state creates global, rapid effects on the morphology and function of multiple tissues and organs, which provides us with a basis for understanding the pathobiology of vitamin A deficiency and provides insight into the observed benefits of vitamin A repletion in humans.
Decades ago it was inferred that vitamin A may influence the genome (content and programming of the nucleus) based on observed cellular and biochemical changes that occur in animals fed a diet lacking vitamin A. In 1925, Wolbach and Howe showed that normal, mucosal cell differentiation and proliferation was altered when animals were made vitamin A-deficient, leading to squamous metaplasia and keratinization. Similar observations were made in infants who had died with apparent vitamin A deficiency. The sequelae attributable to retinol deficiency disappear time-dependently when dietary retinol is restored. Cellular differentiation involves a change in the shape and function of a cell (e.g., mucous-secreting to a keratinized cell) that can be directly traced to the expression of specific genes. Vitamin A appears to act on cell differentiation at every stage of development, including spermiogenesis, oogenesis, fertilization, morphogenesis, organ formation, fetal and perinatal growth, development, organ maturation, and adult function.

The cell nucleus is a major target site for the action of the natural retinoids, retinol, and retinoic acid, which appear to affect the expression of specific gene products through either activation or repression of transcription. Adequate amounts of retinol (specifically all-trans retinol), when derived from dietary sources as esters or provitamin A-carotenoids, can satisfy all vitamin A requirements for growth and development. However, it may be that retinoic acid, an oxidative intermediate of retinol metabolism, plays a more direct and active role in the nuclear activities of the cell.

Gene regulation involves complex mechanisms whereby primary ribonucleic acid (RNA) is transcribed with coded information from gene sequences on the chromosomal deoxyribonucleic acid (DNA) molecule. Messenger RNA (mRNA) transports this genetic code outside the nucleus to cytoplasmic organelles, called ribosomes, where the nucleic acid-derived code is translated into amino acid sequences, providing the template for protein synthesis. The potential exists for the vitamin A molecule (retinol or retinoic acid) to regulate this process at any number of points along the way (e.g., at transcription inside or translation outside the nucleus).

Retinol penetrates the plasma membrane of the cell and is transported through the cytoplasm by a specific, intra-cellular retinol-binding protein (CRBP). Similarly, retinoic acid — formed by oxidation of retinol — is carried through the cell by a retinoic acid binding protein (CRABP). These cellular carrier proteins, the exact function of which is not known, render the fat-soluble retinoids more water-miscible and probably prevent their interaction with cell membranes while delivering their ligands to the nucleus.

In the nucleus, the action of retinoids appears to be similar to that of steroid hormones, thyroid hormone, or vitamin D. In this model, specific nuclear receptor
proteins bind to both the hormone (or vitamin A) and to responsive elements of the DNA sequence on the chromosome to permit direct, hormonal-nuclear interaction. This concept has gained strong support in recent years with the discovery of several genes that code specifically for nuclear retinoic acid receptor proteins (RARs) that are synthesized outside of the nucleus and translocated from the cytoplasm.\textsuperscript{26-30} RARs can be induced by their ligand (i.e., retinoic acid) to bind to nucleic acid sequences on the DNA molecule (i.e., retinoic acid response elements) that initiate transcription, leading either to activation or repression of transcription of specific genes. Different "isoforms" of RARs exist (e.g., RAR-\(\alpha\), RAR-\(\beta\), etc.). These isoforms exhibit varying affinities for retinoic acid and attach to the DNA at different locations. Some RAR isoforms share similar amino acid sequences across species, suggesting that they may also share common functions across species. These and other RAR attributes may explain, in part, the tissue-specificity (e.g., keratinization of tracheal epithelium\textsuperscript{29-30} vs. morphologic but non-keratinizing effects in the testes\textsuperscript{31}) and temporal and spatial effects of vitamin A deficiency. Interestingly, RARs appear not to interact with retinol, for which an approximate 100-fold higher concentration is needed to induce receptor activity, supporting the notion that retinoic acid is the active form of vitamin A in the nucleus.

How ubiquitous is the genomic influence of vitamin A? A recent survey of the literature indicated that vitamin A (either as retinol or retinoic acid) affects the expression of more than 140 gene products.\textsuperscript{19} This represents < 2\% of all genomic products, since the DNA of a eukaryotic cell (one with a "true nucleus") appears to be capable of encoding for more than 30,000 proteins. A recent census indicates that the number is much higher, reaching, at the time of this presentation, at least 300 (F. Chytil, unpublished). The actual number affected by vitamin A is likely to be many times the above number. For example, studies employing computer-assisted, two-dimensional gel-electrophoresis have shown that, within two hours, more than 300 instances of protein inhibition and over 100 activations were observed among the nearly 1,000 cytosolic proteins synthesized and detached in the testes of vitamin A-deficient rats.\textsuperscript{32} Notably, refeeding of the rats with retinol or retinoic acid did not produce equivalent responses, suggesting that retinol and retinoic acid may work but not by identical mechanism(s). Thus, it is likely that \textit{in vivo} vitamin A affects the synthesis of hundreds of proteins.

Evidence from studies in whole animals and cell cultures indicates that changes in gene expression induced by retinoic acid and retinol are time-dependent, sequential, and very rapid.\textsuperscript{19} At a minimum, "early" and "late" response phases may be distinguishable. Early changes in both systems can be obtained within 4 hours after exposure of vitamin A-deficient rats or cells to retinol and especially retinoic acid, corresponding to a time period in which nuclear metabolism would likely be altered. Rapid effects may be mediated by (so far, unidentified) retinoid-responsive genes which would, in turn, produce gene products responsible for time dependence. Late changes (beyond 4 hours after exposure) representing changes in cell shape (\textit{i.e.}, differentiation) are observed much more frequently and may occur as the consequences of early nuclear and subcellular effects.\textsuperscript{19}
In summary, vitamin A — and perhaps more correctly, its oxidative product, retinoic acid — influences nuclear gene expression in animals by direct interaction with the genome. Recently discovered nuclear retinoic acid receptors that are structurally related to steroid hormone receptors appear to mediate the activation or suppression of gene expression induced by retinoic acid. Many questions remain about mechanisms of tissue-specific action, activation by retinoic acid of the cellular retinol-binding protein gene, and regulation of RAR expression. However, it is apparent that vitamin A exerts rapid-acting, time-dependent, and tissue-specific switching on and off effects on gene expression, providing a potential molecular basis for the wide variety of physiologic responses observed in the human organism to changes in vitamin A nutriture.

CONSEQUENCES OF VITAMIN A DEFICIENCY

A. Catherine Ross

Animal Models of Vitamin A Deficiency

Although rodent species have been most frequently studied, the effects of vitamin A deficiency on growth, epithelial cell differentiation, bone remodeling, reproductive function, retinol metabolism, vision, and immunity have been studied in a variety of other species, including the pig, chick, and calf. Observations have differed somewhat, depending on a number of variables: the species studied, the age (time during life cycle) when vitamin A deprivation began, the type of tissue examined, the dietary protocol used to produce deficiency, and the mode of housing, particularly as it determines exposure to infectious organisms. This review examines the models which have been used to produce vitamin A deficiency, followed by a discussion of the specific symptoms associated with vitamin A deficiency with the goal of drawing a time line of events during the course of vitamin A depletion and progression of deficiency.

• The weanling model

Animals are fed a vitamin A-free diet beginning at the time of separation from the mother. This model has been used most often, resulting in the earliest signs of vitamin A deficiency. Some investigators place pregnant animals on a vitamin A-free diet during pregnancy or lactation to restrict vitamin A transfer across the placenta and mammary glands and to control the type of solid food that may be available to the young. A frequent concern for investigators is that “weanling rats” obtained from commercial vendors may arrive with substantial reserves of vitamin A.

• The retinoic acid cycling model

Introduced by Lamb and colleagues, this model is a dietary protocol in which a retinol-free diet is fed until near the time of onset of vitamin A deficiency symptoms,
then retinoic acid is provided in cycles to sustain growth while tissues are depleted of remaining retinol. This model permits distinction between symptoms due to a deficiency of retinol versus retinoic acid and, because retinoic acid is not stored appreciably, results in a rapid and relatively synchronous onset of total vitamin A deficiency when retinoic acid is withdrawn.

Animals on different dietary regimens may be kept in "conventional" housing (defined as non-barrier, but highly variable in quality of cleanliness), barrier-reared (microisolator or barrier-room housing), inoculated with only specific flora, or kept under germ-free conditions (though whether truly gnotobiotic or barrier-reared is not always clear). Animals also vary in quality among vendors and even among specific facilities of a particular vendor.

Symptoms of Vitamin A Deficiency

- **Growth cessation**
  
  In the rodent, the requirement for vitamin A has been shown to be related to the rate of growth and is usually the first outward (clinical) sign of vitamin A deficiency. Requirements vary somewhat among strains of rat and are greater in the male, whose rate of growth is also greater.

  For conventionally reared animals maintained on a vitamin A-deficient diet from the time of weaning, the rate of weight gain decreases as vitamin A reserves become exhausted and tissue vitamin A becomes inadequate. With the weanling rat, growth retardation is apparent after about 4-6 weeks on a vitamin A-free diet. The decline in growth rate is accompanied by a decrease in food intake. Evidence also suggests decreased utilization of protein and/or other nutrients. Exhaustion of liver vitamin A reserves precedes the growth plateau. There also tends to be a reproducible decrease in plasma vitamin A before growth (weight gain) slows.

  Rats maintained under germ-free conditions survive longer than conventionally housed rats but still reach a weight plateau. Thus, the depression in weight gain is not simply a secondary manifestation of (subclinical) infection. Germ-free rats maintain their plateau while, in comparison, conventionally housed rats typically begin to lose weight and then succumb.

  In the retinoic acid cycling model, rats normally reach the weight plateau after approximately 4 weeks on the vitamin A-free diet, but do not become blind until 3-4 months. These observations give a picture of the time needs for depletion of the last amounts of available retinol in an isolated, peripheral tissue.

- **Increased cerebrospinal fluid pressure**
  
  Elevated CSF pressure has been demonstrated in a number of species (rat, pig, calf, chick). It appears to be related to development of the dura mater, as it occurs before the weight plateau is reached in rats with chronic vitamin A deficiency (weanling model) but after the weight plateau in rats made acutely vitamin A-deficient (somewhat later in
life). In humans, elevated CSF has been rarely reported as a sign of vitamin A deficiency, but has been described more often as a sign of hypervitaminosis A.

- **Alterations in the differentiation / morphology of epithelial and mesenchymal tissues**

  Much of the current work and excitement about retinoids is related to their ability to control cellular differentiation through the expression of specific genes, beginning early in development. Early research demonstrated changes in the morphology of epithelial tissues during vitamin A deficiency. Changes are seen in both conventional and germ-free animals, and are usually reversed after treatment with retinoic acid or retinol. The specific observations depend on the normal pattern of epithelial development (Table 4.1).

- **Reproductive failure**

  Spermiogenesis in the male and normal gestation in the female require vitamin A; both processes appear to require retinol as opposed to retinoic acid for at least some functions. The female’s vitamin A requirement increases as a result of transplacental transfer and the demands of nursing. Females with a very low vitamin A status sometimes conceive but pregnancy ceases or delivery is delayed and difficult if vitamin A is inadequate.

  The transfer of retinol from mother to young during pregnancy may be sub-normal during vitamin A deficiency but, over a range of sufficient vitamin A stores, is essentially constant. The normal neonate’s reserves are low at birth. Transfer from mother to young during lactation reflects maternal status and/or recent intake to a greater extent. Maternal diet can significantly affect vitamin A stores in neonates.

<table>
<thead>
<tr>
<th>Table 4.1</th>
<th>Changes Observed in Epithelial Tissues during Vitamin A Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal morphology:</strong></td>
<td><strong>Typically changes to:</strong></td>
</tr>
<tr>
<td>Columnar epithelium with goblet cells (cornea, conjunctiva, respiratory, urinary, and genital tracts; salivary glands, trachea)</td>
<td>Loss of goblet cells, squamous cells replace columnar epithelium</td>
</tr>
<tr>
<td>Mucous-secreting non-keratinizing (intestine)</td>
<td>Loss of goblet cells and mucous production</td>
</tr>
<tr>
<td>Non-mucoid epithelia (skin, hair)</td>
<td>Hyperkeratinized (dry, squamous, thickened, scaly, rough)</td>
</tr>
</tbody>
</table>
• **Impaired vision**

Vitamin A is required for the maintenance of the eye's epithelial tissue (cornea, conjunctiva) as well as its retina. The former are likely to be maintained by retinoic acid, while only retinaldehyde can form the visual pigment rhodopsin. Changes in vision occur late in vitamin A deficiency. The pools of vitamin A (retinyl esters, retinol, retinal) in the retinal pigment epithelium appear to be protected or isolated in that, once established, there is very slow exchange with or release to the plasma retinol pool. Among the earliest animal experiments, eye signs tended to occur late in deficiency, preceded by poor growth and, often, death.45

**Biochemical Studies**

• **Retinol turnover**

The vitamin A molecule circulates from the liver, plasma, and other tissues many times before being disposed. The irreversible disposal rate of retinol decreases as the (liver) reserves of retinol approach depletion,46 signaling a conservation response. Plasma retinol concentration begins to decline before the weight plateau is reached.46

• **Liver retinol reserves**

No biochemical signs of vitamin A deficiency are known to precede the exhaustion of liver retinol reserves. Thus, when available, this measurement most accurately reflects status. The exact level of liver stores at which other changes begin is not well established and may differ among species. Disturbances in hepatic retinol metabolism, including secretion of retinol-binding protein (RBP), occur when liver total retinoid (retinol plus retinyl ester) falls below 10 μg/g liver.47 Accumulation of liver retinyl esters shortly after birth is the norm. The capacity for storage in stellate cells is high and ester levels up to 1 mg/g liver, when accumulated gradually, appear to be consistent with good health.

• **Retinol-binding protein (RBP) synthesis and secretion**

The hepatic synthesis of RBP continues during vitamin A depletion but secretion of holo-RBP is reduced.47 Whether apo-RBP continues to be secreted is less clear, but based on work with cultured liver cells, it is likely that this occurs. Within 1-2 hours of retinol being available to a vitamin A-deficient rat liver, holo-RBP is secreted. Plasma levels of retinol-RBP are reestablished within 2-8 hours of oral repletion with vitamin A.48

• **Tissue retinoid-binding proteins**

Studies have examined whether the protein mass or messenger RNA content of the cellular retinol-binding proteins, CRBP and CRBP II, differ with vitamin A status. Generally, these remain relatively constant as retinol status is varied.5
Liver esterification of retinol (storage)

The enzyme considered of major importance for intestinal retinol esterification during absorption and for deposition of liver retinyl ester reserves is lecithin: retinol acyltransferase (LRAT). During vitamin A depletion, the activity of this enzyme decreases in the liver. In contrast, LRAT activity in the intestine, where it esterifies newly absorbed retinol, is preserved. The change in liver LRAT occurs early during depletion and nearly parallels the decrease in plasma retinol. This mechanism (decreased liver LRAT, normal intestinal LRAT) appears to be appropriately adaptive in that the intestine’s absorptive capacity is retained, while hepatic storage is curtailed, presumably so that more retinol is available for oxidation and secretion.

INFLUENCE OF VITAMIN A ON THE IMMUNE RESPONSE

A. Catherine Ross

In their classic treatise on the synergism of malnutrition and infection, Scrimshaw, Taylor, and Gordon wrote in 1968 that “no nutritional deficiency is more consistently synergistic with infectious disease than that of vitamin A.” Despite the strong association, studies exist which both do and do not support a relationship. It seems most likely that vitamin A is a significant factor, but is only one factor among others that determines outcome of infection. Interacting variables include (a) the model, (b) nature of challenge, (c) type of immune response elicited, and (d) concomitant factors (age, general nutritional status, environment).

Animal models have been helpful in producing a “single nutrient” deficiency. Three main dietary models have been studied: (a) chronic deficiency induced by a vitamin A-free, post-weaning diet (used in most studies on immunity), (b) deficiency induced by a vitamin A-free diet introduced later in life, and (c) cyclic deficiency (retinol deficiency with cycles of retinoic acid supplementation). It is not yet known whether these models produce the same effects on the immune system.

Vitamin A Deficiency and Resistance to Infection

Numerous studies have documented decreased resistance to experimental and natural infection in the vitamin A-deficient animal (Table 4.2). Few of these have examined the immune response explicitly, but changes in immunity could underlie many of the observations.
### Table 4.2 Some observations on resistance to infection in vitamin A-deficient animals

<table>
<thead>
<tr>
<th>Observations</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathologic evidence of infection</td>
<td>rat</td>
<td>Green, 1928(^{54})</td>
</tr>
<tr>
<td>↑ resistance to typhoid, anthrax and paratyphoid bacilli</td>
<td>rat</td>
<td>Lassen, 1930(^{55})</td>
</tr>
<tr>
<td>↑ resistance to endogenous infection</td>
<td>rat</td>
<td>Ongsakul, 1985(^{56})</td>
</tr>
<tr>
<td>↑ resistance <em>E. coli</em> infection</td>
<td>chick</td>
<td>Friedman, 1991(^{57})</td>
</tr>
<tr>
<td>Longer survival of vitamin A-deficient animals when germ-free or given antibiotics</td>
<td>rat</td>
<td>Bieri, 1968(^{36})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raica, 1970(^{37})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anzano, 1979(^{30})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rodgers, 1970(^{38})</td>
</tr>
<tr>
<td>↑ corneal inflammation, ulceration and necrosis after herpes virus infection</td>
<td>rat</td>
<td>Nauss, 1985(^{58})</td>
</tr>
<tr>
<td>↑ leukocyte infiltration and corneal ulceration after <em>P. aeruginosa</em> infection</td>
<td>rabbit</td>
<td>DeCarlo, 1981(^{59})</td>
</tr>
<tr>
<td>Impaired clearance of parasites and ↑ worm burden</td>
<td>rat, mouse</td>
<td>Parent, 1984(^{60})</td>
</tr>
<tr>
<td>Intestinal villus destruction in vitamin A deficiency combined with rotovirus infection</td>
<td>mouse</td>
<td>Ahmed, 1990(^{61})</td>
</tr>
<tr>
<td>↓ clearance of <em>E. coli</em> and <em>in vitro</em> phagocytic activity</td>
<td>rat</td>
<td>Ongsukul, 1985(^{56})</td>
</tr>
<tr>
<td>↓ antibody response to <em>Schistosoma mansoni</em> infection</td>
<td>rat</td>
<td>Parent, 1984(^{60})</td>
</tr>
<tr>
<td>↓ antibody response to Newcastle disease virus or <em>Salmonella pullorum</em> antigen</td>
<td>chick</td>
<td>Sijtsma, 1990(^{62})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Panda, 1963(^{63})</td>
</tr>
<tr>
<td>No difference in worm burden or replication following infection with <em>Trichinella spiralis</em> but decreased anti-I(_G) response</td>
<td>mouse</td>
<td>Carman, 1991(^{64})</td>
</tr>
</tbody>
</table>

### Gross Changes in the Immune System

Changes in lymphoid organ mass, cellularity, and histology have been reported, but consistency is not strong. Observations seem to depend greatly on the species and the stage of vitamin A deficiency.
**Antibody (B-cell) Responses**

Recent evidence supports a role of retinol in growth and differentiation of B lymphocytes. Generally, Ig production by B cells appears to be maintained during vitamin A deficiency, and Ig levels may even be elevated. In the face of normal total IgM and IgG, specific responses may still be significantly depressed.

Poor antibody responses have been documented to some antigens, but not to others. A possible generalization is that type 2 antigens (e.g., bacterial polysaccharides with repeating subunit structure) and T-cell dependent (TD) antigens elicit a poor response in the vitamin A-deficient animal. In contrast, the antibody response to type 1 antigens (e.g., LPS structure) appears to remain normal. Enhancement of the antibody response by vitamin A has been documented in a number of systems, usually with TD antigens (see below). Effects range from true adjuvant properties to stimulation of expected responses. Examples include responses to IgG and to tetanus toxoid.

The particular class of antibodies affected (IgM, IgG, IgG isotypes) is somewhat controversial and probably depends on both the animal model and the antigen studied.

**T-cell Responses (Cellular Immunity)**

Cell proliferation in response to mitogen stimulation has been examined most frequently. Changes in proliferation of splenic lymphocytes stimulated with Concanavalin A, a T-cell mitogen, have been reported most consistently (whether this represents an overall reduction in response or delayed kinetics of response is not clear). For other mitogens and cells of other lymphoid tissues, the effects of vitamin A deficiency are less consistent. Other changes reported with vitamin A deficiency that implicate T-cells include decreases in T-cell-mediated cytotoxicity, delayed-type hypersensitivity (DTH) and T-cell dependent antibody responses.

Non-specific (non-antigen-antibody) responses to changes in vitamin A nutriture involve the neutrophil respiratory burst, macrophage phagocytosis, and natural killer (NK) cell activity. Decreased macrophage phagocytosis in the deficient state is a possible cause of decreased killing/clearance of microorganisms; little is known of changes in neutrophil responses, considered to be an early first-line defense. Decreased NK cell activity effects surveillance of abnormal cells, but also has implications for regulation of specific immunity (antibody production, antibody-dependent cellular cytotoxicity).

**Stimulation of the Immune System by Retinoids**

Adjuvant effects of vitamin A on T-cell and B-cell responses include increased antibody production, enhanced macrophage phagocytosis, and increased bacterial killing. Effects of supplemental retinoids on non-specific immunity in experimental models are strik-
ing and consistent. Phagocytosis in vitro and bacterial clearance in vivo have been demonstrated.\textsuperscript{68} Retinoid supplementation has induced rejection of immunogenic tumors (thought to involve CD8 T-cell and NK responses).\textsuperscript{69,70}

**Important Gaps in Knowledge**

As (or more) important than what is known is what remains unknown in the complex relationship between vitamin A and the immune response. Therefore, it seems appropriate to summarize this section with a series of questions, intended to provoke further discovery:

- What are the effects of concurrent vitamin A administration on immune responses (e.g. antibody production?) To what antigens and in what age groups do they apply?
- Are immune responses compromised during marginal vitamin A deficiency? How do observations relate to the severity of vitamin A depletion?
- Is mucosal immunity affected?
- Are the responses of polymorphonuclear leukocytes and phagocytes reduced during vitamin A deficiency?
- Is the fundamental ability to respond to immunologic challenges retained in vitamin A deficiency? If so, what stimuli can induce a normal response? (Are signaling mechanisms intact? What signals are missing/defective?)
- How are the adjuvant properties of retinoids mediated? For which antigens can retinol/retinoids be effective adjuvant?
- How quickly can immune responses be rehabilitated? Does rehabilitation take place at the level of hematopoiesis, or more distally?
REFERENCES


Assessment of Vitamin A Status

Alfred Sommer

If one assumes there is a fully normal state of vitamin A nutrirture then, as poor dietary intake and other demands such as growth (normal) or infection (abnormal) deplete nutrirture, suboptimal vitamin A stores develop, followed by physiological (e.g., cellular, biochemical) consequences of deficiency. In this classical progression, xerophthalmia would develop, followed by blindness from keratomalacia and eventually death. However, there appears to be an increased risk of morbidity and mortality well before the xerophthalmic stage of deficiency. With this new “model,” the challenge of assessment becomes to accurately identify populations that are experiencing early or marginal vitamin A deficiency, since preventive measures at that point on the continuum can markedly reduce the risk of mortality. The emphasis is, by necessity, on the population rather than the individual since even suspected cases of vitamin A deficiency, once in contact with the health system, can be readily and presumptively treated with vitamin A with little or no harm but a great deal of potential benefit. Alternately, community control requires much larger and sustainable program interventions.

One leading parameter of impending risk would be a deterioration in dietary vitamin A intake. Prolonged dietary deficiency would produce a change in hepatic vitamin A stores that, once depleted, would cause a fall in circulating vitamin A levels. Abnormal cytology would occur at this stage in deficiency and eventually gross clinical signs would appear. At present, there are various indicators that reflect each of these stages of deficiency, each with its own strengths and limitations.

Dietary Intake

Dietary assessment provides an early, indirect measure of risk of vitamin A deficiency. Common techniques include food intake frequencies, histories, and direct observation. The strengths of dietary assessment include observing the early occurrence of dietary deterioration on the continuum of deficiency, as well as the applicability of dietary assessment for community use. Its weaknesses include problems with accuracy, preci-
Liver and Circulating Levels of Vitamin A

Hepatic stores can be measured directly by biopsy or, more commonly, by surrogate measures such as the relative dose response (RDR) curve. This technique measures the change in vitamin A concentration from two serum retinol determinations after oral challenge with vitamin A. The strength of the RDR is that it attempts to measure the adequacy of vitamin A reserves in the liver, where over 90% of the vitamin is stored in the body. A study several years ago in Brazil very nicely demonstrated the responsiveness of the RDR. It reflected liver store repletion after dosing with vitamin A and apparent depletion of hepatic levels after an outbreak of chicken pox in a high-risk group of children. The modified RDR follows the same principle but is based on dosing and measuring vitamin A-2 and, thus, requires only one blood draw.

As an alternative, circulating levels of vitamin A (which decline after hepatic stores deplete) can be measured by a single serum determination. Serum vitamin A determination has long provided the biochemical standard for status assessment with cut-off values that are well-known and, on a population basis, tend to correspond to clinical and other aspects of deficiency.

Despite apparent strengths of these biochemical methods, the disadvantages when blood is drawn under field conditions include an increased health risk to children in terms of sterility and safety. Secondly, the current AIDS pandemic increases concern about worker and subject safety when dealing with needles and blood products in endemically AIDS-infected areas. Lastly, there are a number of practical problems related to the storage, handling, and standardization of analysis of serum samples that render biochemical assessment of populations a difficult and uncertain process.

Physiologic (Cytologic) Status

Epithelial metaplasia occurs early in the progression of vitamin A deficiency in multiple organs of the body. This process, involving epidermoid changes of columnar epithelium and loss of mucous-secreting cells, can be detected on the conjunctival surface of the eye by conjunctival impression cytology (CIC), a technique that has been introduced for vitamin A assessment over the past decade. The advantages of CIC are that it aims to detect early, pre-xerophthalmic metaplastic change; it is non-invasive; it detects changes that are approximately 10 times more prevalent than mild xerophthalmia and therefore requires a smaller sample size to assess a population; and its storage and handling require no cold chain or light protection. Specimen collection, processing, and interpretation are becoming increasingly standardized with a manual in wide use and with the development of a new specimen applicator.
The principal disadvantage is that the CIC-abnormal state tends to respond poorly to changes in vitamin A intake and nutrure, similar to a non-responsive Bitot's spot. Thus, while CIC can provide a reliable estimate of baseline vitamin A status in a population, it is less useful for demonstrating change after vitamin A intervention. Thus, it would appear to be inappropriate for surveillance purposes. It is also difficult to obtain conjunctival specimens in very young children (i.e., under 2 years of age).

Clinical Signs and Symptoms

Clinical expression of deficiency can be detected by a history of night blindness with reasonable sensitivity and specificity, especially where a local term exists for the condition. A history of night blindness given by a mother appears to be highly reproducible and has often been corroborated by low serum vitamin A levels where the two have been simultaneously measured. Objective tests of night blindness are also under development based on dark adaptometry. For example, at the Dana Center for Preventive Ophthalmology at Johns Hopkins University, a hand-held, cupped device with a variable power source has recently been developed that is placed over one eye, providing a “Gonzfeld stimulus” (total-eye, standardized light pulse) to that eye. With the delivery of a pulse in the dark-adapted state, the fellow eye is observed for pupillary constriction. Thus, very early night blindness may become more detectable in the future.

Signs of more advanced vitamin A deficiency include the classic xerophthalmic changes on the conjunctival and corneal surfaces of the eye. Ocular examination may reveal a Bitot's spot, overlying a xerotic and lusterless, keratinized conjunctival surface. It is made up of a nearly pure culture of xerosis bacillus combined with some desquamated keratin. In the absence of a Bitot’s spot or corneal involvement, conjunctival xerosis is a somewhat unreliable clinical sign and is not normally used to estimate the prevalence of xerophthalmia.

Corneal changes that occur in severe xerophthalmia include xerosis, ulceration, and necrosis or full-thickness dissolution of the cornea (keratomalacia). The quiet ulceration that occurs in severe disease with little or no inflammation or infiltration is practically unique to xerophthalmia. In children who survive, healed keratomalacia may produce a bulge or staphyloma, or the ocular contents may have been lost, producing a shrunken globe (phthisis).

Assessment of the relatively infrequent clinical signs of xerophthalmia requires a trained clinician and a large sample size in order to obtain confident estimates of prevalence.
Summary

Each of these indicators measures a different underlying feature of vitamin A status. There is therefore no single "gold standard" against which to assess sensitivity and specificity. Interestingly, at standard cut-offs the biochemical and cytologic indicators often give similar population prevalence estimates of deficiency, even though individual correlation may be low.

Clinical and Observational Studies

Alfred Sommer

1800's – Early 1900's

Numerous reports throughout the 1800's and early 1900's associated corneal melting (keratomalacia) with high childhood and infant mortality. Many of these children also suffered concomitant protein-energy malnutrition.15,16

Early – Mid 1900's

The first three decades of this century were periods of remarkable discovery with respect to the multiple linkages between vitamin A, growth, and infection. As Professors McCollum17 and Osborne18 were revealing the existence of vitamin A by animal experimentation, Professor Bloch, a Danish pediatrician during World War I, was observing diets in two separate wards of an orphanage. He made the observation that orphans who were given foods (i.e., beer-based porridge) that lacked the newly discovered fat-soluble factor A were growing poorly compared to those who were receiving a similar diet but with added whole milk.19 He termed the condition "dystrophia alipogenetica," noting further that the apparently deficient children did not participate in the normal spring growth spurt and were more prone to infection, especially urinary tract infection (UTI). This led to vitamin A being referred to as an "anti-infective" factor.20

In the early twenties, the landmark observations of epithelial metaplasia and keratinization of the respiratory tract and other organs were being made in vitamin A-deficient animals by Professors Wolbach and Howe.21 A decade later Dr. Blackfan, with Professor Wolbach, diagnosed vitamin A deficiency solely on the basis of histologic findings at an autopsy of metaplasia. He spotted keratinization of the respiratory tract in 17 malnourished infants who had died of various infections at Boston Children's Hospital.22
Although we have long known the systemic and severe ocular consequences of vitamin A deficiency, it was only in the sixties and seventies that the two began to be more clearly associated with and extended to milder xerophthalmia. Dr. McLaren noted in Lebanon that children with corneal xerophthalmia were more malnourished and were at a higher risk of death than wasted, non-xerophthalmic children.23

Clinical studies in Indonesia in the late seventies showed that children with keratomalacia were also usually severely ill and malnourished. Mortality of these hospitalized children exceeded 25%, considerably lower than a likely 90% case fatality if left untreated. Still, less malnourished children with corneal xerophthalmia experienced a case fatality of 4%.24 It logically followed that less severe, non-blinding (non-corneal) xerophthalmia could be associated with increased health risk to children, but up to the late seventies this had not been clearly demonstrated. The relationship between xerophthalmia and UTI was again noted by Dr. Kenneth Brown and colleagues in Bangladesh in 1978 when nearly 85% of severely malnourished, hospitalized children with non-corneal xerophthalmia had bacteriuria (compared to 12% among similarly malnourished children without eye signs).25

Community-based studies in the past 15 years have greatly advanced our understanding of the health risks attending milder vitamin A deficiency, represented by non-corneal xerophthalmia and subclinical vitamin A deficiency unconfounded by severe protein-energy malnutrition. One longitudinal study of ~4000 rural, preschool Indonesian children investigated antecedent risk factors for incident, mild xerophthalmia.26 Children were examined every 3 months over an 18-month period, including assessment of nutritional and ocular status (e.g., normal, night blind, Bitot's spots), morbidity status, and morbidity history. Their ocular and health status were reclassified at each visit.

Person/time analyses showed a clear dose-response relationship between xerophthalmia status and the risk of child mortality. Children with night blindness died at a rate 3 times higher than that of peers with normal eyes. Children with Bitot's spots died at ~6 times the normal rate. The combined presence of night blindness and Bitot's spots was associated with a nearly 9-fold higher risk of death.26 This finding was supported by observations among survivors: children with mild xerophthalmia incurred higher risks of diarrhea (~3-fold) and respiratory infection (~2-fold),27 two conditions that claim the lives of an estimated 1.3 to 2.5 million children each year.28

The mortality analysis suggested that treating xerophthalmia alone would reduce death rates in this population by 16-20%.26 However, approximately half of children with "normal" ocular status in this population had serum vitamin A levels below 20 μg/dl,26 thus, their own risk of morbidity and mortality was likely to have been above "true" normal. This would suggest that the risk estimates from this study were conservative—a hypothesis that has been borne out by subsequent population-based vitamin A intervention trials.
CLINICAL TRIALS: FOCUS ON VITAMIN A AND MEASLES

Gregory D. Hussey

Measles has been one of the most infectious diseases to inflict the human race. Historically, measles epidemics occurring in immunologically naive populations have been known to effect virtually everyone, producing extremely high case fatality rates (e.g., 30% in Fiji in the 1870's). Today measles remains a major public health problem worldwide, killing an estimated 1.5 million children each year and accounting for 10% of all childhood deaths, despite the availability of an effective vaccine. There is no specific therapy for measles. However, recent studies have indicated that hyporetinemia is an invariable consequence of measles and that vitamin A therapy has a substantial impact on measles-related morbidity and mortality.

Epidemiological Evidence

There is evidence from a number of case-control studies and one longitudinal study describing acute, transient hyporetinemia (i.e., normally <20 μg/dl) in measles which resolved during convalescence without any vitamin A therapy. Low retinol levels in young children have also been found to be significantly associated with an increased mortality. A large number of cross-sectional and case-case control studies have reported measles to be a significant risk factor for the development of corneal xerophthalmia, with estimates of relative risk of 1.6 to 4.6 in Africa and 11 in Indonesia, although an elevation in risk has not been reported in all studies.

The strongest epidemiological evidence for an interaction between measles and vitamin A comes from hospital-based, controlled clinical trials during the past 60 years that have measured the treatment effect of vitamin A on measles morbidity and case fatality (Figure 5.1). An early trial in London in 1932 reduced measles case fatality by 58% by supplementing children with ~20,000 IU of vitamin A per day for 7-21 days of hospitalization. The next published measles trial, carried out fifty years later in Tanzania, showed a 46% reduction in mortality following two consecutive days' treatment with 200,000 IU vitamin A, with the entire impact evident below 2 years of age. A randomized, double-masked trial in South Africa demonstrated an 80% reduction in mortality following receipt of 400,000 IU vitamin A over the first two days of admission. Treated children recovered more quickly from diarrhea and pneumonia and had shorter hospital stays. Significantly less severe illness was reported from a second randomized, double-masked trial in South Africa, attributed to a primary effect on reduced lower respiratory tract infection. This reduction in morbidity was accompanied by increased lymphocyte counts and measles IgG antibody concentrations, both of which closely correspond to measles clinical outcome.

These studies have shown that oral vitamin A treatment has a decisive impact on morbidity (especially with respect to hospital stay and gastrointestinal and respiratory tract infections) and mortality.
Interaction between Measles and Vitamin A Nutriture

The mechanisms of this interaction are not yet fully understood. Measles induces an acute transient hyporetinaemia. Both the measles infection\textsuperscript{43,44} and vitamin A deficiency\textsuperscript{45,46} have adverse effects on both the immune system and epithelial integrity, resulting in immunoparesis and epithelial damage. The consequence of this is impairment of host defence mechanisms and increased susceptibility to secondary infections.

Circulating levels of vitamin A and retinol-binding protein (RBP), the vitamin A-carrier protein, are lowered in the acute phase of illness but both tend to return to normal during convalescence.\textsuperscript{31-34} This return of serum retinol to a normal level in the absence of treatment indicates that often there may be adequate hepatic stores in children with measles. The most likely explanation for the low serum levels is that there is reduced hepatic synthesis, or mobilization of RBP, or there may be increased capillary leakage of RBP as a response to the acute phase of infection. Transient zinc deficiency that occurs with acute stress and that has been reported to follow acute measles\textsuperscript{35} may also be responsible for a decrease in the synthesis of RBP, since zinc may be essential for the formation of RBP.

Vitamin A therapy results in an increase in the serum retinol levels. The replenishment of the active, circulating pool ensures an increased delivery of vitamin A to target organs (\textit{i.e.}, epithelium and cells of the immune system). The beneficial effects of therapy are probably mediated via its action as a nonspecific immunostimulant and through restoration of epithelial integrity and function.
Conclusion

The consistency and strength of association and clear response to vitamin A therapy in measles from epidemiological and clinical studies suggests a causal relationship. Unresolved issues include the nature of the underlying biological mechanism for this interaction and the reason for the efficacy of vitamin A therapy in reducing morbidity and mortality. Although mechanisms are not understood, this should not impede efforts to insure vitamin A availability and use in the standard management of acute measles, even in situations where clinical vitamin A deficiency is not a problem. However, this should not undermine and take precedence over efforts to achieve universal measles immunization, since measles is a preventable disease.

Community-based Trials on Vitamin A and Mortality

Keith P. West, Jr.

An observational study carried out in Indonesia in the late 1970’s described an association between mild xerophthalmia and an increased risk of mortality in children from 1 to 6 years of age. Surviving children with xerophthalmia were observed to incur a higher risk of incident respiratory infection and diarrhea. Noting that these infections are the leading causes of death among preschool children in developing countries and that decades of laboratory research had consistently shown vitamin A-depleted animals to be more susceptible to infection and early death, a hypothesis was formalized in the early eighties: vitamin A deficiency is an underlying cause of preschool child mortality, and improving vitamin A nutriture of children in endemically deficient areas would result in a reduction of their mortality. Six community-based intervention trials carried out in Asia during the past decade have tested this hypothesis and have provided estimates to the degree to which vitamin A supplementation can reduce preschool child mortality.

Vitamin A Mortality Prevention Trials

The vitamin A mortality trials in Asia have varied in the dosage, frequency, and delivery mode for vitamin A, their design, extent of coverage, duration and completeness of follow-up, population characteristics, and parameter of impact estimated (i.e., efficacy or program effectiveness). These studies are briefly summarized and listed in chronological order in Table 5.1. Only the impact of vitamin A on children who were 6 months of age at the time of the baseline are addressed here.
Table 5.1  Summary of community-based vitamin A-intervention trials to reduce preschool child (≥ 6 months of age) mortality

<table>
<thead>
<tr>
<th>Trial</th>
<th>Acelić(^b)</th>
<th>W. Java(^a)</th>
<th>Madurai(^c)</th>
<th>Hyderabad(^d)</th>
<th>Sarlahi(^e)</th>
<th>Jumla(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecology</td>
<td>Tropic</td>
<td>Tropic</td>
<td>Drought</td>
<td>Seasonal</td>
<td>Seasonal</td>
<td>Hills</td>
</tr>
<tr>
<td>Protein-Energy Status</td>
<td>Stunted</td>
<td>Stunted</td>
<td>Wasted</td>
<td>Wasted</td>
<td>Wasted</td>
<td>Wasted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Stunted)(^a)</td>
<td>(Stunted)(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>Form</td>
<td>Dose-iLU</td>
<td>Frequency</td>
<td>Design</td>
<td>Unit</td>
<td>No. Units</td>
</tr>
<tr>
<td></td>
<td>Capsule</td>
<td>200,000</td>
<td>q 6 mo</td>
<td>RCT</td>
<td>Village</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>MSG+VA</td>
<td>620</td>
<td>&quot;Daily&quot;</td>
<td>FT</td>
<td>Village</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Liquid</td>
<td>8,333</td>
<td>Weekly</td>
<td>DMRCT</td>
<td>Cluster</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>Capsule</td>
<td>200,000</td>
<td>q 6 mo</td>
<td>DMRCT</td>
<td>Village</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Capusle</td>
<td>200,000</td>
<td>q 6 mo</td>
<td>DMRCT</td>
<td>Ward</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td>Capsule</td>
<td></td>
<td>Once</td>
<td>RCT</td>
<td>Subdistrict</td>
<td>16</td>
</tr>
<tr>
<td>Age at Baseline in Months</td>
<td>12-71</td>
<td>12-60</td>
<td>&lt;72</td>
<td>12-59</td>
<td>6-60</td>
<td>6-59</td>
</tr>
<tr>
<td>Compliance</td>
<td>82%(^b)</td>
<td>High, passive</td>
<td>91%</td>
<td>~92%(^b)</td>
<td>~92%</td>
<td>88%</td>
</tr>
<tr>
<td>Sample Size(^c)</td>
<td>21,147</td>
<td>8,867</td>
<td>15,141</td>
<td>12,217</td>
<td>28,630</td>
<td>6,139</td>
</tr>
<tr>
<td>Follow-up</td>
<td>96%</td>
<td>NR</td>
<td>(High)(^a)</td>
<td>~90%</td>
<td>96%</td>
<td>100%</td>
</tr>
<tr>
<td>Mortality Rate (per 1000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>4.9</td>
<td>16.9</td>
<td>4.8</td>
<td>5.5</td>
<td>11.5</td>
<td>86.8</td>
</tr>
<tr>
<td>Control</td>
<td>7.4</td>
<td>31.1</td>
<td>10.5</td>
<td>5.85</td>
<td>16.4</td>
<td>122.3</td>
</tr>
<tr>
<td>Relative Risk</td>
<td>0.66</td>
<td>0.54</td>
<td>0.46</td>
<td>0.94</td>
<td>0.70</td>
<td>0.71(^d)</td>
</tr>
<tr>
<td>95% LL</td>
<td>0.44</td>
<td>0.40</td>
<td>0.30</td>
<td>NR</td>
<td>0.56</td>
<td>0.55(^d)</td>
</tr>
<tr>
<td>95% UL</td>
<td>0.97</td>
<td>0.71</td>
<td>0.71</td>
<td>NR</td>
<td>0.88</td>
<td>0.92(^d)</td>
</tr>
<tr>
<td>% Δ Mortality</td>
<td>↓ 34%</td>
<td>↓ 46%</td>
<td>↓ 54%</td>
<td>↓ 6%</td>
<td>↓ 30%</td>
<td>↓ 29%</td>
</tr>
</tbody>
</table>

\(^a\) Inferred from paper.
\(^b\) Proportion of children who received ≥ 1 dose of vitamin A.
\(^c\) Sample size for age groups specified at baseline (≥ 6 months).
\(^d\) Recalculated from paper for 6-59 month olds, assuming 19% increase in variance due to design effect.

DMRCT: Double-Masked, Randomized Community Trial
RCT: Randomized Community Trial
FT: Field Trial
NR: Not Reported
95% LL: Lower Limit of 95% confidence interval
95% UL: Upper Level of 95% confidence interval
• The Aceh Study: Semi-annual, large-dose delivery

A total of 21,147 children 12-71 months of age from 450 villages were enrolled and followed for 12 months, representing 96% follow-up. Two hundred and twenty-nine villages (n = 10,917) were randomly assigned to participate in the government of Indonesia's semi-annual distribution program of 200,000 IU vitamin A to preschool children. The remaining 221 villages (n = 10,230) were assigned to control status. At baseline the two groups were similar with respect to socio-economic status, previous history of mortality, age, sex, nutritional status, and morbidity history. In program villages, 82% of children received at least one capsule during the year; 62% received both capsules. Xerophthalmia rates were significantly lowered in program versus control villages. Mortality rates over 12 months were 4.9 and 7.4 deaths per 1,000 children in the vitamin A program and control groups, respectively, for a relative risk of 0.66, reflecting a 34% reduction attributable to the vitamin A program. The reduction was evident in 4 of 5 age groups and in both sexes. A strong selection bias was evident in program villages, with non-recipient children incurring a 27-fold higher risk of dying than recipients and a 3-fold higher risk of mortality than control children, predicting that the efficacy of vitamin A would be considerably higher with full coverage in that population.

This trial provided the first estimate of mortality reduction in preschool-aged children following periodic vitamin A distribution in the community. Because a program was randomized to villages, with coverage at levels that might be achieved under adequate supervision, the estimate was one of program effectiveness rather than efficacy.

• West Java Study: MSG-A fortification trial

A total of 8,867 children 12 to 60 months of age were enrolled from 10 West Javanese villages. Five villages (n = 4,556) were targeted for monosodium glutamate, fortified with ~2,700 IU vitamin A per gram (MSG+A), to be sold through normal market channels for 11 months. Five nearby villages (n = 4,311) were selected as controls. Previous dietary studies showed that preschool children would consume an average of 0.23 ± 0.20 grams of MSG per day which would provide ~620 IU of vitamin A per day or ~1/2 of the Recommended Dietary Allowance. Baseline dietary intakes, anthropometric data, and iron status were similar in both groups, although vitamin A status tended to be poorer in the treatment villages. MSG+A was effective in improving vitamin A status (reduced xerophthalmia, increased serum retinol in children and in breast milk of mothers). Mortality rates at this age were 16.9 and 31.1 deaths per 1,000 children in the MSG+A and control villages, respectively, for a relative risk of 0.54, or a 46% reduction in preschool child mortality attributable to MSG+A.

The effectiveness of MSG+A fortification in raising vitamin A status above that of controls provided evidence that the vitamin A was consumed by children. Although the potency of the fortified product after 11 months on the shelf was approximately half of
that at the outset, MSG is normally consumed within 4 months of manufacture in Indonesia, thereby allaying concern about shelf losses. These are currently the sole data on the impact of vitamin A fortification on mortality. The findings have stimulated pursuit of a vitamin A-MSG fortification strategy in Indonesia.

- Madurai Study: Weekly doses of vitamin A

A total of 15,419 South Indian children below 6 years of age were enrolled from 206 worker-clusters, with 98% of children being 6-71 months of age at baseline. Clusters were randomized in a double-masked fashion for children to receive weekly doses of vitamin A (8,333 IU) or a placebo. Baseline characteristics (age, sex, vitamin A, and protein-energy status) were reportedly similar although the paper did not present the characteristics by treatment group. Ninety-one percent of all children received 3/4 or more of their intended dosage (at least 37 of 52 weekly doses or 308,000 IU), presumably similar in each group. Mortality rates over 12 months were 4.8 and 10.5 deaths per 1,000 children in the treatment and control groups, respectively, for a relative risk of 0.46, or a 54% reduction attributable to the weekly dose of vitamin A. While stratum-specific estimates showed expected variation, the effect was seen at each age in both sexes and irrespective of anthropometric status.

This study examined the effect that a regular, adequate intake of preformed vitamin A (achieved by weekly supplements) could have on child survival. Although the total dosage was comparable to that of periodic supplementation, the strong protective effect may have been due to the high frequency of low-dose supplementation. Notably, case fatality due to diarrhea, measles, and convulsions (acute, febrile illnesses?) was lower. The trial appeared to have been carried out during the recovery phase of a drought, reflected by high rates of wasting malnutrition at the outset that greatly improved by the end of the trial.

- The Hyderabad Study: Semi-annual large-dose delivery

A total of 84 villages with an estimated 12,217 children 12 to 59 months of age were randomized to receive a large dose of vitamin A (200,000 IU) or a placebo every 6 months. Baseline age distributions were shown to be similar and comment was made about other factors such as income, nutritional status, and crude birth and death rates being similar in both groups. Approximately 90% of children received 1 dose; 58% received two doses. Mortality rates calculated from tabular data in the paper were 5.5 and 5.85 deaths per 1,000 children in the vitamin A and control groups, respectively, for a relative risk of 0.94, or a 6% reduction in mortality, attributable to the intervention which was not statistically significant.

The reasons for the lack of significant effect of vitamin A on child mortality are unknown. Criticism has been raised concerning the differential compliance and follow-up between treatment and control groups. However, a lower-than-expected mortality rate and lack of effect of vitamin A may have been due to the impact of ongoing health and immunization services which had been intensified during the study.
The Sarlahi, Nepal, Study: Tri-annual, large-dose delivery

A total of 28,630 children 6-60 months of age from 261 rural wards were enrolled at baseline, 96% of whom were successfully followed. Wards were randomized for children to receive a large dose (100,000 IU at 6-11 months, 200,000 IU at 12 months or older) (n = 14,143) or a placebo (with 0.5% of this amount of vitamin A) (n = 14,487) every 4 months for a year. Baseline socio-economic status, age, sex, vitamin A status, anthropometric status, and previous history of infant and child mortality were similar in both groups. Approximately 92% of children received their supplement in each group. Mortality rates were 11.5 and 16.4 deaths per 1,000 child-years in the vitamin A and control groups, respectively, for a relative risk of 0.70, reflecting a 30% reduction attributable to vitamin A. The effect was evident across different seasons, at each age (becoming stronger with age), in both sexes, and irrespective of wasting status (by arm circumference).

This trial was carried out in the rural plains of Nepal which is ecologically contiguous with the low-lying Gangetic flood plain of South Asia, during a year of normal seasonal variation in weather, crop production, and illnesses. Consistent reductions in mortality due to measles, diarrhea, dysentery, and acute, febrile illnesses were observed, as were deaths related to malnutrition and other “uncertain” infections (by verbal autopsy). There was little or no apparent effect on mortality due to pneumonia.

The Jumla Study: Single large-dose delivery

Nils M. P. Daulaire

A total of 6,139 children 6-59 months of age in a remote hill district of Nepal were enrolled in a community trial in which half of 16 subdistricts were randomized for children to receive a single 200,000 IU dose of vitamin A (100,000 IU for infants) during the first 5 months of a program (n = 3,239) or thereafter (n = 2,900). Mortality of children in all 16 communities was monitored via an ongoing vital events surveillance system. Baseline characteristics of children in both groups were similar. The mortality rates in the vitamin A program area were 86.8 and in the control area 122.3 deaths per 1,000 child-years, for a relative risk of 0.71 or a 29% reduction in preschool child mortality attributable to the single dose of vitamin A.

This trial in the rural and ecologically and culturally distinct hills of Nepal found a similar, relative impact of vitamin A on mortality as seen in other trials, despite a preschool child mortality rate that was several-fold higher than any other reported study. Reductions in mortality from diarrhea and measles were seen but not from pneumonia — findings that were consistent with the Sarlahi Study. Vitamin A was distributed within the context of an ongoing pneumonia case management program, which rendered an impact on respiratory infection-related deaths more difficult to detect. Finally, the Jumla Study reported the cost of vitamin A distribution to be less than $0.20 US per delivered dose (similar to estimates from Indonesia) and $11.00 US per death averted. The latter cost is partly a function of the expected number of deaths in a population.
Conclusion

Six community trials in Asia have demonstrated mortality reductions of 54% to 6% in preschool children over six months of age. In all studies the effect was evident shortly after supplementation began, paralleling the nearly immediate benefit of vitamin A seen in measles trials. It should be noted that all of the trials were designed to assess the reduction in preschool children as a single group. Differences in impact by age, sex, and other factors offer insight into the consistency of the effect, with the understanding that stratum-specific differences among studies are imprecise. Finally, given the broad range of impact across studies, it is evident that the reduction in mortality achieved by vitamin A supplementation can be expected to vary, depending on the presence and severity of other competing risk factors for child mortality and the presence of preventive programs.

META-ANALYSIS OF PUBLISHED COMMUNITY TRIALS: IMPACT OF VITAMIN A ON MORTALITY

James A. Tonascia

Meta-analysis is a descriptive technique that uses formal statistical methods to combine data from several similar studies in an objective way. This technique was used to arrive at an overall judgement about the effects of vitamin A on child mortality. It is an alternative to narrative summaries that often appear in articles. The technique is useful in that it places studies side by side, examines basic rates, and carefully considers differences in trial design and conduct in order to draw overall conclusions about the outcome effect of the studied intervention.

For this meta-analysis of the impact of vitamin A on child mortality, the results of six vitamin A trials on childhood mortality from very different populations in Asia were considered. Only data from the published papers were used. Reported mortality rates ranged from 5 to over 120 deaths per 1,000 children per year. Over 94,000 children approximately 6 months of age and older were included, of whom 1,152 had died during the various trials.*

Estimates of relative risk (values less than 1.0 favor vitamin A treatment) were plotted on an appropriate scale, with the diamonds on the graph (Figure 5.2) representing the relative weight of each study in the overall estimate. The horizontal lines

* The meta-analysis originally presented at the meeting in Bellagio was for all ages. Subsequently, the range of ages was restricted, and the analysis adjusted, to ≥ 6 months of age at the time of intervention.
extending to either side are the 95% confidence limits. Weights were calculated for each study estimate and were inversely proportioned to the estimated variance of the natural logarithm of each relative risk estimate. In the type of trials considered here, this is equivalent to weighing inversely according to the number of deaths observed. Studies with a high death rate have higher weights than those with lower death rates. Thus, the trials from Hyderabad and Madurai in India assume small weights because of the small number of deaths in those studies. On the other hand, the Jumla and Sarlahi studies in Nepal have the highest weights because of the larger numbers of deaths.

The degree to which individual estimates are statistically consistent affects whether the overall effect can be summarized. The significance test for heterogeneity among trials gives a $p$-value of 0.26, indicating that the estimates are consistent and comparable. This lack of significant heterogeneity among trials enables the individual estimates of relative risk to be pooled to arrive at a summary value. Combining individual estimates, this meta-analysis generated an overall reduction of 34% with very narrow confidence limits (25% to 42%). The corresponding significance test of the null hypothesis of no effect of vitamin A yields an extremely small $p$-value ($p < 7 \times 10^{-10}$).
Conclusions

Notwithstanding the variability in population risk factors, study design, and execution, these trials share several properties that, together, provide strong affirmation that improved vitamin A nutriture can reduce child mortality in developing countries. These properties include:

a) the consistency (lack of heterogeneity) of estimates of impact across a wide range of absolute mortality levels,

b) the strength and statistical coherence of the overall reduction (relative risk ~0.66, 34% reduction) and the attending narrow confidence interval obtained by meta-analysis,

c) the (obvious but essential) correct temporal sequence between the intervention and reduction in preschool child mortality,

d) the predictiveness of impact based on initial observational data,

e) the consistency of the community-based estimates of impact with those reported from measles treatment trials, and

f) the biological coherence of a reduction in child mortality with findings from animal experimentation and other human studies over eight decades that show disturbed host defense mechanisms with vitamin A deficiency.

VITAMIN A DEFICIENCY AND SYSTEMIC MORBIDITY

Betty Kirkwood

Since the effects of improved vitamin A status on reducing child mortality are fairly well established, attention has turned to explaining this impact through intervening effects on the incidence, duration, or severity of infectious morbidity. Although a number of cross-sectional studies have reported associations between a vitamin A-deficient state and morbidity (e.g., diarrhea or respiratory infection), these do not address critical questions of temporal sequence, cause and effect, or involved aspect of morbidity. Findings from longitudinal studies and intervention trials can provide the clearest indications of how vitamin A may influence the risk of morbidity.
Review of Pertinent Studies

Three longitudinal, observational studies — one each in Indonesia, India, and Thailand — provide estimates of the risk of incident acute respiratory infection (ARI) and diarrhea with poor vitamin A status (Table 5.2). In Indonesia, a study examined and reclassified ~4,000 preschool children from West Java every 3 months for 18 months. Children with mild xerophthalmia (night blindness or Bitot's spots) were 1.8- and 2.7-fold more likely to develop these infections, respectively. ARI was clinically diagnosed at the time of examination, while diarrhea occurrence was based on episodic history since the previous visit. In India, a study of similar design was carried out in Hyderabad in which ~1,800 children were re-examined every 6 months. Here also, mildly xerophthalmic children were at a 2-fold higher risk of incident ARI, although no excess risk of diarrhea was observed. In northeastern Thailand, the observational study was different in that serum vitamin A level (in contrast to clinical status) was used to classify children by vitamin A status at baseline. A dose-response association was observed between status and risk of ARI over a 3-month follow-up period. Children of marginal status (serum retinol between 10-19 μg/dl) had a 2.4-fold excess risk; deficient children (serum retinol < 10 μg/dl) had a 3.6-fold higher risk of incident ARI (by history). As in the Hyderabad, India, study, no association was observed with diarrhea.

The observational study in Thailand also included a small intervention trial in which children were randomized to treatment (200,000 IU vitamin A) or control groups.

Table 5.2 Estimated risk of ARI and diarrhea provided by longitudinal studies of vitamin A status and morbidity

<table>
<thead>
<tr>
<th>Study</th>
<th>Vitamin A Status</th>
<th>Relative Risk</th>
<th>ARI</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Java, Indonesia²⁷</td>
<td>Mild xerophthalmia</td>
<td></td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Hyderabad, India⁵⁹</td>
<td>Mild xerophthalmia</td>
<td></td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Northeastern Thailand⁶⁰</td>
<td>Serum retinol:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td></td>
<td>3.6</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Marginal</td>
<td></td>
<td>2.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>
and followed for 4 months. Relative risks of -0.60 for ARI (by history) were recorded throughout for vitamin A recipients; a protective effect was also observed for diarrhea in the first 2 months but not thereafter.

While not specifically designed to address morbidity, the mortality intervention trials offer some evidence on the aspects of infection that may be most affected, or unaffected, by vitamin A (Table 5.3). In Aceh, Indonesia, there were 8%, 11%, and 6% reductions in the 2-week period prevalences of cough, diarrhea, and fever, respectively, among surviving vitamin A program children vs. control village children at the end of the study.\textsuperscript{61} No differences were statistically significant. In Madurai, India, where positive histories of morbidity were recorded every week over a 12-month period, there was no apparent effect of weekly vitamin A supplementation on episodes ARI and diarrhea.\textsuperscript{62} Thus, there was little or no evidence of an impact on morbidity in these two trials despite large and significant reductions in child mortality. In Hyderabad, India, semi-annual vitamin A supplementation had no observed impact on episodes ARI and diarrhea.\textsuperscript{61} Findings from Sarlahi, Nepal, have not yet been published, although preliminary data shown at this meeting suggest that vitamin A modulated the severity of infection (reflected by illness-related mortality) but not prevalence of morbidity (KP West et al., unpublished data).

Recent clinical trials provide data on the effects of vitamin A supplementation on clinically defined rates and severity of infection (Table 5.4). Two randomized trials on hospitalized measles cases showed clear reductions in the severity of morbidity, espe-
Table 5.4 Impact of vitamin A supplementation on rates of severity of infection observed in clinical trials

<table>
<thead>
<tr>
<th>Clinical Trial</th>
<th>Cohort</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Africa 40</td>
<td>Measles patients</td>
<td>Reduced duration of pneumonia and diarrhea</td>
</tr>
<tr>
<td>South Africa 41</td>
<td>Measles patients</td>
<td>Reduced severity score, pneumonia</td>
</tr>
<tr>
<td>Australia 63</td>
<td>Preschool children with history of respiratory illness</td>
<td>19% reduction in incidence of ARI</td>
</tr>
<tr>
<td>Australia 64</td>
<td>Children with history of RSV infection</td>
<td>No impact on ARI</td>
</tr>
<tr>
<td>USA 65</td>
<td>Preterm, low birth weight infants</td>
<td>Reduced incidence of LRI, broncho-pulmonary dysplasia, and airway infections</td>
</tr>
</tbody>
</table>

The first of a pair of studies in Australia reported a 19% reduction in incident ARI with daily vitamin A supplementation, with most of the effect observed in children who were prone to ARI. However, a follow-up trial among children with a previous history of respiratory syncytial viral infection showed no impact of vitamin A on ARI. In the USA, intramuscular injection of 2,000 IU vitamin A into pre-term, low birth-weight neonates markedly reduced lower respiratory tract infection and bronchio-pulmonary dysplasia. Thus, vitamin A supplementation in developed countries may be of value for special high-risk cohorts but, for the most part, remains uncertain.

Ghana Vitamin A Supplementation Child Health Study

Two randomized, double-blind, placebo-controlled trials have been carried out in adjacent areas of the Upper East Region of Ghana, in order to evaluate the impact of 4-monthly high doses of vitamin A on childhood mortality and morbidity (assessed by the incidence, duration, and severity of illness episodes). Both studies are collaborative ventures between London School of Hygiene and Tropical Medicine; the School of Medical Sciences, University of Science and Technology, Kumasi; and the Ministry of...
Health, Accra, Ghana; which were funded by the UK Overseas Development Administration. Preliminary results on the impact on morbidity will be presented here. Results relating to the impact on mortality will be available later this year.

Approximately 1,500 children 6-59 months of age were individually randomized to receive vitamin A (200,000 IU) or a placebo every 4 months and were followed weekly for a total of 60,832 child weeks over a full calendar year. About two-thirds of the study children in both groups were biochemically vitamin A-deficient at baseline, with serum retinol levels below 0.70 μmol/l. The baseline prevalence of night blindness was 1-2% in both groups, although no anterior segment xerophthalmia was observed in the study population. The two groups were comparable at baseline on 66 of 68 (97%) of socioeconomic, demographic, nutritional, and community variables.

There were no significant differences between the vitamin A and placebo groups in the mean daily prevalence of 19 of the 21 morbidity symptoms and conditions (concerning diarrhea, respiratory infection, fever, etc.) ascertained at the home by parental history at the weekly visits. The average daily prevalence of vomiting was, however, 13% lower (1.91% vs. 2.19%, RR = 0.87, p = 0.02), and that of refusing food/latex was 15% lower (1.34% vs. 1.58%, RR = 0.85, p = 0.03) in the vitamin A group.

Although prevalence was little altered over the year, vitamin A supplementation appeared to have a pronounced, positive effect on the severity of illness episodes. Specifically, a lower proportion of episodes of diarrhea among supplemented children reportedly had a high stool frequency (6+ motions/day, RR = 0.92, p = 0.10) and/or associated signs of dehydration (RR = 0.85, p < 0.001). There was a 12% reduction in foamy, frothy, or watery diarrhea and a 14% reduction in field worker-observed noisy breathing in the vitamin A group (p < 0.05).

The rate of clinic attendances was ~2 visits per child/year, with a 12% lower rate in the vitamin A group compared to the placebo group (n = 2518, RR = 0.88, p = 0.002), and the rate of hospital admissions was 38% lower (n = 93, RR = 0.62, p = 0.02).

There appears to have been a slight improvement in the rate of linear growth and in hemoglobin levels in the vitamin A group over the year.

Summary

The results from the longitudinal studies and the supplementation trials carried out in special cohorts suggest that vitamin A deficiency may predispose a child to increased risk of respiratory infection. The picture for diarrhea is less consistent.

To date, mortality trials have failed to demonstrate an impact on common measures of morbidity incidence and duration (and, therefore, prevalence), even in the presence of a large impact on mortality. The results from the Ghana child health study are consistent with these findings. However, this study which, unlike previous ones, was specifically designed to look in detail at vitamin A and morbidity, has revealed an important effect on the severity of illness, and the burden on health facilities (i.e., clinic attendances and hospital admissions).
VITAMIN A DEFICIENCY BEYOND THE PRESCHOOL YEARS

Tara Gopaldas

There is mounting evidence that vitamin A deficiency is a major nutritional problem among school-aged children in underprivileged populations. Nowhere has this been more evident than in India, where considerable work has been under way in recent years to determine the extent of vitamin A deficiency and its consequences in children 5 to 15 years of age. The problem is extensive enough to use the epidemiologic phenomenon of an increased prevalence of mild xerophthalmia with age in older children as a basis to evaluate the preschool vitamin A interventions over time (as the dependence of prevalence on age disappears where successful programs operate).

Studies in underserved areas of Gujarat have reported nearly 80% of school-aged children exhibiting biochemical vitamin A deficiency (serum retinol < 20 μg/dl) accompanied by significantly low intakes of preformed vitamin A and provitamin A carotenoids when compared to the Recommended Dietary Allowances. Between 9% and 12% of those children had xerophthalmia that was clinically responsive to vitamin A therapy, suggesting a state of "true" vitamin A deficiency.

Although mortality is rare beyond the preschool years, frequent morbidity occurs among school-aged children, such as upper respiratory tract and febrile illnesses, parasitism, and diarrhea. A recent clinical trial has shown that 4-monthly vitamin A supplementation could reduce the occurrence and duration of these morbidities, particularly in wasted children. These findings are in agreement with data emerging elsewhere that indicate illness and micronutrient malnutrition to be significant health problems among school-aged children that may be associated with poor growth, learning disabilities, and low attendance. While a clear priority exists to prevent vitamin A deficiency in preschool-aged children, increased attention needs to be given to the extent, severity, and consequences of vitamin A deficiency in the pre-adolescent and adolescent ages as well.
REFERENCES


Recommendations for a Plan of Action Against Vitamin A Deficiency

Toward the end of the meeting, participants were invited to join one of three working groups to discuss and make recommendations on the following topics as they relate to vitamin A deficiency and its control: (a) Assessment, (b) Implementation, and (c) Monitoring and Evaluation.

Assessment of Vitamin A Status

Frederick L. Trowbridge

The assessment of vitamin A status has traditionally focused on the observation of ocular signs and symptoms such as night blindness and various grades of xerophthalmia, reflecting the effects of relatively severe levels of deficiency. However, as the broad mortality and morbidity effects associated with more moderate deficiency are increasingly recognized, indicators that can accurately assess these more moderate deficiency states must be defined.

The following summary provides a brief overview of available indicators for vitamin A assessment, stemming from the discussions of the work group. It is noted that the IVACG has recently developed a monograph (yet to be published) that discusses commonly employed and experimental measures of vitamin A status and intake.

Indicators of Vitamin A Status

- Non-specific indicators

Studies have documented clear associations between vitamin A status and child health indicators, including overall child mortality rates and rates of morbidity and mortality from measles and other infectious diseases. Protein-energy malnutrition (PEM) is also frequently observed in association with vitamin A deficiency. The close association of vitamin A deficiency with these conditions allows them to serve as indirect or non-specific indicators of vitamin A status. Thus, observation of high rates of child mortality, infectious disease mortality, and/or protein-energy malnutrition raises the possibility of vitamin A deficiency as a contributing factor and indicates the need to assess vitamin A status.
Of course, many other factors in addition to vitamin A can affect these child health indicators. For this reason, these non-specific indicators can serve only to identify the possibility of vitamin A deficiency and to stimulate the assessment of vitamin A status using more specific measures.

• Specific indicators

These indicators are more directly related to vitamin A status and can provide a more specific assessment of vitamin A deficiency. However, each of these indicators also has significant limitations that must be recognized when interpreting the information collected.

Community-level data: The physical signs and symptoms of vitamin A deficiency such as night blindness, xerosis, and even keratomalacia may be recognized by communities where vitamin A deficiency is endemic. In such communities, there may be special terms that are used to describe these conditions and there may be locally recognized seasonal or other patterns in their appearance. For example, women in the community may recognize that night blindness often occurs in the later months of pregnancy. Careful questioning in regard to awareness of these conditions may be useful in assessing the likelihood of deficiency in the community.

Dietary intake: Dietary intake of vitamin A by individuals is difficult to assess quantitatively because of inaccuracies associated with recall and food frequency methods. However, estimates of intake of groups are more reliable than individual estimates and can provide insight as to whether the vitamin A intake of a population is likely to be inadequate. Food frequency questionnaires, focusing on vitamin A-rich foods such as leafy green vegetables, may be useful to indicate population-level risk of vitamin A deficiency.

Eye signs and symptoms: Indicators relating to eye signs and symptoms have long been used in the assessment of vitamin A deficiency. An array of indicators has been developed and they are in common use, each detecting different functional consequences of the deficient state. Thus, the combined use of different indicators may not always produce results that are congruent (i.e., high co-positivity and co-negativity). A history of night blindness gathered in a population can serve as a useful indicator of vitamin A deficiency. Dark adaptometry may be measured more quantitatively with equipment that is increasingly applicable to field conditions. Both reflect the adequacy of rod vitamin A nutriture and function. Conjunctival impression cytology (CIC) is a technique that has proven useful for estimating prevalence of mild deficiency in a number of surveys. CIC provides a direct measure of a physiologic consequence of early deficiency as seen at the conjunctival surface. Special training and standardization in specimen reading and interpretation is required.

Readily observed clinical signs on the ocular surface may be viewed as extensions of the early, subtle lesions that are detected by CIC. Bitot’s spots, reflecting metaplasia and keratinization on the conjunctiva, provide a relatively specific indication of active, mild, and non-blinding xerophthalmia, particularly in preschool children. There is some loss of “specificity” when Bitot’s spots are seen in older (school-aged) children, in terms of
correspondence with serum vitamin A levels and response to therapy, possibly reflect- ing (at that point) xerophthalnic lesions that are no longer active.\textsuperscript{5,6}

Corneal xerosis, ulceration, and necrosis (keratomalacia) are highly specific for severe vitamin A deficiency, especially when the extent, bilaterality, and history are characteristic of such lesions. However, the prevalence of corneal disease is very low, owing to its low incidence and short duration (with associated high mortality). Its presence is a clear warning that vitamin A deficiency may be widespread in the community. Children with corneal scars that are properly distinguished from other causes comprise the survivors of severe xerophthalmia. Thus, corneal scarring and destruction (e.g., staphyloma, phthisis) is usually more prevalent than active corneal disease (though much lower than mild xerophthalmia) but allows the same inference to be drawn about the likelihood of severe xerophthalmia and vitamin A deficiency in the community.\textsuperscript{4}

Biochemical assessment: Direct, biochemical assessment of vitamin A deficiency, through measurement of serum retinol, can provide a useful and specific reflection of circulating availability of vitamin A.\textsuperscript{7} At the population level, low mean serum vitamin A levels and an increased prevalence of low values provide a good indication of deficiency. Assessment of a single vitamin A level is more problematic at the individual level because of the lack of a direct relationship between serum levels and liver stores of vitamin A.

The relative dose response (RDR) is a technique that is based on giving an individual a test dose of vitamin A and duplicate determinations of serum retinol approximately 5 hours apart. The RDR provides a basis to estimate liver store adequacy of vitamin A.\textsuperscript{8} A modification of this technique, the MRDR, utilizes a slightly different test compound, vitamin A-2, measures the serum level of vitamin A-2 at 3-5 hours post-dosing, and requires only one blood draw.\textsuperscript{9} Though it is probably the most accurate surrogate indicator of liver stores of vitamin A, the RDR tends to be complex to undertake under field procedures. Simpler but equally reliable field techniques for assessing biochemical vitamin A status, perhaps associated with other micronutrients, are needed.

Data Sources and Target Groups

Useful information to form indicators of vitamin A deficiency requires the identification of data sources and the selection of high-risk target groups for data collection. Potential existing sources of information about vitamin A deficiency within a country include local practitioners, hospital and clinic records, schools for the blind, xerophthalmia and vitamin A deficiency survey reports, etc. These have been summarized in various WHO publications.\textsuperscript{4,10,11} Target groups on whom to focus assessment efforts include preschool children, school-age children, and women of child-bearing age, particularly during pregnancy. Target groups can be selected on the basis of risk level, accessibility, and representativeness.
Using Data to Build Consensus for Action

A primary purpose of assessing vitamin A status is to provide data that can help build consensus on the need to address vitamin A deficiency as a public health problem. The approaches will vary by country. However, there are likely to be several steps or phases that will be common to the consensus-building process in most countries.

Formation of a Vitamin A Task Group: As an initial step, a small group may be formed to outline a strategy for vitamin A assessment and action. Its purpose would be to serve as a focal point for vitamin A-related activities.

Initial Data Collection: Once formed, the Task Group can develop a plan for initial data collection or assembly of available information to define the vitamin A deficiency problem. This effort should make maximum use of existing data from prior surveys or assessments, as well as provide for the rapid collection of additional information from new sources.

Vitamin A Assessment Workshop: Once existing data are compiled, a workshop can be held to provide opportunity for broad discussion of the vitamin A deficiency problem across the sectors that need to be involved to achieve control. Examples of sectors to include in this process are the health, agriculture, education, food industry, and commercial sectors. The purposes of a workshop would be to raise consciousness about vitamin A deficiency, discuss existing data, and lay plans for a more definitive assessment, where warranted, in order to more fully understand the extent of the problem.

Broad-based Data Collection: Should the workshop indicate a reason for concern with regard to vitamin A deficiency, then (assuming there is a commitment to address the problem) broad-based data collection may be warranted. The assessment might include information on other nutritional deficiencies, consideration of why the deficiency occurs, identification of obstacles to improved status, and initial suggestions on how to overcome existing causes and obstacles.

Developing a National Plan of Action

The final goal of vitamin A status assessment is to have the information utilized to develop an intervention plan. While the approach to developing such a plan will vary by country, the plan should incorporate a strategy for intervention, ongoing monitoring, and applied research.

Planning the Intervention: The intervention strategy developed will depend on the extent of the problem, the target groups identified, and the relative feasibility of different interventions. It is likely that a mix of approaches will be valuable, including supplementation of high-risk groups, fortification of appropriate food with vitamin A, and dietary diversification to include more foods that are natural sources of vitamin A. This mix of approaches will necessarily involve coordination across health, agriculture,
and commercial sectors to exert a maximum impact. A critical component will be community involvement to assure acceptability and long-term sustainability.

**Monitoring the Plan:** It will be important to incorporate monitoring and evaluation components into a national prevention plan from the outset. This will involve identification of feasible indicators that can be tracked over time. It will be useful to consider “process” indicators reflecting the degree to which the program is actually achieving its target output and “outcome” indicators which can detect changes in vitamin A status over time.

**Conducting Applied Research:** Adaptation of vitamin A assessment, intervention, and monitoring methods to the needs of each country will benefit from targeted, applied research. Such investigation can help assure that the methods are functioning properly as they are applied in each particular country setting. This kind of applied research will help to validate the methods used and lead to broader acceptance of results.

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**IMPLEMENTATION OF VITAMIN A PROGRAMS**

*Frances R. Davidson*

The scientific justification to prevent and control vitamin A deficiency and the suffering it causes is now sufficient to strongly encourage governments and international agencies to implement the most appropriate mix of vitamin A interventions on as timely a basis as possible. However, much more information is needed about cost-effective alternatives that can achieve control with varying levels of resources.

National policy needs to articulate the optimal mix of programs and establish a firm commitment to sustain preventive efforts. The goals of such policy and allocation of resources should clearly address vitamin A deficiency control, but should be kept within the broader context of improving micronutrient nutrition as well. However, the lack of a coherent national policy should not delay initiating feasible vitamin A deficiency control programs under existing health and nutrition legislation, targeted to the highest risk groups.

Eliminating vitamin A deficiency is a multi-sectoral challenge that will benefit from coordination. Governments should be encouraged to develop policies to assure that vitamin A deficiency prevention programs are legitimate, rational, and clearly viewed within a development perspective (e.g., inserted into 5-year plans). For example, India’s vitamin A policy is succinctly stated in a booklet of 8 pages, and is available to all government and many other involved agencies.
Coordination

At the international level, the Subcommittee on Nutrition of the Administrative Committee on Coordination of the United Nations (ACC/SCN) should assist in coordinating intergovernmental participation, particularly within regions. This will help to avoid fragmentation and to facilitate cooperation.

A national coordinating committee may be organized around child survival, micronutrients, or other major health initiatives. In the absence of a national committee on nutrition, the Ministries of Health, Agriculture, Industry, or Food may not give high priority to nutrition problems. Special mechanisms can sometimes be developed to cut across, but also draw upon and link, sectoral activities to provide a more “vertical” focus on a problem. A “Mission” on vitamin A deficiency in India illustrates one such example. Alternatively, vitamin A can be included in existing programs that target high-risk groups (e.g., primary health care, MCH, horticulture), drawing on resources currently available to health and nutrition. Within organizational, financial, and infrastructural constraints, each country must adopt an approach uniquely suited to its capabilities and development goals. Integration of non-governmental organizations (NGOs) into the planning, coordination, and execution of national plans and their regional and local adaptations will be important in most countries where vitamin A deficiency is endemic.

There is also an “emergency level” for vitamin A planning and programming. Special populations needing attention include displaced persons cut off from their usual dietary sources, refugees from natural disasters or war, and victims of famine. Such refugee and displaced groups rapidly form large populations that need attention that may be distinct from the host population. Many of these people will be dependent on donated foods, which should include foods that are fortified with vitamin A.

Opportunities for Inclusion of Vitamin A

Vitamin A deficiency was eliminated over the last hundred years in Western and developed nations through an array of interventions that included supplementation (especially cod liver oil), fortification (milk, margarine, and bakery products), health education through schools and the popular media (e.g., in the USA, Professor E.V. McCollum wrote more than 170 articles that were published in popular women’s magazines), in addition to improved diets and economic development. A similar array of short-, medium-, and long-term intervention strategies will likely be needed for developing countries to eliminate vitamin A deficiency as a public health problem. Vitamin A supplementation may be added to immunization campaigns or other programs involving direct delivery of services to high-risk groups. Child and adult education programs, especially for mothers and other caretakers, may offer opportunities to emphasize the value of nutritious, balanced, and adequate diets for child and maternal health. The use of mass media to inform rural populations of food choices is still largely untapped. Market development will facilitate the flow of food within and between
countries and have a stabilizing influence on seasonal and geographic fluctuation in food availability.

For each intervention opportunity, target groups of high priority should be identified by demographic profile and location: preschool children to reduce their mortality, children with health problems such as serious infections, measles, and moderate-to-severe malnutrition to improve their chances for survival, expectant mothers to provide the newborn with vitamin A stores at birth and through breast feeding, school-age children who are about to move into child-bearing age and become parents. Secondly, regions and communities at high risk need to be targeted to maximize efficiency and program specificity. This leads to interventions which are likely to reach the most needy and therefore deliver the greatest benefit at the least cost. Properly timed interventions may also enhance cost-effectiveness: supplementing children just prior to expected seasonal deficiencies, treating ill children immediately upon presentation to a health care facility, seasonally adjusted social marketing initiatives with respect to gardening, preservation, preparation, and consumption of vitamin A and carotenoid-rich foods, as well as feeding and care of children during seasonal illnesses.

Dietary diversification programs need to account for (a) cultural beliefs about appropriate foods for different age groups, (b) economic barriers to incorporation of vitamin A or pro-vitamin A-containing foods, (c) the availability of new food types (e.g., ivy gourd in Thailand), and (d) unique opportunities that may exist, such as school feeding and garden programs.

Although limited at present, opportunities to fortify foods with vitamin A will become more available in the future. Thus, there is a need to be watchful for potentially fortifiable foods that will appear in developing country markets over the next decade while efforts continue to resolve technical problems related to color, stability, potency, and other potential obstacles to fortification.

**MONITORING AND EVALUATION**

*Keith P. West, Jr., and Nils M. P. Daulaire*

Monitoring and evaluation follows on the adoption of program strategies for a given country. Its major purpose is to allow policy makers and program managers to assess status and progress towards decreasing vitamin A deficiency in target populations by whatever means deemed appropriate. The goals of monitoring and evaluation should be clearly specified and be relevant to both the operational elements and the planned outcome of vitamin A deficiency control. Monitoring and evaluation involve the routine collection and interpretation of data about program operations (relevant to inputs, through-puts, and outputs) as well as periodic, intensive assessment of outcome (e.g., status, practices, intake) in a targeted population.
Program components that are measured should be part of an explicit continuum of action aimed at achieving improved vitamin A availability, intake, and status in the target group(s). These elements should include measuring the net addition of vitamin A into the environment over time, above existing levels prior to intervention.

The evaluation process should take account of the time required to achieve expected benchmarks and outcomes, recognizing that different interventions require shorter or longer periods before which measurable benefit can occur. Recognizing this time-dependent factor is critical for all of those involved in the prevention processes. These groups include donor governments and organizations, recipient governments, planning and program agencies, and the local community.

Monitoring and evaluation should be built in from the start and take place at every level of a program (from micro through macro levels) to improve performance and permit mid-course changes and modifications that are inevitable. That is, monitoring and evaluation should occur throughout the entire program, learning as one proceeds and not just at some defined end-point.

Four broad questions should be addressed in all vitamin A deficiency prevention programs, regardless of the individual strategy. Here, the term “vitamin A” can be high-dose supplements, seeds of vitamin A-rich foods, educational messages, materials and counseling activities that could lead to an increased consumption of vitamin A, availability of vitamin A-rich foods to the community, family, or child, etc.:

1) Are there adequate “vitamin A” supplies to achieve the outcome objectives?

2) Do we have the means to insure delivery of the vitamin A? This refers to the adequacy and performance of a distribution, management, or educational infrastructure.

3) Is the intended target group actually receiving the vitamin A intervention and is the vitamin A reaching the target group in an adequate and consistent fashion?

4) Are we achieving the expected impact on vitamin A status over a reasonable period of time?

These four criteria — supplies, delivery infrastructure, coverage of target group, and improvement in vitamin A status — can be specified to evaluate different program strategies to prevent deficiency (such as dietary diversification, fortification, or supplementation). These criteria are provided in a sample “matrix” (Table 6.1), the cells of which could be filled with specific, measurable indicators of process, output, and outcome.
Table 6.1 Sample matrix for evaluating vitamin A programs

<table>
<thead>
<tr>
<th>Process steps</th>
<th>Dietary diversification</th>
<th>Fortification</th>
<th>Supplementation</th>
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</thead>
<tbody>
<tr>
<td>Supplies</td>
<td></td>
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<td>Delivery infrastructure</td>
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<tr>
<td>Coverage</td>
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<td></td>
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<tr>
<td>Vitamin A status</td>
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REFERENCES


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<th>Name</th>
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<tr>
<td>Dr. Warren Berggren</td>
<td>Director of Primary Health Care</td>
<td>Save the Children</td>
</tr>
<tr>
<td>Dr. Frank Chytli</td>
<td>Professor of Biochemistry</td>
<td>Vanderbilt University School of Medicine</td>
</tr>
<tr>
<td>Dr. Frances R. Davidson</td>
<td>Steering Committee, International Vitamin A Consultative Group; Vitamin A Project Manager, Office of Nutrition</td>
<td>Agency for International Development</td>
</tr>
<tr>
<td>Dr. Nils M. P. Daulaire</td>
<td>Director</td>
<td>Intercept</td>
</tr>
<tr>
<td>Dr. Tara Gopaldas</td>
<td>Professor and Dean</td>
<td>Department for Food and Nutrition</td>
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<td>Dr. Abraham Horwitz</td>
<td>Chairman, ACC/SCN; Steering Committee, International Vitamin A Consultative Group; Director Emeritus, Pan American Health Organization, WHO</td>
<td></td>
</tr>
<tr>
<td>Dr. Gregory D. Hussey</td>
<td>Head, Paediatrics Infections Unit</td>
<td>Department of Paediatrics &amp; Child Health</td>
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<td></td>
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<td>Somerset Hospital</td>
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<tr>
<td>Dr. Festo P. Kavishe</td>
<td>Director</td>
<td>Tanzania Food and Nutrition Centre</td>
</tr>
<tr>
<td>Dr. Betty Kirkwood</td>
<td>Chair, Maternal and Child Health</td>
<td>London School of Hygiene &amp; Tropical Medicine</td>
</tr>
<tr>
<td>Dr. Robert S. Lawrence</td>
<td>Director, Health Sciences Division</td>
<td>Rockefeller Foundation</td>
</tr>
<tr>
<td>Dr. Ellen Messer</td>
<td>Associate Professor (Research)</td>
<td>The Alan Shawn Feinstein World Hunger Program</td>
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<td>Dr. Robert Northrup</td>
<td>Director, Primary Care &amp; Health Services</td>
<td>International Health Institute</td>
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<td>Dr. Jon Rohde</td>
<td>Senior Advisor</td>
<td>UNICEF</td>
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<td>Mr. John M. Palmer</td>
<td>Executive Director</td>
<td>Helen Keller International</td>
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<tr>
<td>Dr. A. Catherine Ross</td>
<td>Professor of Physiology and Biochemistry</td>
<td>Medical College of Pennsylvania</td>
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<tr>
<td>Mr. Richard M. Seifman</td>
<td>Director, Office of Nutrition</td>
<td>Agency for International Development</td>
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<tr>
<td>Dr. Alfred Sommer</td>
<td>Steering Committee, International Vitamin A Consultative Group; Professor and Dean, School of Hygiene &amp; Public Health</td>
<td>Johns Hopkins University</td>
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<tr>
<td>Dr. Ignatius Tarwotjo</td>
<td>Nutrition Research Centre</td>
<td>Bogor</td>
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BELLAGIO MEETING ON VITAMIN A DEFICIENCY & CHILDHOOD MORTALITY

Dr. James A. Tonascia
Professor of Biostatistics and
Co-Director, Center for Clinical Trials
Johns Hopkins University

Dr. Frederick L. Trowbridge
Director, Nutrition Division
Centers for Disease Control

Dr. Keith P. West, Jr.
Associate Professor
Ophthalmology and International Health
Johns Hopkins University

MEETING SECRETARIAT

Dr. Abraham Horwitz
Chair

Dr. Alfred Sommer
Convener

Dr. Keith P. West, Jr.
Rapporteur

Anne L. Ralte
Organizer
Director, Vitamin A
Helen Keller International

Barbara Bodnovic
Information Manager, Vitamin A
Helen Keller International

Krisann Karaviotis
Production Assistant, Vitamin A
Helen Keller International
**Summaries of Bellagio Meeting**


*In French:* Exposé de Bellagio — Carence en Vitamine A et Mortalité Infantile.

*In Spanish:* Informe de Bellagio — Deficiencia de Vitamina A y Mortalidad de la Niñez.

**Reports of Bellagio Meeting’s Conclusions**


