Manual on Vitamin A Deficiency Disorders (VADD)
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Donald S. McLaren, Klaus Kraemer
ACKNOWLEDGEMENTS

The *Sight and Life Manual on Vitamin A Deficiency Disorders* (VADD) is now appearing in its 3rd edition. Although our scope has broadened from “sight”, a single focus on vitamin A, to “life”, a multiple micronutrient focus, we believe that vitamin A remains sufficiently important and interesting for us to dedicate a whole book to this nutrient.

This book not only draws on the latest scientific research and expert insights, but also benefits greatly from the work of previous generations of scientists and policy-makers. As a result, it is almost impossible to acknowledge all those who have contributed their expertise. Key sources include, among others, journal articles, policy statements, reports, fact sheets, guidelines, reference books, websites and – of course – the data and research to be found in *Sight and Life* Magazine itself.

We would like to take this opportunity to acknowledge the support we have experienced during the last couple of years in preparing the 3rd edition of this book. There are many people to acknowledge who have been associated with this project. We would therefore like to start with apologies to those who we may have omitted to mention. Extraordinary thanks go to Svenia Sayer-Ruehmann and Anne-Catherine Frey from the *Sight and Life* team for their skilful technical support, without which this publication would have never been completed. Further, our *Sight and Life* interns Laurence Curty and Sarah Diouf both merit special mention. We continue to appreciate Yvonne Bearne for her proofreading, and the Burger Druck team for laying out the book. We also wish to thank the World Health Organization for its kind permission to reprint the vitamin A deficiency world maps. Last but not least, Royal DSM deserves our special appreciation for its continuous support of *Sight and Life*; in particular, we would also like to thank Stephan Tanda, Chairman of the *Sight and Life* Steering Committee, for writing his encouraging foreword to this volume.
Preface

This Third Edition of the *Sight and Life* Manual on Vitamin A Deficiency Disorders (VADD) is likely to be the last. This is not because there is nothing more to learn about the subject. It is because in recent years there has been a major shift in the approach to the control of vitamin and mineral deficiencies (VMD) or micronutrient deficiencies (MMD) – including VADD. This is largely due to the fact that it is now well recognized that VMD tend to occur together rather than on their own – a tendency that is most likely explained by the fact that most micronutrients are found in the same classes of foodstuffs, especially fruit, vegetables, fish and meat. Until recently, it was general practice to identify and combat the most serious forms of deficiency, usually of a single micronutrient. Prominent among these have been vitamin A, iron, iodine, zinc and folate. Today, although there remains a great deal to be learned about the individual roles and functions of most micronutrients in human nutrition, interest is increasingly focused on their interrelationship, especially as micronutrient intervention programs are on the increase and many are in the process of being scaled-up.

The recognition of the interconnected relationship of the micronutrients is further highlighted by the disbanding of the International Vitamin A Consultative Group (IVACG) and International Nutritional Anemia Consultative Group (INACG), which were set up more than thirty years ago to assist in the control of specific nutritional deficiencies. They have been replaced by the all-embracing Micronutrient Forum (MF), which held its first scientific meeting in April 2007. It is no exaggeration to say that hundreds of nutrition-related scientists, whose knowledge and research efforts were virtually confined to a single micronutrient, will now have to broaden their area of expertise and research. Rightly or wrongly, the demands of the time do not seem to permit the luxury of extreme specialization. Henceforth source materials like this manual will also need to focus more on VMD in general. *Sight and Life* sees this as a key role for itself in the future as we continue to evolve to ensure that we make a valuable contribution.

The Third Edition has not surprisingly increased considerably in size and includes three new chapters. The first of these is specifically titled ‘Foreground’ (as opposed to Background), in order to emphasize, from the start, that the focus of the Manual is on the control of VADD and that this objective should be kept in mind throughout the book. Chapter 2 illustrates how essential and versatile the vitamin A molecules are – together with their close relatives found in nature, the carotenoids. Without them human life, vision, growth and development would not be possible. The bioavailability of carotenoids has become so important that it now requires its own chapter (Chapter 5). In addition, the effect that the acute phase response (APR) has on serum retinol levels is of such significance that, after an introduction to the issue in Chapter 7, it remains a prominent feature in several subsequent chapters. As so often in the field of VMD, the work on APR has been pioneered in relation to VADD, but its effect in relation to other micronutrient deficiencies has received little attention and much more research is necessary.

This edition also includes in Chapter 12 the latest available data on the global occurrence of VAD from the World Health Organization (WHO). Attention is drawn to the inability, over the past several years, to determine accurate prevalence figures for the number of blind persons living in any given country or region of the world or within any vulnerable group etc., due to the deci-
sion to no longer report non-blinding xerophthalmia and blinding xerophthalmia separately. As a consequence, while data on other causes of blindness are becoming increasingly precise, vitamin A deficiency blindness is included under the heading of ‘other causes of childhood blindness’ – something which should be rectified in view of its importance and public health impact.

In Memoriam
Martin Frigg, 1943–2010

While this third edition of the Sight and Life Manual on Vitamin A Deficiency Disorders was in preparation, we received news of the sad death of Martin Frigg, Director-General of Sight and Life from 1994 to 2005. Although Martin did not contribute to the present volume, it would not exist without his invaluable editorial work on the previous two editions.

We gratefully acknowledge Martin’s past contribution and deeply lament his passing.

The Editors

Medicine is often considered to be both a science and an art. In reality it is neither, although it employs both science and art to meet its ends. It is better understood as a human endeavor dedicated to the prevention and cure of disease and the alleviation of suffering. Diseases, like the civilizations that suffer from them, rise and fall, occasionally without the cause(s) or the true effects of the measures employed against them being known. Amongst those diseases attributed to an inadequacy of nutrients in the diet, vitamin A deficiency was shown to hold a prominent place in the middle of the 20th century. At that point, it was the most common cause of blindness in young children worldwide. Within several decades, as a result of research that led to interventions, severe blinding vitamin A deficiency (xerophthalmia) had been brought under control to a large extent. However, at around the same time it was recognized that the problem had several other concerning elements:

- Vitamin A deficiency at a subclinical and so often unnoticed level is extremely widespread in developing countries;
- Even at this subclinical level, it has a significant adverse effect on morbidity and mortality rates;
- Other groups besides young children, such as pregnant and lactating women and school-age children, are also highly susceptible to vitamin A deficiency;
- Vitamin A and both pro- and non-pro-vitamin A carotenoids are increasingly being recognized as playing an important role in diseases which affect all age groups throughout the world, including age-related macular degeneration, cancer, metabolic syndrome and cardiovascular disease; and finally,
- New terminology is now generally accepted for the expanded and enhanced role that vitamin A is now recognized to play – vitamin A deficiency disorders (VADD).

Although many micronutrients are intimately involved in human health and the relationship between the micronutrients is increasingly important, the pioneer in vitamin deficiency research, vitamin A, remains an important and interesting research field. We trust that this Third Edition of the Vitamin A Manual will add to the body of literature that contributes towards interventions that ultimately ensure sustainable and significant improvements in human nutrition, health and well-being.

Donald McLaren
Klaus Kraemer
FOREWORD

A Powerful Commitment

Hidden hunger is the number one cause of death in the world, killing more people than AIDS, malaria and tuberculosis combined. The term describes the malnutrition that results from a lack of essential vitamins and minerals. Malnutrition and hunger in combination currently cause the deaths of 3.5 million children under the age of five every year. The achievement of no fewer than six out of the eight United Nations Millennium Development Goals is contingent on the elimination of hidden hunger.

Among the vitamins essential for healthy growth and development, vitamin A plays a key role. It was the recognition of the link between vitamin A deficiency and blindness in malnourished populations that inspired the founding of Sight and Life in 1986 and, indeed, gave the humanitarian initiative its name.

Since that time much has been learned about the characteristics and effects on the human physiology of a wide range of vitamins and minerals. Moreover, scientists have come to appreciate as never before the complex interactions of these micronutrients. A great deal of original research remains to be done, but the way forward is clear: the elimination of hidden hunger worldwide can only be achieved if scientists, policymakers, non-governmental organizations and the private sector join forces to defeat this public health scourge.

Sight and Life has an important role to play in this effort. It is the role of a committed advocate. Sight and Life maintains close and productive relationships with policymakers, other non-governmental organizations, researchers and private sector organizations to advocate targeted policies and effective programs that bring measurable benefits to some of the world’s poorest and most disadvantaged people. Sight and Life achieves this by invoking an array of approaches. These range from publishing authoritative works such as this volume through disseminating up-to-the-minute information and insights via Sight and Life Magazine to staging conferences and workshops, as well, of course, as funding original research work and field programs on the ground.

The role of Sight and Life is therefore to analyze what is good and what is new in the micronutrient arena but also to be aware of what is tried and tested so that it can effectively advocate good policy- and program-making. I know from my work with them that the Sight and Life team are passionate about the work they do and deeply dedicated to ensuring a sustainable and significant improvement in the health and well-being of some of the world’s poorest and most vulnerable people.

DSM fully supports Sight and Life. In our partnership with the World Food Programme (WFP), which has been running since 2007, Sight and Life plays a key role. It is DSM’s belief that together DSM and WFP can make a difference to the lives of millions of people. Improving nutrition means improving lives: it means breaking the vicious circle of poverty and hunger and malnutrition which leads to suffering, disease and underachievement.

We believe that by improving people’s lives in this way we can make a major contribution to unleashing the untapped human and economic potential of many countries around the globe. As our CEO Feike Sijbesma has often said of DSM, we cannot be successful, nor can we call ourselves successful, in a society that fails. This powerful corporate and personal commitment...
was recognized in October 2010 when the United Nations Association of New York awarded Feike Sijbesma the prestigious 2010 Humanitarian of the Year award for his outstanding commitment to corporate social responsibility (CSR) and in particular for DSM’s partnership with the United Nations World Food Programme. It is an honor of which all of us at DSM are deeply proud. And we will be proud to continue our efforts to improve the lives of millions of people who so urgently need our help.

**Stephan Tanda**
Member of the DSM Managing Board with responsibility for DSM’s Nutrition cluster of businesses
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1 Foreground

The story of how the complex of disorders of human health now known as Vitamin A Deficiency Disorders (VADD) came to be recognized over the course of time is a truly remarkable one. Detailed accounts have been published in recent years (McLaren 1999 and 2004a). Table 1.1 provides the dates of key events in the story.

Table 1.1: Historical background.

<table>
<thead>
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<th>Year</th>
<th>Event</th>
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<tr>
<td>1816</td>
<td>Xerophthalmia in experimental animals</td>
</tr>
<tr>
<td>1904</td>
<td>First large human epidemic in Japan</td>
</tr>
<tr>
<td>1913</td>
<td>“Fat soluble A” discovered in the USA</td>
</tr>
<tr>
<td>1930</td>
<td>Formulae of β-carotene and vitamin A discovered (Karrer, Switzerland)</td>
</tr>
<tr>
<td>1947</td>
<td>Industrial vitamin A synthesis (Isler at Roche, Switzerland)</td>
</tr>
<tr>
<td>1964</td>
<td>First global survey of VADD</td>
</tr>
<tr>
<td>1980s</td>
<td>Importance of vitamin A in child survival shown</td>
</tr>
<tr>
<td>1990s</td>
<td>Maternal mortality reduced with retinol or β-carotene supplements Discovery of retinoid receptor gene family</td>
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</table>

It is intended that detailed and up-to-date information on every relevant aspect of VADD will be systematically covered in the chapters that follow, with attention being focused in particular on providing an outline of their setting, nature, and significance. This outline is meant to introduce the subject in such a way that a degree of confidence, involvement and concern is instilled in the reader from the outset.

SETTING OF VADD

Vitamin A deficiency is just one among many vitamin deficiencies that occur in humans. The form that damages the eye and vision, known as xerophthalmia, has been documented since ancient times. Other vitamin deficiencies, similarly long since recorded, include beriberi (thiamine deficiency), pellagra (niacin deficiency), scurvy (vitamin C deficiency), rickets and osteomalacia (vitamin D deficiency).

Vitamins and certain minerals make up the micronutrients – substances required for health in very small amounts, i.e. just a few milligrams or micrograms per day. There are also several lipids, known as essential fatty acids, which are needed in the diet in small amounts.

Finally, there are macronutrients, the fats, proteins, and carbohydrates needed in relatively large amounts to help provide the body with energy and fulfill its structural requirements. Some of the building blocks of proteins, amino acids, are “essential” nutrients.

Varying combinations of deficiencies in all these substances lead to what is probably the most widespread nutritional deficiency disease of all: protein-energy malnutrition (PEM). In its most severe clinical forms this syndrome includes kwashiorkor and marasmus, but the majority of cases are characterized by failure to thrive and underweight.

The latest press release on global young child annual mortality data is that of UNICEF (10 September 2009). According to these data the abso-
lute number of child deaths in 2008 declined to an estimated 8.8 million, from 12.5 million in 1990. The data show that global under-five mortality has decreased steadily over the past two decades. The average rate of decline from 2000 to 2008 is 2.3%, compared to a 1.4% average decline from 1990 to 2000.

Table 1.2 lists the five main nutritional deficiency diseases. When the WHO first named four major deficiency diseases as being of global public health magnitude (Anon 1972), zinc deficiency in humans had been barely recognized (see Chapter 10). It is rather depressing to have to note that after more than 30 years the original four deficiency diseases still remain major problems and have been joined by a fifth.

**Table 1.2**: Major nutritional deficiency diseases.

<table>
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<th>1.</th>
<th>Protein-Energy Malnutrition (PEM)</th>
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<td>Vitamin A Deficiency Disorders (VADD)</td>
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<td>3.</td>
<td>Nutritional Anemias (especially Iron)</td>
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<tr>
<td>4.</td>
<td>Iodine Deficiency Disorders (IDD)</td>
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<tr>
<td>5.</td>
<td>Zinc Deficiency</td>
</tr>
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</table>

More than a third of all children under the age of five in developing countries are affected by PEM: by international criteria they are either wasted, stunted, or underweight (de Onis, Monteiro, Akre et al. 1993). It is probable that many of them also suffer from VADD, but statistics are not available because in recent years the terms PEM, kwashiorkor and marasmus for the severe clinical forms of childhood malnutrition have been abandoned and there are no records of their prevalence, trends, or mortality worldwide.

Another important feature of the setting of VADD is the frequency of the infectious diseases that accompany them. The relationship is synergistic: VAD weakens the defenses of the body and has specific damaging effects on the immune response (see Chapter 6). Infections in turn often impair vitamin A status by decreasing appetite, increasing demands on the body, and sometimes causing loss of the vitamin through the urine (see Chapter 14).

Recent estimates by WHO (Bryce, Boschi-Pinto, Shibuya et al. 2005) suggest that approximately 10.6 million children under the age of five die annually, most in developing countries. In the same report, pneumonia, surprisingly, turned out to be the leading killer of children, responsible for about one third of all deaths under five (Figure 1.1).

Undernutrition was an underlying cause in 53% of all deaths presented here. Further details are not available, but it would be reasonable to assume, from work referred to below (see Bryce, Black, Walker et al. 2005), that VAD and zinc deficiency play an important role. However, it appears that the term “undernutrition” only applies to the presence of wasting and/or stunting. Growth retardation is associated with deficiencies of vitamin A or zinc, and possibly with other micronutrient deficiencies. In 2002 a groundbreaking study was published which showed the global and regional burden of disease and the 26 selected major risk factors responsible (Ezzati, Lopez, Rodgers et al. 2002). The burden of disease was measured as death and disability, represented by a unit termed “disability-adjusted life year” (DALYs). Child and maternal underweight were the fourth highest cause of mortality, with high blood pressure as the first. When deficiency of iron, zinc and vitamin A are added to underweight, to form a category of malnutrition of their own, the outcome is then second overall. For DALYs, undernutrition is the single leading global cause of loss of health constituting 16% of the total burden. For iron, vitamin A, and zinc deficiency, the DALYs are 2.4%, 1.8%, and 1.9%, with iodine deficiency only 0.1%.

In 2006 (Lopez, Mathers, Ezzati et al.) an update of these data showed some changes. Child underweight for age became the fourth attributable cause of deaths, with zinc, vitamin A, and iron deficiencies in 12th, 14th and 15th positions.
respectively. For the attributable disease burden (% global DALYs), child underweight for age was first, with zinc, iron, and vitamin A deficiencies 11th, 13th and 14th respectively. In the first of a series of papers, known as The Lancet Series, devoted to the latest assessment of “Maternal and child malnutrition”, Black, Allen, Bhutta et al. (2008) dealt with global and regional exposures and health consequences. It was estimated that in children under five years in developing countries stunting, wasting, and IUGR (intra-uterine growth retardation) together account for 22% of deaths and 21% of DALYs. In the same age group VAD and zinc deficiency account for 0.6 million and 0.4 million deaths respectively, and combined for 9% DALYs.

The aim of a recent study (Bryce, Black, Walker et al. 2005) was to calculate the cost of saving the lives of 6 million children each year throughout all of the 42 countries in which 90% of child deaths occurred in 2000. Both vitamin A and zinc supplementation were among the 18 measures chosen. The total annual cost of new resources required was estimated at US$ 5.1 billion.

**NATURE OF VADD**

The term VADD has been defined as “a comprehensive term that covers all aspects of the deficiency state of vitamin A and includes adverse effects on health, survival and vision.”

Until quite recently, vitamin A deficiency, in the form of xerophthalmia, was considered to constitute a major cause of preventable blindness in developing countries. Similarly, iron deficiency was regarded as the most widespread form of anemia, mainly in children and women of child-bearing age. Iodine deficiency was recognized as the primary cause of endemic goiter. Rickets and osteomalacia were acknowledged

Pneumonia causes 19% of all under-5 deaths. This figure, however, does not include deaths caused by pneumonia during the neonatal period. It is estimated that 26% of neonatal deaths, or 10% of under-5 deaths, are caused by severe infection. A large proportion of these infections is caused by pneumonia/sepsis. If these deaths were added to the overall estimate, pneumonia would account for up to 3 million, or as many as one-third (29%) of under-5 deaths worldwide.

**Figure 1.1:** Pneumonia is the leading killer of children worldwide. This figure shows global distribution of cause-specific mortality among children under five in 2004 (Bryce, Boschi-Pinto, Shibuya et al. 2005).
forms of bone disease, in young children and elderly adults respectively, caused by vitamin D deficiency.

It has become evident that even mild degrees of these and all other forms of nutritional deficiency have important adverse implications for health. And as a consequence, these deficiencies are much more widespread than previously thought, and are thus of much greater public health importance.

In the specific case of VADD, much progress has been made in the identification of the subclinical stages of deficiency (see Chapter 7). Following upon the great efforts that were made in recent decades to combat xerophthalmia (see McLaren 1999), this severe form of the disease has been greatly reduced. Even so, there remains much more work to be done (Table 1.3).

A further aspect of the nature of nutritional diseases that is becoming increasingly apparent is the fact that, at the subclinical level, most of the underprivileged members of developing countries, of all ages, are subject to a lifetime of dietary inadequacy that affects many nutrients. In such cases multi-micronutrient (MMN) deficiency becomes the rule. This situation, which is complex, serious, and urgent, poses a new challenge to those concerned with human health and welfare (see Chapter 11).

**SIGNIFICANCE OF VADD**

Just over four decades ago xerophthalmia was demonstrated to be of worldwide significance (Oomen, McLaren, Escapini 1964). Latest available health statistics show the impact of VADD on the health of young children (Table 1.3) and the impact of vitamin A intervention on child survival:

“Improvement of vitamin A status in young child populations… leads to a reduction in all-cause mortality rates of about 23%.” (Beaton, Martorell, Aronson et al. 1993).

“Improved vitamin A nutriture would be expected to prevent approximately 1.3–2.5 million deaths annually among children aged under 5 years.” (Humphrey, West, Sommer 1992).

In recent years attention has been focused on vitamin A deficiency in women. It transpires that they are indeed a susceptible group (Table 1.4).

Recently estimates were made of the loss of DALYs attributable to the present prevalence of VAD in 0–4 year old children worldwide (Table 1.5) (Mason, Musgrave, Habicht 2003). Another group (Levine, Pollit, Galloway et al. 1993) calculated the return on investments from VAD control in US$ (Table 1.6). These data are indeed impressive, concerning both the potential benefit from the reduction in disease burden from elimi-

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**Table 1.3**: Impact of vitamin A deficiency on health of preschool-age children (latest estimates of global numbers affected).

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>190 million</td>
<td>with low vitamin A status indicated by serum retinol &lt;0.70 μmol/L (WHO 2009)</td>
</tr>
<tr>
<td>5.7 million</td>
<td>with night blindness (XN) (WHO 2009)</td>
</tr>
<tr>
<td>4.424 million</td>
<td>with clinical xerophthalmia (one or more of WHO 1982 xerophthalmia classification eye signs present) (West, Darnton-Hill 2008)</td>
</tr>
<tr>
<td>0.4 million</td>
<td>blind due to VAD (no data being collected, but previous studies suggested about 10% of those with clinical xerophthalmia. Also responsible for about 10% of all blind children. See previous editions of this book).</td>
</tr>
</tbody>
</table>
nating VADD in 0–4 year old children, and also the possible return on investments from VAD control.

This brief overview may be concluded on an optimistic note. As long ago as 1993, in its Development Report, Investing in Health, the World Bank published a study in which the benefits and costs of 47 health interventions were evaluated. As Figure 1.2 shows, vitamin A supplementation was among the very best performers in both regards. In 2008, the Copenhagen Consensus concluded that for each US$ spent on vitamin A and zinc supplementation a return on investment of US$ 17 can be expected (see also Chapter 15).

In 2008 (Rosenbloom, Kaluski, Berry) a new concept was introduced, called the global nutritional index (GNI), modelled on the human development index. For each country it is based on three indicators of nutritional status: deficits, excess and food security, and for the first time provides a single statistic for each country according to its overall level of nutrition.

Table 1.4: Impact of VADD on health in pregnancy (from WHO 2009). *

<table>
<thead>
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<th>Prevalence</th>
<th>DALYs lost</th>
<th>% Decrease</th>
</tr>
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<tr>
<td>19.1 million with serum retinol &lt;0.70 μmol/L</td>
<td>20.10</td>
<td>19.26</td>
</tr>
<tr>
<td>9.75 million with night blindness (XN)</td>
<td>1.30</td>
<td>3.76</td>
</tr>
</tbody>
</table>

*no data given for xerophthalmia or blindness

Table 1.5: Reduction in disease burden from eliminating VAD in 0–4 year old children through decrease in mortality risk (from Mason, Musgrave, Habicht 2003).

<table>
<thead>
<tr>
<th>Region</th>
<th>Prevalence of clinical VAD (%)</th>
<th>DALYs lost from VAD (millions)</th>
<th>% Decrease in DALYs</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>1.00</td>
<td>20.10</td>
<td>19.26</td>
</tr>
<tr>
<td>China</td>
<td>0.20</td>
<td>1.30</td>
<td>3.76</td>
</tr>
<tr>
<td>Other Asia</td>
<td>0.76</td>
<td>6.60</td>
<td>12.22</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>0.93</td>
<td>20.80</td>
<td>15.59</td>
</tr>
<tr>
<td>Latin America</td>
<td>0.25</td>
<td>1.20</td>
<td>5.00</td>
</tr>
<tr>
<td>Middle East Crescent</td>
<td>0.28</td>
<td>4.10</td>
<td>6.75</td>
</tr>
</tbody>
</table>

Table 1.6: Return on investments from VAD control in US$ (from Levine, Pollitt, Galloway et al. 1993).

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Cost of lives saved</th>
<th>Discounted value of productivity gained/program</th>
<th>Cost per DALY gained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplementation (children &lt;5 years)</td>
<td>0.50</td>
<td>47.50</td>
<td>1.40</td>
</tr>
<tr>
<td>Fortification</td>
<td>154</td>
<td></td>
<td>4.20</td>
</tr>
</tbody>
</table>
Figure 1.2: Benefits and costs of 47 health interventions. Dots include ages under and over 15. (Reproduced with permission from World Bank).
Vitamin A in Nature

Substances closely related to vitamin A abound in both plant and animal life and take part in a most amazing variety of processes. In one way or another, many of these processes are associated with the action of light. Without light there would be no life as we know it. As far as is known, there is no other molecular group in nature that matches the versatility of vitamin A and its relatives.

LIGHT

Light rays are composed of small packets of energy called photons. In that very small part of the electromagnetic spectrum that can be detected by our photoreceptors, termed the visual spectrum, the energy level of photons specifies a color, ranging from red to violet. The longest wavelength (red) has the lowest intensity of brightness, or flux, and the shortest wavelength (violet) has the highest intensity (Figure 2.1). Thus light can be conceived of in two ways; as quanta, or photons of varying flux, and as waves of varying wavelength.

CAROTENOIDs AND RETINOIDs

Each molecule addressed here belongs to one of two related classes of substances, carotenoids or retinoids. Members of each are found in both plants and animals, but carotenoids predominate in plants and retinoids in animals. The majority of retinoids – perhaps all – are derived from carotenoids, which appear to have originated in archebacteria several billion years ago towards the beginning of evolutionary time, long before plant life proper. They were part of the process that provided our previously anoxic earth with its oxygen-rich atmosphere (Margulis, Sagan 1995).

The chemical structure of carotenoids and retinoids is very similar (Figure 2.2). Carotenoids have a basic C40 skeleton which is made up from successive additions of C5 isoprene units. Retinoids approximate to half of the carotenoid molecule, consisting of four isoprenoid units joined together in a head-to-tail manner and customarily containing five conjugated double bonds. The term vitamin A is used as a generic descriptor for retinoids that qualitatively exhibit the biological activity of all-trans retinol.

Most readers are probably unaware that recently electrophysiology as the standard method for investigating neuronal signaling is being replaced by various optical probes as a result of new...
forms of microscopy. Among these amazing new probes the type includes forms of rhodopsin (e.g., channelrhodopsin and holorhodopsin) that will appear several times later (Scanziani, Häusser 2009).

Examples of some non-provitamin A carotenoids appear below (Figure 2.3).

Carotenoids are yellow, orange or red pigments. Over 600 have been identified and the list is constantly growing. Of the 600 over 50 have a structure that enables them to be converted into vitamin A, which includes at least one unsubstituted β-ionone ring and a polyene side chain. The other end of the molecule may have a cyclic
or an acyclic structure. It may be lengthened but not shortened to less than an 11-carbon polyene chain. Chain lengthening decreases activity. Ultimately, all vitamin A in nature originates from one or other of these provitamin carotenoids. All-trans \(\beta\)-carotene is by far the commonest but \(\alpha\)-carotene, \(\gamma\)-carotene and \(\beta\)-cryptoxanthin contribute to lesser extents (Table 2.1).

### Table 2.1: Relative provitamin A activity of various carotenoids (Simpson, Tsou 1986).

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-carotene</td>
<td>100</td>
</tr>
<tr>
<td>(\alpha)-carotene</td>
<td>50–54</td>
</tr>
<tr>
<td>(\gamma)-carotene</td>
<td>42-50</td>
</tr>
<tr>
<td>3,4-dehydro-(\beta)-carotene</td>
<td>75</td>
</tr>
<tr>
<td>(\beta)-carotene-5,6-epoxide</td>
<td>21</td>
</tr>
<tr>
<td>(\alpha)-carotene-5,6-epoxide</td>
<td>25</td>
</tr>
<tr>
<td>3-oxo-(\beta)-carotene</td>
<td>52</td>
</tr>
<tr>
<td>3-hydroxy-(\beta)-carotene</td>
<td>50–60</td>
</tr>
<tr>
<td>(cryptoxanthin)</td>
<td></td>
</tr>
<tr>
<td>4-hydroxy-(\beta)-carotene</td>
<td>48</td>
</tr>
<tr>
<td>(\beta)-2’-apo-carotenal</td>
<td>active</td>
</tr>
<tr>
<td>(\beta)-8’-apo-carotenal</td>
<td>72</td>
</tr>
<tr>
<td>lycopene</td>
<td>inactive</td>
</tr>
<tr>
<td>lutein</td>
<td>inactive</td>
</tr>
<tr>
<td>3,3’-dihydroxy-(\beta)-carotene</td>
<td>inactive</td>
</tr>
<tr>
<td>(zeaxanthin)</td>
<td></td>
</tr>
</tbody>
</table>

The most characteristic feature of the carotenoid structure is the long system of alternating double and single bonds, usually 9–13 in number, the polyene chain that forms the central part of the molecule. It is this that gives carotenoids their distinctive molecular shape, chemical reactivity and light-absorbing properties. Usually, the most stable form of the polyene chain is a linear, extended conformation, as in lycopene.

Each of the many carotenoid conjugated double bonds can form vast numbers of geometric isomers; theoretically hundreds of thousands. The presence of a \(cis\)-double bond predisposes the isomer to increased thermodynamic instability. The ability of carotenoids to absorb visual light is related to the presence of delocalized \(pi\) electrons. In plants, energy can transfer from excited carotenoids, generating excited chlorophyll, which is active in photosynthesis (see below). In leaves carrying out photosynthesis, the physical structure of the chloroplast, the subcellular structure containing the carotenoid, facilitates this transfer of energy to chlorophyll (see Figure 2.4) (Britton 1995).

Under conditions of high light intensity chlorophyll in the triplet state can accumulate and cause damage. Carotenoids can counteract this effect in two ways: by deactivating the triplet state of chlorophyll or by converting single-state oxygen to its ground triplet state and again allowing release of the transferred energy as heat (Pascal, Liu, Broess et al. 2005). With the advance of global warming, this protective function is increasingly significant.

Because of their high degree of unsaturation, carotenoids can extract or donate electrons, resulting in radical anions and cations which can react with oxygen or other molecules; they show both antioxidant and prooxidant properties under various conditions (Britton 1995). These properties are being investigated in relation to the prevention or treatment of various diseases (see Chapter 14). Of special interest has been the discovery that high concentrations of two carotenoids, zeaxanthin and lutein, are normally deposited in the human retina. Low concentrations appear to be associated with development of age-related macular degeneration (see Chapter 14).

The attractive red, orange, and yellow colors of many autumn leaves are due to their xanthophyll content. Xanthophylls are non-provitamin A carotenoids that have one or more oxygen molecules on the ring or in the chain. It has been suggested that these changes in color amount to a warning to pests that the tree wouldfight any infestation (Hamilton, Brown 2001). Abscisic acid, an anti-growth hormone and carotenoid derivative, controls the fall of leaves in autumn. Xantho-
Phylls also contribute to the striking plumage of many birds. Both provitamin and non-provitamin carotenoids are responsible for the appealing red and yellow colors of fruits and vegetables, and both classes are of great nutritional and health significance. Carotenoids also are found in algae, fungi, yeasts, moulds, mushrooms, bacteria and in all classes of plants and animals.

Carotenoids and retinoids are nonpolar and hydrophobic. The formation of complexes with proteins allows them access to aqueous media, such as plasma. The many activities of carotenoids have been classified into functions, actions and associations (Olson 1996) (see Chapter 4).

**PHOTOSYNTHESIS**

By far the best known function of carotenoids is the part they play in photosynthesis, which in simple terms is the process whereby plants, in the presence of light, cause carbon dioxide (CO₂) from the atmosphere to combine with water (H₂O) to form sugar or starch, with oxygen (O₂) as a by-product.

The green pigment chlorophyll, in particular chlorophyll-a, is responsible for most light absorption, but the red, orange or yellow carotenoids, covering other parts of the visual spectrum, may account for about one third of total photosynthesis (Figure 2.4).

![Figure 2.4: Photosynthesis at different wavelengths. (A) The ability of light of different wavelengths to support photosynthesis; (B) The absorption spectra for three photosynthetic pigments; chlorophyll a, chlorophyll b, and β-carotene. (Adapted from Lodish, Baltimore, Berk et al. 1995).](image-url)
Photosynthesis takes place in an organelle known as a chloroplast (**Figure 2.5**). Like the mitochondrion it has been shown to have its own DNA, different from that of the cell, and it is now accepted to be an example of eusymbiosis, where early in evolution one organism has been incorporated into another for the mutual benefit of both (Margulis, Sagan 1995).

**RHODOPSIN WHERE LEAST EXPECTED**

Most readers are familiar with rhodopsin, or visual purple, as the visual pigment in the rods of the retina responsible for vision under conditions of restricted illumination, or night vision. They might be surprised to learn that there are many hundreds of different forms of rhodopsin found in nature, many in simple organisms of the plant kingdom. In all instances, rhodopsin consists of two parts: 1) a membrane protein known as a G-protein which differs slightly in each case; and 2) a prosthetic group, or chromophore, which is a special form of vitamin A and responsible for the unique process of phototransduction (see below for details) (**Figure 2.6**).

The importance of the class of proteins known as G-proteins has been discovered only in recent

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**Figure 2.5:** The structure of a chloroplast with simplified membrane structure. (Adapted from Hopkins, Huener 1995).

**Figure 2.6:** Rhodopsin molecule. The dashed circle in the centre of the transmembrane region indicates the site and plane of 11-cis-retinal to the lysine residues at position 206 and 209. (Adapted from Forrester, Dick, McMenami et al. 1996).
years. Many receptors linked to effector systems by trimeric guanosine triphosphate (GTP)-binding proteins (G-proteins) have been described. These proteins regulate the activity of a specific plasma membrane enzyme or ion channel (as in the case of rhodopsin).

BACTERIORHODOPSIN

Of the many plant rhodopsins, the first discovered and the best understood is bacteriorhodopsin, produced by the archebacterium *Halo bacterium halobium* (Stryer 1995). In the presence of abundant oxygen, this organism oxidizes fuel molecules to generate ATP, as do most aerobic organisms. However, when oxygen is scarce, it synthesizes large amounts of bacteriorhodopsin, which takes part in a light-driven proton pump (Figure 2.7).

The essence of this process is the photoisomerization of the all-trans retinal chromophore to the 13-cis form, which causes a bend in the all-trans molecule that amounts to only 2 Å (1 angstrom = a hundred millionth of a centimeter). At the same time, a proton (H⁺ atom) is pumped from the cytosol (inside of the cell) to the outside.

Something very similar happens in the visual cycle in animals (see below).

A “TYPICAL” EYE

The entire visual process is highly complex and requires lengthy description. In the present context, we limit our discussion to the relevant involvement of vitamin A in the initial process of phototransduction (conversion of light energy into nerve cell energy). Differences between in-

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Figure 2.7: Proposed mechanism of light-driven proton pumping by bacteriorhodopsin. (Adapted from Mathies, Lin, Amed et al. 1991).
vertebrates and vertebrates and certain special cases will be commented upon, but the human eye is here presented as a generally “typical” case, particularly with regard to phototransduction. The emergence in evolution of the earliest true eye (as opposed to primitive luminescent “eye spots”) is believed to have occurred in a trilobite and to have triggered the Cambrian explosion about 544 million years ago (Parker 2003).

The retina is composed of ten layers of specialized cells, and in mammals the photoreceptors form the outermost of these underneath the retinal pigment epithelium (RPE) (Figure 2.8). This strange arrangement, which means that before phototransduction can take place light has to pass through all other nine layers, has never been fully explained. Even so, it does not seem to interfere with the clarity of images.

There are two types of photoreceptor: rods, for vision in poor light; and cones, for vision in bright light (where colors can be distinguished). Cones and rods have differences in both structure and function (Figure 2.9). The chromophore is the same in both, 11-cis-retinal (not 13-cis-retinal as in bacteriorhodopsin – see above), but the G-proteins differ. Rhodopsin is in the rods, and in man there are three slightly different versions of a similar G-protein, iodopsin, in the cones. These cone photoreceptors absorb either blue, green or red light, giving man, and all other mammals, trichromatic vision. It has been proposed that complex color vision evolved to facilitate the gathering of colored fruits and young leaves for food (Sumner 2002). Some animals have fewer types of cones, some have more (up to four), while others, including birds, can even see ultraviolet light.

The photoreceptors of the human retina are so sensitive that one can be triggered by a single photon. In a rod this generates a current of ap-

![Figure 2.8: Schematic diagram of the ten layers of the retina. (Adapted from Cormack 1987:691).]
approximately 1–3 picoamperes; in a cone it generates only about 10 femtoamperes or about one hundredth that of a rod. However, the response time of a cone is about four times faster than that of a rod. Cones are better suited for discerning rapidly changing events; rods for low-light visual acuity.

The process of phototransduction begins in the outer segment of the rods and cones and the signal generated passes down a fine cilium to the inner segment. In the human eye, there are about 100 million rods and only 3 million cones.

The primary event in visual excitation is the isomerization of the 11-cis-retinal to the all-trans form. This markedly alters the geometry of retinal. The Schiff base linkage of retinal moves approximately 5 Å (angstrom) in relation to the ring portion of the chromophore (Stryer 1995) (Figure 2.10).

Before light excitation, 11-cis-retinal locks the receptor protein (opsin) in its inactive form. The energy in a photon has been converted into the energy of atomic motion to the extent of 5 Å. In the case of bacteriorhodopsin (see above) the 13-cis-isomer is used and the energy shift is 2 Å and not 5 Å. Much of the isomerization of retinal takes place within 200 femtoseconds, one of the fastest reactions in nature, giving rise to a series of tran-
sient intermediates. One of these, metarhodopsin II, called photoexcited or activated rhodopsin, triggers an enzymatic cascade that activates a G-protein (transducin) cascade leading to cyclic GMP hydrolysis, which closes cation-specific channels to eventually generate a nerve signal.

In this process, the rhodopsin molecule alters its light-absorbing properties, from magenta through orange to yellow and ultimately to white or “bleached.” At this stage it has become opsin and dissociated all-trans-retinal. The opsin undergoes regeneration by binding another molecule of 11-cis-retinal. In addition to restoring light-absorbing capacity, this is critical for shutting off the pigment’s catalytic activity and thus allowing full dark adaptation to occur.

Another protein, RPE65 in the RPE, performs a key role in vision. The palmitoylation of RPE65 serves to switch off the visual cycle in darkness and to switch it on in light (Wolf 2005). Recent work shows that another function taking place in human RPE cells is cleavage of β-carotene by carotenoid 15,15’-monooxygenase (CMO1) (Chichili, Nohr, Schaeffer et al. 2005).

VARIATIONS IN CHROMOPHORES

Throughout the animal kingdom hundreds of visual pigments have been characterized by their absorption maximum (gamma max). The gamma max range from 432 nm for the green rod of the frog to 625 nm for the red-absorbing cone of the goldfish. The G-protein (opsin family) variation, and not the chromophore, is responsible for most of these differences.

The main chromophores of the visual pigments are derived from one of the two forms of vitamin A: vitamin A1 (all-trans-retinol) or vitamin A2 (all-trans-3-dehydroretinol). The chromophores are respectively retinal (the great majority) and 3,4-didehydroretinal. So far only three other retinal congeners have been identified throughout the animal kingdom. All are versions of hydroxyretinal and occur mainly in insects. These chromophores are thought to be derived from the cleavage of β-carotene to retinal followed by hydroxylation within the retina (Seki, Isono, Ozkaki et al. 1998).

The visual pigments of freshwater fish differ from those of marine fish and almost all other animals. Freshwater fish and some amphibians have 3-dehydroretinal as chromophore and porphyropsin for visual pigment, with gamma max near 540 nm (compared with all-trans-retinal and rhodopsin, with gamma max near 500 nm).

There is evidence that the retinas of freshwater fish and some amphibians can convert retinal into 3-dehydroretinal. This process may be regulated largely by properties of light in the natural environment and is sometimes under hormonal control. Age and diet also play a part. The difference in the gamma max of the different chromophores appears to be an adap-
tation that allows fish to make use of the longer wavelengths of light found in fresh water as compared with sea water.

Euryhaline fish (those that tolerate a wide range of salinity in water), have a mixture of both rhodopsin and porphyropsin. In the frog *Rana pipiens* the chromophore in the tadpole is 11-cis-3-dehydroretinal. In the adult, this is replaced by 11-cis-retinal.

### PHOTOENTRAINMENT

The term photoentrainment applies to the phenomenon of regulation and control of a wide variety of metabolic and other processes in which light entering the eyes brings about daily or other regular changes also known as circadian rhythm or biological clock (Foster, David-Gray, Lucas 2001).

In mammals this clock is located within a paired nucleus in the brain above the crossing of the optic nerves, known as the suprachiasmatic nucleus or SCN. Until recently, it was assumed that photoentrainment was carried out by the photoreceptors, the rods and cones. Recently it has been shown that so-called intrinsically photosensitive retinal ganglion cells (ipRGCs), composing one of the ten layers of the retina, contain an opsin/11-cis-retinal-based photopigment called melanopsin (Dkhissi-Benyaha, Rieux, Hut et al. 2006). Consequently, the circadian processes that melanopsin controls are sensitive to vitamin A deprivation. In recent years one of these processes, the contraction of the pupil, known as the pupillary threshold, has been exploited in assessment of vitamin A status (see Chapter 7, p. 72).

### VITAMIN A FUNCTIONS UNRELATED TO LIGHT

The involvement of light has been absolutely essential for the widely differing functions of vitamin A and its relatives described above, but for most functions of vitamin A derivatives throughout the mammalian body, all-trans retinoic acid (RA), light plays no part at all.

Vitamin A itself (all-trans retinol) has no known biological activity. The different retinoid forms present in the body are generated mainly through modifications to the terminal polar end of the molecule. Retinol and retinyl esters (mainly palmitic, oleic, stearic, and linoleic) are the most abundant forms. Formation of retinyl esters makes the retinoid less toxic and permits its storage, mainly in the liver, within intracellular droplets.

Aside from in the eye (see above), retinal has no known function other than serving as an intermediate in the synthesis of retinoic acid (RA). All-trans RA is formed through the irreversible oxidation of all-trans-retinal.

The all-trans- and 9-cis-isomers of RA are transcriptionally active and account for the gene regulatory properties of retinoids within most cells and tissues. RA can bind three retinoid A receptors (RARα, RARβ, and RARγ) and three retinoid X receptors (RXRα, RXRβ, and RXRγ). Well over 500 genes may be regulated by RA.

Because there are no known enzymes that can reduce RA to retinal, excess RA must be catabolized. This probably accounts for the presence of small amounts of many other forms of retinol and RA.

This is only an outline of the subject and more details of certain aspects are given in Chapters 4–6, which consider the roles vitamin A and its relatives play in health.
3 Methods of Analysis

ANALYTICAL PROCEDURES

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

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

The use of dried blood spots after collecting small amounts of blood (about 15-20 μL whole blood) has recently been perfected (Erhardt 2005). A standard sandwich ELISA technique can be used for measuring retinol binding protein (RBP) for vitamin A status, and also at the same time C-reactive protein (CRP) and α-1 glycoprotein (AGP) as indicators of acute and chronic infections. The need for this has only been recognized in recent years, and is discussed later in several sections (see especially Chapter 7 and the acute phase response).

In recent years, notable advances have been made in the application of stable isotope techniques for the assessment of vitamin A status (Ribaya-Mercado, Solomons 2003). A principle similar to that of Fick’s, known for decades in experimental physiology, uses measurement by mass spectrometry of deuterium-labeled vitamin A (non-radioactive) which is swallowed and allowed to equilibrate throughout the body before being measured again. The technology involved is highly complicated. When a subject’s vitamin A status is low, less dilution of an oral dose of labeled vitamin A by endogenous vitamin A occurs. Under these circumstances, a relatively high ratio of labeled to nonlabeled retinol is found in the serum. Application of this technique in human populations is discussed later (Chapter 7). Using isotopes to determine bioavailability or bioconversion has been critically reviewed by van Lieshout, West, and van Breeman (2003) and Yeum and Russell (2002).

Double stable isotope tests are being used to estimate the bioavailability of β-carotene and other carotenoids (see Chapter 5).

Using the very sensitive technique of accelerator mass spectrometry (Burri, Clifford 2005) it has been possible to feed very small doses of C-14-labeled β-carotene to volunteers and to study its metabolism. Interesting variations are being found in healthy subjects, and the implications of this are discussed later (see Chapter 5).

BIOASSAY PROCEDURES

In the past, biological tests played an important part in assessing vitamin A activity. These
include the classical growth response tests in vitamin A deficient rats, liver storage assays in rats and chicks, and the vaginal smear technique. Cell culture systems have been introduced for assessing the biological activity of retinoids and carotenoids (Bertram 2004; Bertram, Vine 2005).

More recently, cell culture models, using CaCo-2 cells, have been established for studying intestinal uptake and metabolism of carotenoids (During, Hussain, Morel et al. 2002).

CAROTENOID ANALYSIS IN FOODS

The technology of carotenoid analysis is becoming increasingly complex. This is not the appropriate place to enter into detail, but there are authoritative sources for those who need them (Yeum, Booth, Sadowski et al. 1996; Pfander 2002; Britton, Liaaen-Jensen, Pfander 2004).

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

One specific technique for determination of carotenoids in tissues is of particular interest. Raman spectroscopy has been applied to the measurement of carotenoids in the macula of the eye in relation to diagnosis and treatment of age-related macular degeneration (see Chapter 13) and also in the skin (Gellermann, Zidichouski, Smidt et al. 2005).

For retinoid research, cell reporter assays or transgenic reporter animals are increasingly used (Gundersen 2006).
4 Food Sources

INTRODUCTION

As far as it is known, no species has been unable to survive and reproduce due to their natural habitat being so impoverished of the required nutrients. Changes in climate, overpopulation, predation, or any of several inimical circumstances have developed later to bring many flourishing species to an end. Inadequate dietary intake of nutrients, rather than of overall food, has rarely been the cause. Man has been no exception to this.

All living things are required to consume in some way an adequate amount of the elements and molecules of which their own bodies are made. That this process usually takes place in a balanced manner is nothing short of a marvel of nature. We have no idea how organisms select their diet, either quantitatively or qualitatively, for health.

It was made clear in Chapter 2 that through the processes of photosynthesis and the absorption of water and nutrients from the soil and atmosphere, all the members of the plant kingdom from trees, bushes and flowers to moulds and bacteria, are maintained. Examples are known, for instance, of inadequate mineral content of soils and of resultant plant nutritional deficiency disease. Most plant and animal species seem to have evolved successfully by consuming a rather uniform diet.

However, in the case of humans, as we have seen (Chapter 1), nutritional deficiencies of one kind and another have developed through subsistence on the very varied dietaries that have been consumed by mankind over the span of recorded time. In recent times, along with everything else, food has become increasingly globalized. In hard economic times this brings its dangers (see Preface).

There is no single standard ideal diet for the whole of mankind; all we know is that some are more healthy than others, while some are frankly nutritionally inadequate. The surprising thing is that early man had the abilities to find edible wild-growing vegetables and fruits that are not fatally toxic and eventually to cultivate some of these and to domesticate and cross-breed some grasses as cereals and some animals, with no understanding whatsoever of the science of nutrition.

The origins of both non-human and human nutritional deficiencies are buried deep in evolutionary time. The fact remains that man is required to consume adequate amounts of more nutrients, numbering about 40, than any other species. Almost all of these are required in only very small amounts. They are the micronutrients; the vitamins and essential elements. Most classes of foodstuffs (cereals, vegetables, fruits, lentils, nuts and fish, meat and dairy products) contain some micronutrients, but some are devoid of or have very low concentrations of them. In addition, the amount of a foodstuff consumed is important. For instance, for most people in the West, bread and potatoes, which have very low concentrations of thiamine and ascorbic acid respectively, are the major source of each vitamin because they are consumed in large amounts.

In the present context, the sources in food of provitamin A carotenoids and preformed vitamin A are of paramount importance. It should be recognized that the underlying single major reason for the existence of the problem of VADD, and consequently the impetus behind the writing of this book, is the fundamental and widespread deficiency in the basic diet in sources of vitamin A in certain parts of the world and under certain circumstances. Vitamin A itself has been known for almost 100 years (see Chapter 1). Knowledge of how to cure the deficiency almost
as long, yet VADD in various forms (Chapter 12) persists, as do the defective diets that cause them.

There are several fascinating questions that still remain unanswered regarding why humans, especially young children, have proved to be so susceptible to becoming vitamin A deficient. For instance, the liver stores much of the vitamin A ingested, and the level in the blood usually rises steadily with increasing age, whereas most other vitamins are readily excreted. The normal concentration of provitamin A carotenoids, especially in different vegetables and fruits, varies enormously. Substituting one for another in the diet might prove disastrous. In particular, rice, the staple food of a large proportion of the world’s population, has no provitamin A carotenoid activity in any of its hundreds of different varieties in nature. Only through genetic manipulation has so-called ‘Golden Rice’ been produced (see Chapter 15).

For decades the scientific community remained in over-optimistic ignorance of the true difference in relative vitamin A activity between preformed vitamin A and provitamin A carotenoids (see later in this Chapter). Consideration of the impact of this scientific knowledge on human health is delayed until Chapter 12. Vitamin A activity in the regional and world food supplies (see Figure 12.1) is then dealt with as a fitting background for the global occurrence of VADD.

Human communities rely on a very wide range of plant and animal foods to meet their dietary requirements for vitamin A. Ovo-lacto vegetarians and those who eat no foods of animal origin (vegans) can usually obtain all their nutritional needs from plant sources alone, with the exception of vitamin B₁₂. Animal products are usually expensive and are rarely relied on almost exclusively to meet requirements. From what is known of the carotenoid composition of common fruits and vegetables and surveys on carotenoid intakes, the intake of non-provitamin carotenoids is greater than that of provitamin carotenoids.

There are several parts of the rest of this book where dietary carotenoid intake will be shown to be of very great significance. Firstly, in Chapter 14 the consumption of some of the non-provitamin A carotenoids will be shown to be related both to the prevention and/or treatment of some of the common chronic or degenerative diseases in general medicine. As an example, analysis of the third NHANES data in the United States has revealed interesting population determinants of serum lycopene concentrations (Ganji, Kafai 2005). All told there are more than 40 carotenoids present in the human diet, of which 25 are known to be absorbed, metabolized, or utilized by the body. The formulae and activities of these metabolites are under active investigation (Khachik 2005). Secondly, in Chapter 12 on Global occurrence of VADD, in the section on Vitamin A in global food supply, Figure 12.5 shows, by region of the world, the daily per capita supply of vitamin A and the proportion of the total from provitamin A. There are important differences between regions which will be commented on in Chapter 12 and also in relation to VAS in newborns in Chapter 15.

**UNITS OF VITAMIN A ACTIVITY**

Earlier analytical methods failed to distinguish between individual carotenoids, and as a result non-provitamin carotenoids were frequently included along with those with vitamin A activity in concentrations reported in food composition tables for fruits and vegetables. For example, one analysis showed that as little as 7% of the carotene value estimated was actually β-carotene or provitamin A. The introduction of HPLC paved the way for the solution to this problem, but it has taken time for accurate analyses to be made and for the results to replace the older, inaccurate values in food composition tables. The 1998 USDA–NCC Carotenoid Database for US Foods (see Appendix) and McCance and Widdowson (Paul, Southgate eds. 1978) can be used as reliable sources for values of individual carotenoid contents of foods.
Another problem that has to be addressed with regard to the vitamin A activity of foodstuffs is the need to make allowances for the differences in activity between retinol itself and β-carotene, and also the need to differentiate between the activity of β-carotene and other provitamin carotenoids. The reasons for these differences will be considered later (see Chapter 5). The generally accepted position on the relationships between different expressions of vitamin A activity at the present time is as shown in Table 4.1.

Table 4.1: Retinol activity equivalent (RAE).

<table>
<thead>
<tr>
<th>Retinol activity equivalent (RAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 μg RAE = 1 μg retinol</td>
</tr>
<tr>
<td>2 μg β-carotene in oil</td>
</tr>
<tr>
<td>12 μg β-carotene in mixed foods</td>
</tr>
<tr>
<td>24 μg other provitamin A carotenoids in mixed foods</td>
</tr>
</tbody>
</table>

The rationale for the introduction of the new unit, retinol activity equivalent (RAE), is discussed further in Chapter 5. In practice, it means that provitamin A activities of mixed foods have been halved. Some researchers maintain that for dark green leafy vegetables the reduction should be even greater (West, Eilander, van Lieshout 2002).

The values for provitamin A activity are approximations because the bioavailability and bioconversion of carotenoids may be influenced by many different factors. The doubt that has recently been cast on the efficacy of dark green leaves and some other sources of provitamin A has stimulated much research activity in this field (see Chapter 5).

PROVITAMIN A CAROTENOID SOURCES

Dark green leafy vegetables, yellow fruits, orange roots – mainly carrots – and the oils of palms are the main sources of provitamin A. Among leaves, only those that are dark green are good sources. This is because the carotenoid content in chloroplasts is roughly proportional to the concentration of photosynthesis-associated chlorophyll (see Chapter 2). Edible dark green leaves are readily available in most areas where VADD are a problem. The species vary considerably from place to place and Table 4.2 shows just a few examples of typical provitamin A values for some that are commonly consumed and have proved useful in intervention programs. More exhaustive lists of similar data on a national or regional basis are becoming increasingly available.

Table 4.2: Examples of common vegetable/fruit carotenoid sources.

<table>
<thead>
<tr>
<th>μg RAE/100g edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango (golden)</td>
</tr>
<tr>
<td>Papaya (solo)</td>
</tr>
<tr>
<td>Cucurbita (mature pulp)</td>
</tr>
<tr>
<td>Buriti palm (pulp)</td>
</tr>
<tr>
<td>Red palm oil</td>
</tr>
<tr>
<td>Carrot</td>
</tr>
<tr>
<td>Dark green leafy vegetables</td>
</tr>
<tr>
<td>Tomato</td>
</tr>
<tr>
<td>Apricot</td>
</tr>
<tr>
<td>Sweet potato, red and yellow</td>
</tr>
</tbody>
</table>

Vegetables

Current analyses tend to report the content of each carotenoid identified, irrespective of presence or absence of provitamin activity. This has the additional value of indicating the value of the foodstuff as a source of antioxidant activity. Table 4.3 shows the lutein and β-carotene concentrations in green vegetables.

β-carotene and lutein are the major carotenoids in leafy vegetables, and together account for over 80% of all carotenoids. α- and γ-carotene, cryptoxanthin and lycopene occur in lower concentrations.
Carrots are increasingly being grown in parts of the developing world and may vary considerably in carotenoid content (Table 4.4). A characteristic feature is the relatively high concentration of \( \alpha \)-carotene, usually about half of that of \( \beta \)-carotene.

### Table 4.3: Lutein and \( \beta \)-carotene concentrations in green vegetables (\( \mu g/100 \text{ g fresh weight} \)) (Ong, Tee 1992).

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Lutein</th>
<th>( \beta )-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green, leafy* (4 types)</td>
<td>–</td>
<td>330–5,030</td>
</tr>
<tr>
<td>Green, nonleafy (6 types)</td>
<td>–</td>
<td>217–763</td>
</tr>
<tr>
<td>“Cruciferous” vegetables (5 types)</td>
<td>280–34,200</td>
<td>80–14,600</td>
</tr>
<tr>
<td>Leafy vegetables (32 types)</td>
<td>–</td>
<td>1,000–44,400</td>
</tr>
<tr>
<td>Tuberous vegetables and beans (16 types)</td>
<td>–</td>
<td>40–1,700</td>
</tr>
<tr>
<td>Green, leafy* (7 types)</td>
<td>250–10,200</td>
<td>1,000–5,600</td>
</tr>
<tr>
<td>Other vegetables (19 types)</td>
<td>trace–440</td>
<td>11–430</td>
</tr>
<tr>
<td>Green, leafy* (27 types)</td>
<td>73–29,900</td>
<td>97–13,600</td>
</tr>
<tr>
<td>Green, nonleafy (8 types)</td>
<td>142–460</td>
<td>74–569</td>
</tr>
</tbody>
</table>

* Values from different references

### Table 4.4: \( \alpha \)- and \( \beta \)-carotene concentrations in carrots (\( \mu g/100 \text{ g fresh weight} \)) (Ong, Tee 1992).

<table>
<thead>
<tr>
<th>Carrot</th>
<th>( \alpha )-Carotene</th>
<th>( \beta )-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>2,000–5,000</td>
<td>4,600–12,500</td>
</tr>
<tr>
<td>Canned</td>
<td>3,200–4,800</td>
<td>7,000–11,000</td>
</tr>
<tr>
<td>Frozen</td>
<td>8,400–8,800</td>
<td>26,000–28,100</td>
</tr>
<tr>
<td>Raw</td>
<td>3,790</td>
<td>7,600</td>
</tr>
<tr>
<td>Raw, A+ hybrid</td>
<td>10,650</td>
<td>18,350</td>
</tr>
<tr>
<td>Freshly cooked, A+ hybrid</td>
<td>15,000</td>
<td>25,650</td>
</tr>
<tr>
<td>Canned</td>
<td>2,800</td>
<td>4,760</td>
</tr>
<tr>
<td>Line B6273</td>
<td>Lyophilized</td>
<td>3,400</td>
</tr>
<tr>
<td>Raw</td>
<td>3,200</td>
<td>5,200</td>
</tr>
<tr>
<td>Frozen</td>
<td>3,100</td>
<td>5,100</td>
</tr>
<tr>
<td>Line B9692</td>
<td>Lyophilized</td>
<td>6,100</td>
</tr>
<tr>
<td>Raw</td>
<td>6,600</td>
<td>11,700</td>
</tr>
<tr>
<td>Frozen</td>
<td>6,600</td>
<td>11,600</td>
</tr>
<tr>
<td>HCM line</td>
<td>Lyophilized</td>
<td>20,300</td>
</tr>
<tr>
<td>Raw</td>
<td>20,600</td>
<td>25,100</td>
</tr>
<tr>
<td>Frozen</td>
<td>20,400</td>
<td>25,500</td>
</tr>
<tr>
<td>Raw</td>
<td>2,200–4,900</td>
<td>4,600–10,300</td>
</tr>
<tr>
<td>19 cultivars</td>
<td>3,410</td>
<td>6,770</td>
</tr>
</tbody>
</table>
In tomatoes, lycopene usually far exceeds the concentration of β-carotene, although some varieties rich in β-carotene have been developed. Tomato and tomato products are by far the best source of lycopene, which is of considerable interest in relation to some prevalent diseases worldwide (see Chapter 14). The main carotenoids in pumpkin are α-carotene and β-carotene.

**Fruits**

The vitamin A activity of fruits is generally lower than that of leafy vegetables and their carotenoid content is more complex. Their greater acceptability, especially to young children, is an advantage as far as intervention programs are concerned (see Table 4.5). The “sweet paste” of the buriti (*Mauritia vinifera*) fruit in north and central Brazil is almost as rich in provitamin A carotenoids as is red palm oil (see below).

Currently, quite extensive analysis is underway in search of antioxidant and phytonutrient properties among fruits that are less well-known in the West, but commonly consumed for putative health reasons by more traditional societies (Burke, Smidt, Vuong 2005).

**Table 4.5:** Carotenoid concentrations in fruits (µg/100 g fresh weight) (Ong, Tee 1992).

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Lutein</th>
<th>Cryptoxanthin</th>
<th>Lycopene</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana</td>
<td>20–40</td>
<td>0</td>
<td>0</td>
<td>60–160</td>
<td>40–100</td>
</tr>
<tr>
<td>Berries, grapes, black currant</td>
<td>20–200</td>
<td>0</td>
<td>0</td>
<td>0–60</td>
<td>6–150</td>
</tr>
<tr>
<td>Mango</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Orange, mandarine</td>
<td>20–30</td>
<td>7–300</td>
<td>–</td>
<td>20</td>
<td>25–80</td>
</tr>
<tr>
<td>Papaya, watermelon</td>
<td>0</td>
<td>450–1,500</td>
<td>2,000–5,300</td>
<td>0</td>
<td>228–324</td>
</tr>
<tr>
<td>Starfruit</td>
<td>60</td>
<td>1,070</td>
<td>0</td>
<td>0</td>
<td>28</td>
</tr>
</tbody>
</table>

**Table 4.6:** Carotenoid concentrations in selected roots and tubers (µg/100 g fresh weight) (Ong, Tee 1992).

<table>
<thead>
<tr>
<th>Root or tuber</th>
<th>Lutein</th>
<th>Cryptoxanthin</th>
<th>Lycopene</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet potato</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Different varieties</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>5–551</td>
</tr>
<tr>
<td>Yellow variety</td>
<td>25</td>
<td>0</td>
<td>42</td>
<td>19</td>
</tr>
<tr>
<td>Orange variety</td>
<td>7</td>
<td>27</td>
<td>147</td>
<td>1,140</td>
</tr>
<tr>
<td>Cassava</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>White variety</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>40–790</td>
</tr>
<tr>
<td>Yellowish</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3–40</td>
</tr>
<tr>
<td>Potato</td>
<td>13–60</td>
<td>Trace</td>
<td>Trace</td>
<td>3–40</td>
</tr>
<tr>
<td>Taro</td>
<td>3–31</td>
<td>1</td>
<td>1–3</td>
<td>2–16</td>
</tr>
</tbody>
</table>
Vegetable oils

Carotenoids are present in most oils that are consumed but usually in low concentration. Red palm oil (*Elaeis sp.* see Table 4.7) contains the highest carotenoid concentration in the vegetable kingdom and has been extensively studied, for its unsaturated fatty acid content as much as for its high β-carotene content. It is also a rich source of other dietary substances such as vitamin E, ubiquinones and phytosterols. Different species have different concentrations, as do different oil extracts. It is the main cooking oil in most regions of west and central Africa, but the tree is also cultivated in parts of Asia and of South America. Red palm oil used in cooking imparts a distinctive taste to food that may not be readily accepted by some people. Highly refined red palm oil is increasingly being made available and is much more readily acceptable than the crude form. Many authorities see a major role for this oil in the control of VADD in the future (Kritchevsky 2000; Nagendran, Unnithan, Choo et al. 2000; Rao 2000 and Radhika, Bhaskaram, Balakrishna et al. 2003; see also Chapter 15).

Table 4.7: Carotenoid contents of palm oil extracts (mg/kg) (Ong. Tee 1992).

<table>
<thead>
<tr>
<th>Carotenoid contents of palm oil extracts (mg/kg) (Ong. Tee 1992).</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude palm oil</td>
<td>630–700</td>
</tr>
<tr>
<td>Crude palm olein</td>
<td>680–760</td>
</tr>
<tr>
<td>Crude palm stearin</td>
<td>380–540</td>
</tr>
<tr>
<td>Second pressed oil</td>
<td>1,800–2,400</td>
</tr>
<tr>
<td>Residual oil from fiber</td>
<td>4,000–6,000</td>
</tr>
</tbody>
</table>

Other sources

Hens’ eggs are often considered to be a rich source of provitamin A because of the rich color, but the major pigments are lutein and zeaxanthin, and β-carotene makes up less than 7% of the total. Some fish flesh is brightly colored, but most of the pigment is xanthophyll.

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

PREFORMED VITAMIN A

Mention has already been made of fish liver oils as highly concentrated sources of vitamin A. They are used as pharmaceuticals rather than as diet items. Fish liver is often discarded along with other soft organs, but if utilized could form a valuable source of the vitamin in many parts of the developing world. The storage form is vitamin A1 alcohol (retinol) in saltwater fish. In freshwater fish it is vitamin A2 alcohol (3-dehydroretinol), which has about 40% of the activity of retinol. Because of the teratogenic effects of large doses of vitamin A, the consumption of liver is contraindicated during pregnancy (Ministry of Agriculture, Fisheries and Food, UK 1995). Most mammalian livers consumed, such as those of calves, ox, lambs or chicken, have concentrations that are

Table 4.8: Examples of common animal vitamin A sources (μg retinol/100 g edible portion).

<table>
<thead>
<tr>
<th>Fatty fish liver oils</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Halibut</td>
<td>900,000</td>
</tr>
<tr>
<td>Cod</td>
<td>18,000</td>
</tr>
<tr>
<td>Shark</td>
<td>180,000</td>
</tr>
<tr>
<td>Herring and mackerel</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dairy produce</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter</td>
<td>830</td>
</tr>
<tr>
<td>Margarine, vitaminized</td>
<td>900</td>
</tr>
<tr>
<td>Eggs</td>
<td>140</td>
</tr>
<tr>
<td>Milk</td>
<td>40</td>
</tr>
<tr>
<td>Cheese, fatty type</td>
<td>320</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meats</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver of sheep and ox</td>
<td>15,000</td>
</tr>
<tr>
<td>Beef, mutton, pork</td>
<td>0–4</td>
</tr>
</tbody>
</table>
comparable to those in cod liver oil. Milk, butter, cheese and eggs are all moderate sources. **Table 4.8** gives a selection of preformed vitamin A concentrations of some common foods.

Over the years vitamin A has been added successfully to many foods. What is termed food fortification (or occasionally nutrification) is one of the major long-term approaches to the control of the problem of VADD, and is discussed further in Chapter 15.
INTRODUCTION

Major progress has been made in recent years in the research of bioavailability of carotenoids. Largely because of the important implications the subject has for measures aimed at controlling VADD (see Chapter 15) and the increasing attention given to it by research groups, it is logical to devote a separate chapter to it.

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

In fairness, it should be recalled that in the 1960s and long thereafter attention in this field was focused on xerophthalmia and its prevention. Intermittent large-dose supplementation was the prevailing control measure for this emergency situation. The growing and consumption of dark green leaves and yellow fruits could protect against severe VAD leading to xerophthalmia, it might not be capable of preventing subclinical vitamin A deficiency in a substantial proportion of the young child and maternal populations of developing countries.

The group at the Department of Human Nutrition at Wageningen, Netherlands, was at the forefront of the investigations into the bioavailability of dietary carotenoids, non-provitamin A and provitamin A. They also led the way in the study of the many complex factors that determine bioavailability.

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

**RECENT ADVANCES IN TECHNOLOGY**

The methodology for the accurate determination of provitamin A carotenoid bioactivity in man is still in an early and rapidly growing stage of its development. There are two main approaches: techniques that rely on changes in one or more indicators of vitamin A status, and those that provide a more direct estimate of absorption and/or conversion efficiency of single doses of provitamin A carotenoids (Parker 2000; Yeum, Russell 2002; van Lieshout, West, van Breemen 2003). It seems unlikely that precise values will be set, as in the past, but it should be possible eventually to provide reliable, practical information for application in interventions for the control of VADD. There is evidently a great deal more painstaking research that needs to be done (Traber 2000).

**ISOTOPE DILUTION TECHNIQUES**

Equimolar doses of labeled β-carotene and retinyl acetate were administered orally to a single healthy subject (Hickenbottom, Lemke, Dueker 2002). The results suggested that 8.5 mol of β-carotene were equivalent to 1 mol of preformed vitamin A. On a mass basis 15.5 μg of β-carotene was equivalent to 1 mg retinol. β-carotene was found to be 55% absorbed. This gave precise confirmation of the low bioefficacy of β-carotene.

Vitamin A itself increases the absorption of β-carotene (Lemke, Dueker, Follett et al. 2003). One nmol of [14C]-β-carotene was given to two human volunteers. Vitamin A supplementation (10,000 IU/day) was given from day 53 and a second dose of [14C]-β-carotene was administered on day 74. There were three main effects: 1) increased apparent absorption; 2) 10-fold reduction in urinary excretion; 3) a lower ratio of labeled retinyl ester/β-carotene concentrations in the absorptive phase. While there was less cleavage of β-carotene during vitamin A supplementation, higher absorption resulted in larger molar vitamin A values.

A new phenomenon has been revealed as a result of the introduction of isotope dilution techniques. It has become apparent that healthy people have an extremely wide range of ability to convert β-carotene into vitamin A. For example, 15 healthy adult Chinese were studied for 55 days after receiving on day one isotope-labeled β-carotene and retinyl acetate (Wang, Yin, Zhao et al. 2004). Plasma samples were collected at frequent intervals. It was found that four of the subjects had conversion factors of >29:1; they were labeled “poor converters.” Eleven were “normal converters” with conversion factors of 2.0:1 to 12.2:1 (mean 4.8). The post-intestinal absorption conversion was estimated to be about 30% of the total converted retinol.

Similar results were obtained by Hickenbottom, Follett, Lin et al. (2002). Out of 11 healthy men only six had sufficient measurable plasma concentrations of D6 β-carotene and D3 retinol. Burri (2001) has reviewed the subject and pointed out its important implications for the control of VADD in developing countries. The possible reasons for these interesting results are not fully established. Variable intestinal absorption, variable conversion to vitamin A, or accelerated clearance from the blood are possibilities. Recently, it has been demonstrated that the β-carotene low responder phenotype could be caused by genetic polymorphisms in the β-carotene 15,15′-monooxygenase gene (Leung, Hessel, Meplan et al. 2009). Two common non-synonymous SNPs exist in the human carotenoid 15,15′-monooxygenase (CMO1) gene that occur at high frequencies and that alter β-carotene metabolism. Female volunteers carrying either the 379V or the combined 267S + 379V variant alleles showed a reduced ability to convert β-carotene to retinol. The results provide a putative explanation for the poor responder phenotype in β-carotene bioconversion.
It has recently been shown in a healthy man that excentral cleavage of β-carotene can occur in vivo (Ho, de Moura, Kim et al. 2007). The fate of an oral dose of all-trans [10,10', 11,11'- 14C]-β-carotene (1.01 nmol; 100 nCi) and of its metabolites was followed. This process had previously been shown to occur in vitro and in animal models.

Tang, Gu, Hu et al. (1999) in China studied the effect of a two vegetable diet on vitamin A body stores of children 3–7 years old by a double stable isotope method (see below). Vitamin A labeled with two different kinds of deuterium (D4 and D8) was administered before and after the intervention to estimate the occurrence of changes in total body vitamin A stores. One group received their daily customary intake of 56 g of green-yellow vegetables and 224 g of light-colored vegetables, and the other received 238 g of green-yellow vegetables and 34 g of light colored vegetables. Only the latter diet maintained adequate vitamin A stores. It was calculated from the isotope studies that provitamin A carotenoids (mainly β-carotene) provided an estimated vitamin A equivalence of 27:1 on average (see above). It should be noted that to achieve this effect more than 250 g of vegetables was consumed each day by these children, as young as 3 years old. Another study of the bioavailability of β-carotene from oriental vegetables found higher bioavailabilities, but still much less than from purified β-carotene beadlets (Huang, Tang, Chen et al. 2000).

FACTORS AFFECTING THE BIOAVAILABILITY OF CAROTENOIDS

In very general terms it has been possible to rank different vegetable and fruit sources of carotenoids, allowing for the influence of the food matrix and food processing (Boileau, Moore, Erdman Jr 1999) (Figure 5.1).

The Wageningen group has introduced a mnemonic (SLAMENGHI) to assist in the discussion of this complicated subject. It should be pointed out that the question of bioavailability applies not

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**Figure 5.1:** Effect of food matrix and processing on bioavailability of carotenoids (after Boileau, Moore, Erdman Jr, 1999).
only to provitamin A carotenoids, but also to non- provitamin A carotenoids, such as lycopene, lutein and others whose possible role in prevention or treatment of a number of diseases is discussed in Chapter 14 (Chung, Rasmussen, Johnson 2004; Boileau, Boileau, Erdman Jr 2002).

Before clarifying the mnemonic SLAMENGHI, it is first necessary to mention another term. Bioconversion is not the same as bioavailability: it relates to the efficiency with which the carotenoid in question is converted to vitamin A in the body. There is little information on this subject. The 1976 FAO/WHO Expert Group assumed that β-carotene has twice the efficiency of other provitamin A carotenoids.

‘S’: Species of carotenoids

The naturally occurring configuration of carotenoids in plants is usually the all-trans isomer. All-trans β-carotene is more readily absorbed in man than the 9-cis form. A significant proportion of 9-cis β-carotene is converted to the all-trans form before entering the bloodstream. Cis-lycopene, however, is preferentially absorbed compared with trans-lycopene (Boileau, Boileau, Erdman Jr. 2002).

‘L’: Molecular linkage

Esters of carotenoids are common in fruit and vegetables, but there has been little study of their absorption. Lutein esters and the free form have been shown to be equally bioavailable (Chung, Rasmussen, Johnson 2004).

‘A’: Amount of carotenoids consumed in a meal

This has been studied in many human experiments, and the consensus is that multiple dosing in a day is the most effective way to raise plasma levels.

‘M’: Matrix in which the carotenoid is incorporated

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

‘E’: Effectors of absorption and bioconversion

Dietary components influence absorption. A minimum amount of fat is required for adequate micelle formation. This appears to be sufficient for optimal absorption of α-carotene, β-carotene and vitamin E, but lutein esters require higher amounts of fat (Roodenburg, Leenen, van het Hof et al. 2000). Using stable-isotope-dilution methodology, Ribaya-Mercado, Maramag, Tengco et al. (2007) fed provitamin carotenoid-rich meals with differing amounts (7, 15, or 29 g fat/day) to groups of more than thirty school children in the Philippines. Minimal fat intake enhanced the vitamin A body pool size as much as did the higher fat levels.

Bioavailability of carotenoids is higher if ingested with food than without, or with a low amount of fat (Dimitrov, Meyer, Ullrey et al. 1988). Adequate protein and zinc intake assists in the maintenance of vitamin A status. Vitamin E, as an antioxidant, protects vitamin A from being oxidized. Fiber, chlorophyll and non-provitamin carotenoids, which are commonly present in the diet, tend to reduce bioavailability. There is evidence that alcohol ingestion interferes with the conversion of β-carotene to vitamin A. Dietary
intake of vitamin A itself has been shown to influence absorption and retinol equivalence of \( \beta \)-carotene in humans (Lemke, Dueker, Follet et al. 2003).

‘N’: Nutrient status of the host

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

‘G’: Genetic factors

As clinical genetics advances it is likely that there will be further reports of cases of rare genetic defects as the cause of vitamin A deficiency. To date, three types of defect are known: enzymatic failure to cleave \( \beta \)-carotene in the small intestinal mucosa (McLaren, Zekian 1971), heterozygotic reduction of plasma RBP (Matsuo, Matsuo, Shiraga et al. 1988), and mutations in the gene for retinol-binding protein (Biesalski, Frank, Beck et al. 1999); (see also Chapter 14).

‘H’: Host-related factors

The serum response to \( \beta \)-carotene is higher in women than in men. In several ways, men are more susceptible to VAD than women. Age does not appear to be a factor. Diseases that interfere with intestinal absorption, especially of lipids, are likely to impair carotenoid bioconversion. In terms of public health, especially in developing countries, intestinal parasites such as \textit{Ascaris lumbricoides} and \textit{Giardia lamblia} are of great importance (see Chapter 13).

‘I’: Mathematical interactions

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

In recent years, further contributions have been made to our understanding of the complexities involved in the bioavailability of carotenoids. In particular, the important effects the food matrix has on carotenoid absorption have been detailed by Schwartz (2004). In summary, the type of matrix, the physical state of carotenoids, effects of processing, co-consumption of fat, carotenoid interactions, and structural considerations of the molecules all have profound effects on absorption.

To date, one of the most revealing studies in this regard is that of Tang, Qin, Dolnikowski et al. (2005). By feeding intrinsically deuterated spinach or carrots to volunteers, it was shown that the average provitamin A carotenoid to vitamin A conversion efficiency is 14.8:1 by weight from carrot \( \beta \)-carotene, and 20.9:1 from spinach. These values are much less than those used previously, although significant amounts of vitamin A can be provided from these sources. The great importance of the nature of the matrix was confirmed, and the difference between the values of spinach and carrots is probably explained by the difference in the food matrix. \( \beta \)-carotene is contained in spinach leaves in the form of pigment proteins located in chloroplasts. In carrots, it is in the form of crystals in chromoplasts and more readily released in the digestive tract.

At the XXII International Vitamin A Consultative Group (IVACG) Meeting, Wongsiriroj, Paik and Blaner (2005) presented evidence for a new hypothesis to explain how tissues regulate vitamin A formation in response to need. They have found that the presence of \textit{apo}-cellular retinol-binding protein 1 (\textit{apo}-CRBP-I) strongly stimulates the conversion of \( \beta \)-carotene to vitamin A. The presence of \textit{apo}-CRBP-I in tissues such as
liver, lung, testis, eye and kidney indicates that the tissues have insufficient vitamin A. This has the effect of stimulating the formation of vitamin A to meet normal cellular and tissue functions. Thus tissues can use CRBP-I as a sensor to assess vitamin A status.

OTHER TECHNIQUES

*In-vitro* techniques are also being introduced. A relationship has been shown to exist between the β-carotene content of a diet and the β-carotene content of exfoliated colonic epithelial cells obtained from a subject consuming that diet (Gireesh, Nair, Sudhakaran 2004). A similar technique using the Caco-2 cell line from human intestine is being extensively applied (Garrett, Failla, Sarama 1999; Liu, Glahn, Liu 2004). Simple *in-vitro*, rapid, cost-effective screening techniques like these may contribute important information in a field where much remains to be discovered.

CONVERSION FACTORS

In response to new studies of bioavailability and bioconversion of carotenoids the US Institute of Medicine made new recommendations (2001) (see also Chapter 4). The factors for β-carotene (1:12) and other provitamin A carotenoids (1:24) are both half of previous values. They are both equivalent to 1 μg of all-trans retinol, and all are equivalent to 1 μg of the new unit that has replaced the retinol equivalent (RE), called Retinol Activity Equivalent (RAE) (*Table 5.1*). West, Eilander, and van Lieshout (2002) were of the view that these estimates might be overoptimistic. From their work, the bioefficacy for dark green leafy vegetables was about 1:26 or 1:28. For fruit the bioefficacy was 1:12. This suggests that, with a mixture of vegetables and fruits in a ratio of 4:1, typical for both developing and developed countries, the bioefficacy of β-carotene from a mixed diet would be only 1:21. Attention is now being focused on processing and other methods that may considerably improve bioefficacy, the term that is tending to replace bioavailability.

**Table 5.1:** Vitamin A conversion factors.

<table>
<thead>
<tr>
<th>1 μg retinol =</th>
<th>0.00349 μmol retinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.15 μg retinyl acetate</td>
<td></td>
</tr>
<tr>
<td>1.83 μg retinyl palmitate</td>
<td></td>
</tr>
<tr>
<td>3.33 IU (1 IU = 0.3 μg)</td>
<td></td>
</tr>
<tr>
<td>1 Retinol Activity Equivalent (RAE)</td>
<td></td>
</tr>
<tr>
<td>1 Retinol Equivalent</td>
<td></td>
</tr>
</tbody>
</table>

In animal experiments (Deming, Baker, Erdman 2002) it has been shown that the presence of cis-isomers of β-carotene in a diet may affect the effectiveness of β-carotene as a vitamin A precursor.
6 Vitamin A in Health

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

DIGESTION AND ABSORPTION

Dietary preformed vitamin A and carotenoids are released from protein in the stomach by proteolysis. They then aggregate with lipids and pass into the upper part of the small intestine. Dietary fat and protein and their hydrolytic products stimulate, through the secretion of the hormone cholecystokinin, the secretion of bile. This emulsifies lipids and promotes the formation of micelles which have lipophilic groups on their inside and hydrophilic groups on their outside. This facilitates the absorption of fat. Bile salts stimulate pancreatic lipase and other esterases that hydrolyze retinyl esters in intestinal mucosal cells (enterocytes). Retinol, the product of the hydrolysis, is well absorbed (70–90%) by intestinal mucosa cells.

A proportion of provitamin A carotenoids in the diet passes into the intestinal mucosal cells. A fraction of these, along with non-provitamin A carotenoids, passes through unchanged into the lymph and blood. The remainder of the provitamin A carotenoids undergoes cleavage with the formation of vitamin A. The key enzyme in this process, carotenoid 15,15'-monooxygenase (CMO1) was cloned independently by two research groups (von Lintig, Vogt 2000; and Wyss, Wirtz, Woggon et al. 2001). This process can also take place within the liver and some other tissues (Goodman, Huang, Shiratori 1966). Symmetrical cleavage of the β-carotene molecule yields two molecules of retinal, which is mostly reduced and esterified to retinyl ester. Some cleavage is asymmetrical and less retinal is produced (von Lintig, Voigt 2004; see Figure 6.1). In practice, β-carotene and other provitamin A carotenoids have only a fraction of the activity of retinol, as was pointed out earlier (see Chapter 3).

There is evidence from its structural and biochemical properties that CMO1 belongs to an ancient family of carotenoid-modifying enzymes.

Figure 6.1: Carotenoid cleavage enzymes in E. coli. (Adapted from von Lintig and Vogt 2004).
first described in plants (Giuliano, Al-Babili, von Lintig 2003).

Within the mucosal cells retinol is esterified before incorporation into chylomicrons (see Figure 6.2). In this process a specific cellular retinol-binding protein (CRBPII) carries the lipid-soluble retinol through the aqueous media and delivers it to the enzyme lecithin:retinol acyltransferase (LRAT) (Ong, Kakkad, MacDonald 1987). This enzyme appears to be the main intestinal enzyme that normally esterifies retinol and then delivers it to the chylomicrons. In mice lacking LRAT, only trace amounts of retinyl ester are stored in liver, lung and kidney tissues, but adipose stores are greatly elevated (O’Byrne, Wongsriroj, Libien et al. 2005).

In terms of intake of vitamin A in the diet, about 10% is not absorbed, 20% appears in the feces through the bile, 17% is excreted in the urine, 3% is released as CO₂ and 50% is stored in the liver (Olson 1994).

**TRANSPORT TO THE LIVER**

Chylomicrons consist of aggregates of thousands of molecules of triglycerides and phospholipids with fat-soluble vitamins, including retinyl esters and carotenoids, cholesterol esters and some apolipoproteins (see Figure 6.3).

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

---

**Figure 6.2:** Absorption and metabolism of carotenoids.
METABOLISM IN THE LIVER

In the liver (see Figure 6.4), most vitamin A from chylomicron remnants in the form of retinyl esters is taken up by hepatic parenchymal cells (hepatocytes). There the esters are hydrolyzed and after processing in endosomes retinol is transferred to the endoplasmic reticulum. There it binds to retinol-binding protein (RBP) and after the complex enters the Golgi complex it is secreted from the cell.

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

Most of the retinyl ester taken up by hepatocytes from chylomicron remnants is transferred as retinol attached to RBP to another type of liver cell called stellate cells (Wake 1994). Storage of vitamin A as retinyl esters seems to be one of the main functions of this type of liver cell. They also produce collagen type III, which when excessive may contribute to a form of cirrhosis of the liver (see also Chapter 13). 50–80% of vitamin A in the body is in the liver. 90–95% of this is in the stellate cells and 98% of this is in the form of retinyl esters, mostly as palmitate. This store is normally sufficient to last for several months. Bound to RBP, retinol is released from both stellate and parenchymal cells. When liver reserves of vitamin A are low parenchymal cells are the major site of storage (Batres, Olson 1987). Some parenchymal cells contain much more vitamin A than others. These are respectively termed “heavy” or “light” according to their density (Batres, Olson 1987a).

Cells identical to the liver stellate cells also occur in many other tissues of the body, including the lung, intestine and kidney (Nagy, Holven, Roos et al. 1997). It has been shown that adipose tissue is an important storage site for retinol, as well as for β-carotene (Wei, Lai, Patel et al. 1997). About 15–20% of the rat’s store of retinol is in adipocytes. This comes from chylomicron-bound esters, and not from circulating RBP as is the case of that in the liver. Furthermore, free retinol is secreted rather than retinol bound to RBP. The secretion of vitamin A from the liver is a complicated process and by tracer kinetics in animal studies several pools have been identified (Green, Green 1996). Green and Green (2005) have very recently reviewed this topic in relation to the
assessment of vitamin A status in humans. Vitamin A storage and action in the liver is the subject of a recent review by Wongsiriroj and Blaner (2004).

**TRANSPORT BETWEEN CELLS**

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

In well-nourished adults total RBP concentration in plasma is 1.9–2.4 mmol/L (40–50 mg/mL). The value for children is about 60% of that of adults. Protein-energy malnutrition, infections and parasitic infestations lower concentrations of RBP in plasma and in these cases caution must be used in assessing vitamin A status (see Chapter 7).

RBP is a member of the lipocalin superfamily, which includes proteins that bind to other lipid-soluble molecules (Table 6.1). RBP
is synthesized in hepatocytes, but probably also in many other tissues, the knowledge of which is rapidly growing.

Mean values and ranges of serum retinol in healthy subjects as a function of age and sex are shown in Table 6.2. In most age ranges, levels are higher in males.

Retinol is extensively recycled between plasma, liver and other tissues. At present it is thought that most of the irreversible utilization

### Table 6.1: Properties and functions of some selected lipocalins (after Sivaprasadarao, Findlay 1994).

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

* Number of amino acids

### Table 6.2: Serum retinol concentrations (µmol/L) as a function of age and sex in American residents, 1971–74* (Olson 1996).

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

* The 5th and 95th percentile values are given in parentheses. (Derived from Pilch, 1987)
of retinol is a kind of detoxification, and only a small fraction is functional. Small amounts of other retinoids can be detected in plasma, including all-trans retinoic acid.

There are several molecules for the extracellular transport of retinoids and carotenoids (Table 6.3). It should be noted that there are no specific carrier proteins for carotenoids.

Concentrations of carotenoids in plasma (see Figure 6.5) are highly dependent on diet. In the case of provitamin A carotenoids, normal levels in plasma are significantly higher in women than in men (Olmedilla, Granado, Blanco et al. 1994), in contrast to retinol.

**CELLULAR RETINOL-BINDING PROTEINS (CRBP) (LI, NORRIS 1996)**

Some retinol may enter cells by diffusion but the evidence is that most is taken up by membrane RBP receptors which at the present time are being characterized (see Figure 6.6 and Table 6.4). In bovine pigment epithelial cells a multitransmembrane protein, STRA6, has been identified as a

<table>
<thead>
<tr>
<th>Approximate MW (kD)</th>
<th>Main ligand</th>
<th>Suggested function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons and their remnants</td>
<td>20,000–200,000</td>
<td>Retinyl esters and carotenoids</td>
</tr>
<tr>
<td>VLDL and LDL</td>
<td>2,000–20,000</td>
<td>Carotenoids</td>
</tr>
<tr>
<td>RBP</td>
<td>21</td>
<td>Retinol</td>
</tr>
<tr>
<td>IRBP</td>
<td>140</td>
<td>Retinol, retinal</td>
</tr>
<tr>
<td>EBP</td>
<td>20</td>
<td>Retinoic acid</td>
</tr>
</tbody>
</table>

**Table 6.3: Extracellular transport molecules for retinoids and carotenoids (Blomhoff 1994).**

**Figure 6.5: Typical carotenoid pattern in plasma.**
specific membrane receptor for RBP (Kawaguchi, Yu, Honda et al. 2007). Recently, malformations in humans have been reported in association with mutations in STRA6. Carotenoids are mainly carried in plasma by low-density lipoproteins (LDLs) and enter cells bearing the LDL receptor.

Within cells there are many proteins that specifically bind retinoids and direct them to specific enzymes (see Table 6.4). These proteins, like RBP, also belong to a family with other members. In recent years mounting evidence has established key functions for CRBP in retinoid metabolism. It acts as a kind of chaperone in protecting retinol within the cell and interacts with certain retinoid-metabolizing enzymes (Napoli 2000).

**Table 6.4: Intracellular retinoid-binding proteins (Blomhoff 1994).**

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

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

**Figure 6.6: Hypothesis for cellular RBP metabolism.** RBP-retinol may be recognized by a cell surface receptor. Retinol may be transferred to CRBPs either at the cell surface or after internalization into endosomes. CRBP may also deliver normal retinol to newly synthesized RBP in the endoplasmic reticulum (Blomhoff 1994).
GENE FUNCTION AND RETINOIDS

It is known that the functions of vitamin A in different tissues, with the exception of the visual process (see later), are mediated by its acid derivatives (Pfahl, Chytil 1996). All-trans retinoic acid (RA) is noncovalently bound to three nuclear RA receptors (RARα, RARβ, RARγ) in gene transcription regulation. When activated, the nuclear receptors for vitamin A are able to bind to “response elements” in specific genes to increase or decrease the level of expression of the gene. The response elements are nucleotide sequences which in the DNA compose the genes.

Soon afterwards it was shown that 9-cis RA binds and activates three other nuclear receptors: RXRα, RXRβ, RXRγ. RA only binds RAR receptors, whereas 9-cis RA can bind all six nuclear receptors, but from recent work it seems that it is not the endogenous ligand for RXR (Wolf 2006). The expression of these six receptors varies between cells, but most if not all cells express at least one of these receptors. Several hundred genes have so far been shown to be induced or repressed by retinoids. Table 6.5 gives a representative listing of vitamin A responsive genes thought to be activated through the action of RARs and/or RXRs.

Table 6.5: Partial and representative listing of vitamin A responsive genes thought to be regulated through the action of RARs and/or RXRs (Blaner 1998).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene function/role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytocin</td>
<td>Reproduction</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Growth</td>
</tr>
<tr>
<td>Phosphoenol pyruvate carboxykinase</td>
<td>Gluconeogenesis</td>
</tr>
<tr>
<td>Class I alcohol dehydrogenase</td>
<td>Alcohol oxidation</td>
</tr>
<tr>
<td>Transglutaminase</td>
<td>Cell growth and cell death</td>
</tr>
<tr>
<td>Laminin B1</td>
<td>Cellular interactions</td>
</tr>
<tr>
<td>Matrix Gla Protein</td>
<td>Bone growth and strength</td>
</tr>
<tr>
<td>Keratin</td>
<td>Skin</td>
</tr>
<tr>
<td>Cellular retinol-binding protein type I</td>
<td>Vitamin A metabolism</td>
</tr>
<tr>
<td>RARβ</td>
<td>Vitamin A action</td>
</tr>
<tr>
<td>Hox 1.6</td>
<td>Fetal development</td>
</tr>
<tr>
<td>Dopamine D2 receptor</td>
<td>Central nervous system</td>
</tr>
</tbody>
</table>
between 100 and 1000 times smaller. Even so, it is RA that is responsible for the vast majority of vitamin A actions in the body. Most of the RA has to come from retinol and its esters by way of oxidation to retinal. It is proposed that enzymes in two families are of key importance in this process; the SDR (short-chain dehydrogenase/reductase) family, and the ADH (medium-chain alcohol dehydrogenase) family. The second and final step of retinal oxidation to RA is carried out by several retinal dehydrogenases (RALDH). This subject has recently been reviewed (O’Byrne, Blaner 2005).

Cytochrome P450 (CYP) enzymes constitute a large superfamily with hundreds of members. They catalyze the oxidative metabolism of many endogenous compounds as well as numerous foreign chemicals. It has been proposed that P450s regulate steady-state levels of ligands for the nuclear hormone receptor superfamily which includes retinoids (Sonneveld, van der Saag 1998). RA is metabolized through oxidation to more polar metabolites. Recently, a novel cytochrome P450 enzyme (CYP26) with specific RA 4-hydroxylase activity has been cloned from man and some animal species. It fulfils all the requirements of an enzyme which could control levels of active retinoids in cells and target tissues. CYP26 may play a role in embryonic development (see below and Chapter 14) and in cancer (see Chapter 14). The great advances made in the understanding of the hormonal control retinoids exert over gene function have highly significant implications for the management and treatment of many diseases that occur throughout the world (see Chapter 14).

### FUNCTIONS OF VITAMIN A

In recent years knowledge of the functions of vitamin A has increased greatly. Table 6.7 indicates the major functions of vitamin A as far as man is concerned. In view of the fact that nuclear receptors for retinoids have been identified in virtually every type of cell it could be argued that in some way vitamin A plays a part in every bodily process. For our present purposes we concentrate upon those included in Table 6.7.

#### Vision

In recent years, many details of the story of the role of vitamin A and its metabolites in photo-

| Table 6.6: Partial listing of members of the “Steroid-Thyroid-Retinoid Superfamily” of nuclear receptors and their activators (Blaner 1998). |
|---|---|
| Nuclear receptor | Activating substance |
| Retinoid X receptor (RXR) | 9-cis retinoic acid |
| Retinoic acid receptor (RAR) | all-trans retinoic acid |
| Vitamin D receptor (VDR) | 1,25 dihydroxy vitamin D |
| Thyroid hormone receptor (TR) | Triiodo-thyronine (T3) |
| Estrogen receptor (ER) | Estrogen |
| Progesterone receptor (PR) | Progesterone |
| Glucocorticoid receptor (GR) | Cortisol |
| Mineralocorticoid receptor (MR) | Aldosterone |
| Androgen receptor (AR) | Testosterone |
| Lipid X receptor (LXR) | Cholesterol metabolite(s) |
| Peroxisome proliferator-activated receptor (PPAR) | Putative fatty acid metabolite |
reception have been filled in. The main aspects of the functions of these compounds in different aspects of vision have been described and illustrated in Chapter 2 and need not be repeated here.

It has recently been shown (Wolf 2004) that the visual cycle in cones differs significantly from that in rods. The main difference is in the mechanism of isomerization of all-trans-retinol to 11-cis-retinol, and the latter’s oxidation to 11-cis-retinal. When light strikes the retina a series of complex biochemical changes takes place, resulting in the generation of a nerve impulse. The initial event in visual excitation is the isomerization of 11-cis retinal to the all-trans form (see Figure 2.10).

Vitamin A, in the form of all-trans retinol, is delivered by the blood to the retinal pigment epithelium (RPE) where it is either esterified for storage, or isomerized to 11-cis retinol, which is then oxidized to 11-cis retinal. This is transported to the photoreceptor rod or cone cells where it combines with the protein opsin to form rhodopsin or iodopsin, respectively, which are light sensitive. On exposure to light, 11-cis retinal is isomerized back to all-trans retinal and the nerve impulse is generated. On release from the protein all-trans retinal is reduced to all-trans retinol and is transported back to the RPE to complete the cycle. As in the liver, lecithin:retinol acyltransferase (LRAT) is essential for accumulation of all-trans-retinyl esters in the RPE (Batten, Imanishi, Maeda et al. 2004).

The latest scheme of the visual cycle is illustrated in Figure 6.7 (Pepperburg, Crouch 2001). This shows in outline a new, recently elucidated, visual cycle by which a protein found in the RPE called retinal G-protein-coupled receptor (RGR) can supplement during intense illumination the 11-cis retinal required to regenerate rhodopsin. In addition, it has been shown that the protein RPE65 performs a key role in the trans-cis isomerization of retinol. The palmitoylation of RPE65 switches off the visual cycle in darkness and switches it on in the light (Wolf 2005).

The space between the RPE and the layer of photoreceptor cells is taken up mostly by a large glycoprotein called the interphotoreceptor retinoid-binding protein (IRBP), the function of which is to transport two different molecular species of vitamin A to and from two different cell types, as outlined above. Chen and Noy (1994) have proposed mechanisms to account for this process. Another retinoid-binding protein is also unique to the eye. It is cellular retinaldehyde (retinal)-binding protein (CRALBP). It binds closely to 11-cis retinal and to 11-cis retinol, and protects the former from photoisomerization.

It has recently been shown that β-carotene may be converted to retinol in the RPE, thus providing an alternative pathway of supply of retinol (Reddy, Nohr, Schaeffer et al. 2005).

In the developing embryo RA is highly concentrated in certain tissues (Dräger, Wagner, McCaffery 1998). These patterns are generated

---

### Table 6.7: An outline of functions of vitamin A.

<table>
<thead>
<tr>
<th>Vision</th>
<th>Photopic and color, scotopic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular differentiation morphogenesis</td>
<td>Gene transcription</td>
</tr>
<tr>
<td>Immune response</td>
<td>Non-specific, cell metabolism</td>
</tr>
<tr>
<td>Hemopoiesis</td>
<td>Iron metabolism</td>
</tr>
<tr>
<td>Growth</td>
<td>Skeletal</td>
</tr>
<tr>
<td>Fertility</td>
<td>Male and female</td>
</tr>
<tr>
<td>Thyroid metabolism</td>
<td>Regulation of TSH secretion</td>
</tr>
</tbody>
</table>
by the local expression of RA-synthesizing aldehyde dehydrogenase enzymes. The eye, especially vulnerable to vitamin A deficiency, is one of the RA-richest regions in the embryo. In the mature eye the pattern of these enzymes is stable, but the amount of RA synthesized is variable and is dependent on ambient light levels. This results from changing levels of the RA precursor retinal, which is released from illuminated rhodopsin. Thus this is a mechanism whereby light can directly influence gene expression. This phenomenon of light-induced release of retinal from rhodopsin occurs only in vertebrate, photoreceptors. It might be speculated that this may have accelerated the rapid evolution of RA-mediated transcriptional regulation, at the transition from invertebrates to vertebrates. It may also explain the prominent role of RA in the eye. Figure 6.8 shows a scheme of vitamin A usage illustrating just how close the links between its role in vision and in transcriptional control are.
Cellular differentiation

Retinoic acid can combine with the nuclear retinoic acid receptor leading to growth inhibition as in some tumors. RA can also bind to the orphan nuclear receptor peroxisome proliferator-activated receptor β/δ (PPAR-β/δ). This results in stimulation of cell growth and inhibition of apoptosis. To bind to RAR, RA is carried into the nucleus by the cytosolic RA-BP-II. To bind to PPAR-β/δ, it is transported into the nucleus by the cytosolic fatty-acid-binding protein 5 (Wolf 2008).

In vitamin A deficiency keratin-producing cells replace mucus-secreting cells in many epithelial tissues of the body. This is the basis of the pathological process termed xerosis that leads to the drying of the conjunctiva and cornea of the eye (see Chapter 8). The process can be reversed by vitamin A. It has become clear recently that vitamin A, mainly in the form of RA, plays a key, hormone-like role in cell differentiation throughout the tissues and organs of the body (Figure 6.9).

Glycoprotein and glycosaminoglycan synthesis

Glycoproteins are polypeptides with short chains of carbohydrates. Glycosaminoglycans are related compounds that are cell surface molecules. Retinoids have been shown to control the expression of enzymes involved in the synthesis of some of these compounds (Vahlquist 1994). Impairment of this function by VAD may contribute to lack of mucin secretion and liquefaction of the cornea seen in xerophthalmia (see Chapter 8).

Embryogenesis

Severe vitamin A deficiency on the one hand, and excessive dosing with vitamin A and also RA on the other, result in malformations of the embryo affecting most organs of the body in many vertebrate species. The embryo is only sensitive to teratogenic influences, that is to say those that induce congenital malformations, during a rela-
tively short period after conception. After that period, known as the organogenetic period, has passed, usually the first three months of fetal life in man, there is no longer any risk in this regard to the fetus (Gerster 1997; Wiegand, Hartmann, Hummler 1998; Ward, MacGowan, Hornby et al. 2000). To date it has not been conclusively demonstrated that human VAD causes congenital malformations. A number of individual case reports have been published but the association might well be coincidental and not causal. Although some of the synthetic retinoids are clearly teratogenic in man, there is very much less evidence for a similar effect of large doses of retinol (see Chapter 14). Some other aspects of this topic are considered later in relation to the safe use of vitamin A, especially in the form of all-trans-retinoic acid via specific nuclear transcription factors, the retinoid receptors, may be appreciated by the following sampling of some of the recent experimental contributions in this field.

Zile (2004) and colleagues have concentrated on cardiovascular development in the early avian embryo during neurulation, corresponding to the first 2–3 weeks of human pregnancy. Several developmental genes, linked to those events, are regulated by vitamin A. They include homeobox genes HoxB-1 and Msx-1; the cardiogenic transcription factors GATA-4 and GATA-6 and the heart asymmetry genes nodal, snail and Pitx-2. RARα 2, RARγ and RXRα have

Recent animal work (Quadro, Hamberger, Gottesman et al. 2005) has shown that retinol-RBP is the primary contributor to fetal development, whereas retinyl esters are mainly responsible for accumulation of fetal retinoid stores.

An indication of the fundamental importance of vitamin A, especially in the form of all-trans-retinoic acid via specific nuclear transcription factors, the retinoid receptors, may be appreciated by the following sampling of some of the recent experimental contributions in this field.

Zile (2004) and colleagues have concentrated on cardiovascular development in the early avian embryo during neurulation, corresponding to the first 2–3 weeks of human pregnancy. Several developmental genes, linked to those events, are regulated by vitamin A. They include homeobox genes HoxB-1 and Msx-1; the cardiogenic transcription factors GATA-4 and GATA-6 and the heart asymmetry genes nodal, snail and Pitx-2. RARα 2, RARγ and RXRα have

**Figure 6.9:** Suggested roles of CRBPs and CRABPs. See text for details (Blomhoff 1994).
been identified as the retinoid receptors essential for transducing the RA signal during the critical RA-requiring developmental window. One study (LaRue, Argraves, Zile et al. 2004) demonstrated that vascular abnormalities observed under conditions of retinol deficiency are due to a reduction in the number of angioblasts. This leads to an insufficiency in the number of endothelial cells required to build complex vascular networks.

The addition of retinol in vitro to bovine embryo culture media was shown to have a significant positive effect on early embryonic development (Lima, Oliveira, Goncalves et al. 2004).

The key role played by retinoids in lung development and regeneration (Maden 2004) gives hope that they may provide the first treatment for diseases such as emphysema and chronic obstructive pulmonary disease.

Excessive dietary intake of retinoic acid induces hindlimb and eye deformities in the frog (Alsop, Brown, Van Der Kraak 2004).

During embryogenesis the bilateral symmetry and orderly formation of somites is signaled by retinoic acid (Kawakami, Raya, Raya et al. 2005).

Many other tissues and organs are under active study along similar lines; all provide increasing evidence for the universal importance of the presence of an appropriate concentration of RA in precise areas of the body and at critical times in embryogenesis for normal development to occur.

**Immune response**

Many of the epithelial tissues are important barriers to infection and VAD impairs this function in a nonspecific way. In addition, vitamin A is known to be involved in maintaining immunocompetence in more specific ways. It helps to maintain the lymphocyte pool. Vitamin A also functions in T-cell-mediated responses. RXRα has an essential role in the development of TH2 responses (Du, Tabeta, Mann et al. 2005).

Some aspects of the immune response previously considered unrelated, such as immunoglobulin production, are now known to be affected by retinoids (Semba 1998). It has been suggested (Buck, Ritter, Dannecker et al. 1991) that a third oxidized metabolite of retinol, 14-hydroxy-4,14-retro retinol is the active molecule in the immune system, but how it acts is not known. Recently it has been shown that retinoic acid regulates factors known to be required for Ig class switch recombination and modulates the population dynamics of ligation-stimulated B cells (Chen, Ross 2005).

Mucida, Park, Kim et al. (2007) have shown that RA is a key regulator of TGF-β-dependent immune responses, capable of inhibiting the IL-6-driven induction of pro-inflammatory TH17 cells and promoting anti-inflammatory Treg cell differentiation. Thus RA is capable of regulating the balance between pro-inflammatory and anti-inflammatory immunity (see Table 10.2).

Much of the evidence for the functioning of vitamin A in the immune response comes from studies in both experimental animals and human subjects who are vitamin A deficient. This subject is pursued further in Chapters 9 and 10.

**Thyroid function**

Thyroid-stimulating hormone (TSH) of the anterior pituitary gland is regulated by the binding of the thyroid hormone activated thyroid receptor to the TSH gene. At the same time, the binding of the 9-cis-retinoic acid activated retinoid X receptor to the same gene occurs. Both interactions, separately or simultaneously, can suppress and thus regulate the expression of the TSH gene (Wolf 2002).

**Reproduction**

It used to be thought that vitamin A in the form of retinoic acid could not prevent signs of vitamin A deficiency in the organs of the reproductive system, as is the case in the eye. This area remains under-researched, but certain aspects
have become clearer in recent years (Ross 2005).

In males, failure of gametogenesis, reduced testosterone production, and loss of fertility occur in the presence of vitamin A deficiency. All three main types of testicular cells, Sertoli, germinal and Leydig cells, undergo morphologic changes in vitamin A deficiency. A recent study in mouse testis suggests that spermiation requires RXRβ/RARα heterodimers in Sertoli cells, whereas spermatogonia proliferation involves, independently of RXR, two distinct RAR-mediated signaling pathways in both Sertoli cells and spermatogonia (Vernet, Dennefeld, Rochette-Egly et al. 2006).

In females, vitamin A is required for normal estrus cycle and implantation of the blastocyst after conception. The role of retinoic acid in regulating gene expression and morphogenesis in the developing embryo was covered earlier in this chapter.

In the human, there is evidence (see Chapter 9; West, Katz, Khatry et al. 1999) that suggests that vitamin A or β-carotene supplementation during pregnancy has a beneficial effect on certain aspects of gestation. The mechanism(s) is not understood at present.

**Hemopoiesis**

VAD in man and in experimental animals is consistently associated with an iron deficiency type of anemia. It has been repeatedly shown that in these circumstances, in addition to iron, vitamin A is required for a full hematologic response. The mechanism remains unclear. VAD might interfere with the absorption, transport or storage of iron. On the other hand, it might act directly on hemopoiesis, although that seems less likely (Sommer, West 1996:150–162). The demonstration that RA is necessary for erythrocyte differentiation suggests that it may control the usage of iron (Pfahl, Chytil 1996; see also Chapter 11).

**Growth**

Several aspects of this subject have received further attention in recent years. It is probable that there are many factors responsible for growth in early life; it is likely that many have not been discovered yet. In addition, the long-term and large-scale studies that are necessary to provide definite answers have not yet been undertaken. In most instances the aspect of growth that has been chosen for study has been height, rather than weight or some other parameter. Height, compared with weight, has the advantage that it is short-term less readily influenced by such factors as infectious diseases. On the other hand, it is subject to much less variation so alterations are much more difficult to recognize.

Deficiency of certain nutrients, including vitamin A, are likely to be partly responsible for the widespread occurrence throughout developing countries of height retardation or “stunting”. Another important factor seems to be the effect of seasonal variation. This probably results from a combination of low nutrient intake and occurrence of outbreaks of some infectious diseases at the particular seasons when growth retardation is maximal. Several recent studies illustrate the difficulty and magnitude of the problem.

Retinoic acid is known to play its hormone-like function in the control of growth and development of tissues in the musculo-skeletal system, just as it does elsewhere. In communities subject to widespread VAD, the occurrence of many other adverse factors has made the demonstration of the retarding effect of VAD difficult (Sommer, West 1996:163–188; see Chapter 10). One possible mechanism for the influence on growth is the recent demonstration that both vitamin A and retinoic acid produce rapid release of cyclic AMP (adenosine monophosphate) and human growth hormone secretion (Djakoure, Guibourdeuche, Porquet et al. 1996).
Other functions

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

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

Gap junctional communication
Gap junctions are narrow, hydrophilic pores connecting the cytosol of two adjacent cells. There is evidence that gap junctions play a role in regulation of morphogenesis, cell differentiation, secretion of hormones, and growth. Effects on gap junctional communication might be involved in carcinogenesis and teratogenesis (Hix, Vine, Lockwood et al. 2005). RA and its analogues exert their effect by acting as ligands of nuclear receptors RAR or RXR (see earlier and Stahl, Sies 1998). It has also been shown that carotenoids and other micronutrients, as well as their metabolites and cleavage products, are also involved (Stahl, Sies 2005).

Signal transduction
Evidence for a cytoplasmic role for retinoids as an effector in signal transduction has been reviewed recently (Hoyos, Hammerling 2005). Oxygen is among a group of inorganic gases, including also ROS (reactive oxygen species), with intracellular messenger properties. The role of retinol can be seen as a device for the efficient and accurate channeling of ROS to the zinc-finger domains of serine/threonine kinases involved in signal transduction.

Mitochondria and apoptosis
Recent in-vitro studies have shown that various synthetic retinoids appear to target mitochondria to trigger apoptosis in transformed cells. This may be important for their anticancer potential (Hail, Lotan 2005).

ACTIVITIES OF CAROTENOIDS
Olson (1999) has classified the many activities of carotenoids into functions, actions, or associations, but these divisions are not strict and rigid. In Table 6.8 an attempt is made to bring them together in outline form. The underlying mechanisms of some of these activities of carotenoids have been discussed in Chapter 5.

Functions usually denote a more or less clearly defined purpose. The conversion of provitamin A carotenoids to vitamin A in the animal body is the most evident one. Others, as indicated in Table 6.8, are less well understood in chemical terms. Actions, both biological and chemical, are readily demonstrated but their consequences are not so clear. Associations of carotenoids are mostly known from epidemiological studies and usually have their importance in relation to occurrence of diseases (see Chapter 14).

Evidence suggests that carotenoid cleavage products may increase the risk of cancer development by impairing mitochondrial function. Depletion of mitochondrial sulfhydryl groups and impaired oxidative phosphorylation may induce oxidative damage in DNA molecules and nuclei.
(Siems, Wiswedel, Alija et al. 2005). On the other hand it has been shown (Zhao, Aldini, Johnson et al. 2006) that carotenoid supplementation by mouth, with a combination that includes lutein, \( \beta \)-carotene and lycopene, can exert protection against DNA damage.

Laboratory studies are contributing to our understanding of the antioxidant activity of carotenoids in terms of quenching of singlet oxygen, as mentioned in Table 6.8. Very recently it has been shown that carotenoids normally present in human serum are capable of inhibiting cellular proliferation (McDevitt, Tchao, Harrison et al. 2005).

The biological activity of carotenoid metabolites has recently been reviewed (Krinsky 2005). It has to be appreciated that nutrients, such as carotenoids, are by no means the only dietary constituents that are capable of preventing cellular oxidative damage. Many studies have shown that flavonoid components of fruit and vegetables, such as anthocyanins and flavonols, also have this ability (Prior 2003).

### HUMAN REQUIREMENTS

Along with those of other nutrients, the requirements for vitamin A for humans of either sex, of different ages and in various physiological states, such as pregnancy and lactation, are kept under regular review. As mentioned earlier (see Chapters 4 and 5) it is now customary to express the vitamin A activity of a diet in terms of retinol activity equivalents (RAE) to take account of the different activities in the body of retinol and the provitamin carotenoids which have recently been altered. Table 6.9 gives criteria and dietary reference intake values for vitamin A for children and adults.

Tables like these are designed to indicate how deficiency may be prevented and how a safe intake for the large majority of a population (covering about ±2 SDs from the mean) may be provided. They were originally meant to ensure that, if met, deficiency would not occur, although a considerable proportion of a population might consume more than they needed to.

However, evidence has accumulated in recent years for a protective effect against some chronic diseases of increased amounts of antioxidant nutrients, such as the carotenoids (see above and Chapter 14). These possible beneficial effects have not previously been taken into consideration when recommended dietary allowances (RDAs) have been formulated. The usual advice being given is to increase the intake of fresh fruits and vegetables.

---

#### Table 6.8: Functions and actions of carotenoids.

<table>
<thead>
<tr>
<th>Functions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Some carotenoids are the only known precursors of vitamin A and its derivatives</td>
<td></td>
</tr>
<tr>
<td>• Accessory pigments in energy transfer in photosynthesis</td>
<td></td>
</tr>
<tr>
<td>• Phototropism in simple and higher plant forms</td>
<td></td>
</tr>
<tr>
<td>• Photoprotective role in bacteria and also man</td>
<td></td>
</tr>
<tr>
<td>• Plant growth regulation</td>
<td></td>
</tr>
<tr>
<td>• Reproduction regulation in fungi</td>
<td></td>
</tr>
<tr>
<td>Actions</td>
<td></td>
</tr>
<tr>
<td>• Quenching of singlet oxygen</td>
<td></td>
</tr>
<tr>
<td>• Color attractants in flowers for insect pollination</td>
<td></td>
</tr>
<tr>
<td>• Coloration of food for mankind</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.9: Criteria and dietary reference intake values for vitamin A by life-stage group.

<table>
<thead>
<tr>
<th>Life-stage group</th>
<th>Criterion</th>
<th>EAR* (μg RAE/d)</th>
<th>RDA* (μg RAE/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>0–6 mo</td>
<td>Average vitamin A intake from human milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–12 mo</td>
<td>Extrapolation from 0–6 mo AI**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–3 y</td>
<td>Extrapolation from adult EAR</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>4–8 y</td>
<td>Extrapolation from adult EAR</td>
<td>275</td>
<td>275</td>
</tr>
<tr>
<td>9–13 y</td>
<td>Extrapolation from adult EAR</td>
<td>445</td>
<td>420</td>
</tr>
<tr>
<td>14–18 y</td>
<td>Extrapolation from adult EAR</td>
<td>630</td>
<td>485</td>
</tr>
<tr>
<td>&gt;18 y</td>
<td>Adequate liver vitamin A stores</td>
<td>625</td>
<td>500</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–18 y</td>
<td>Adolescent female EAR plus estimated daily accumulation by fetus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19–50 y</td>
<td>Adult female EAR plus estimated daily accumulation by fetus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–18 y</td>
<td>Adolescent female EAR plus average amount of vitamin A secreted in human milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19–50 y</td>
<td>Adolescent female EAR plus average amount of vitamin A secreted in human milk</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Estimated average requirement (EAR) and recommended daily allowance (RDA) according to the Food and Nutrition Board (FNB)
** Adequate intakes (AI) according to FNB
INTRODUCTION

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

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

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

Nutritional status always concerns one particular aspect of the state of the body and consequently there are different types of test possible for the assessment of status. Clinical signs in the eye, several aspects of eye function, histological changes in the conjunctiva, serum level of retinol, and indirect assessment of liver reserve or body store are most commonly used in the case of vitamin A.

The use of somatic measurements, such as weight and height, is the mainstay in the detection of protein-energy malnutrition under field conditions. Although there is evidence that vitamin A deficiency does have an effect on growth (see Chapter 10), there are many other nutritional factors and infections which act similarly. There is no aspect of growth retardation which can be linked specifically to vitamin A deficiency. In fact, to describe a community as “underweight” or “stunted” contributes no understanding of the nature of the underlying causes.

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

Several years later the first national point prevalence survey of vitamin A deficiency was carried out in Indonesia (Sommer 1982). A second WHO Expert Group on the Control of Vitamin A Deficiency and Xerophthalmia (WHO Expert Group 1982) was largely influenced by this study to make several alterations in the classification and in the public health criteria (Tables 7.1 and 7.2).

The criteria shown in Table 7.2 were widely adopted and used successfully over the past 30 years or so. However, as the more severe eye lesions and very low serum retinol levels gradually became less common, changes were advocated (Sommer and Davidson 2002). These are shown
in Table 7.3. Table 7.3 has several weaknesses and does not appear to have come into general use.

### Table 7.1: Xerophthalmia Classification by ocular signs (WHO Expert Group, 1982).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Minimum prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night blindness (XN)</td>
<td>1.0%</td>
</tr>
<tr>
<td>Conjunctival xerosis (X1A)</td>
<td>0.5%</td>
</tr>
<tr>
<td>Bitot’s spot (X1B)</td>
<td>0.01%</td>
</tr>
<tr>
<td>Corneal xerosis (X2)</td>
<td>0.05%</td>
</tr>
<tr>
<td>Corneal ulceration/keratomalacia &lt;1/3 of corneal surface (X3A)</td>
<td>0.05%</td>
</tr>
<tr>
<td>Corneal ulceration/keratomalacia ≥1/3 of corneal surface (X3B)</td>
<td>5.0%</td>
</tr>
<tr>
<td>Corneal scar (XS)</td>
<td></td>
</tr>
<tr>
<td>Xerophthalmic fundus (XF)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 7.2: Criteria for assessing the public health significance of xerophthalmia and vitamin A deficiency, based on the prevalence among children less than six years old in the community (WHO Expert Group, 1982).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Minimum prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical (primary)</td>
<td></td>
</tr>
<tr>
<td>Night blindness (XN)</td>
<td>1%</td>
</tr>
<tr>
<td>Bitot’s spot (X1B)</td>
<td>0.5%</td>
</tr>
<tr>
<td>Corneal xerosis and/or ulceration/keratomalacia (X2 + X3A + X3B)</td>
<td>0.01%</td>
</tr>
<tr>
<td>Xerophthalmia related corneal scars (XS)</td>
<td></td>
</tr>
<tr>
<td>Biochemical (supportive)</td>
<td></td>
</tr>
<tr>
<td>Serum retinol (vitamin A) less than 0.35 μmol/L (10 μg/dL)</td>
<td>5%</td>
</tr>
</tbody>
</table>

### Table 7.3: Recently proposed prevalence criteria indicating significant VAD within a defined population.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Minimum prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Plasma retinol 0.7 μmol/L (20 μg/dL)</td>
<td>in &gt;15%</td>
</tr>
<tr>
<td>1Night blindness (XN)</td>
<td>≥5% in women</td>
</tr>
<tr>
<td>2Total xerophthalmia (XN, X1B, X2, X3, XS)</td>
<td>&gt;1%</td>
</tr>
</tbody>
</table>

In addition a simplified approach has been advocated. This is to sum all the eye signs and if the total exceeds 1% a xerophthalmia problem exists. A regrettable consequence of this simplification is the inability to distinguish between blinding and non-blinding xerophthalmia. If this practice is generally applied it will no longer be possible to estimate the role of VAD as a cause of blindness (see also Chapter 12).

At the present time there seems to be a tendency to abolish the division traditionally set between clinical and subclinical deficiency. Although there is no clear-cut threshold, this concept seems to have worked especially well in the case of VAD. “Subclinical” implies that there is no clinical evidence of disease. The subject has no complaints of ill health and the examiner is unable to elicit any physical signs of disease. Some indicators of vitamin A status span this borderline between subclinical and clinical. This is the case both for impairment of retinal rod function and for abnormal bulbar conjunctival histology. Both of these will be discussed in detail later, but it is useful at this point to see how they span the subclinical-clinical divide (see Tables 7.4 and 7.5). These tables show how impairment of function and abnormality of structure, respectively, increase progressively with increasing degree of deficiency.

Although the following chapters deal with the ocular lesions of xerophthalmia, after looking at the subclinical stages of vitamin A deficiency and the tests that have been developed for them it is important to recognize that in historical terms the eye signs were studied first. This is because xerophthalmia, attributable to severe deficiency,
was the first to be recognized as a serious public health problem. It was only with the recognition of xerophthalmia as the most common cause of blindness in young children around the world (see Chapter 12), that all the efforts expended to control it from the 1960s onwards could be justified.

When, in the 1980s, it became evident that subclinical deficiency was associated with a significant increase in young child mortality (see Chapter 9), what might be termed a second wave of concern resulted. This realization provided the impetus for research into reliable methods for the assessment of vitamin A status at the subclinical level.

**INDICATORS AT THE SUBCLINICAL LEVEL**

Nearly thirty years on there is a variety of techniques on offer for assessment of VAD at this level. It will be necessary to explain the varying state of popularity that each enjoys at the present time. In addition, recent research has shown that the whole area of early detection of VAD is influenced by accompanying subclinical and clinical infections or trauma. Assessment of the status of some other micronutrients is also similarly affected.

**Acute phase response (APR)**

In the two previous editions of this Manual the phenomenon known as the acute phase response (APR) was referred to and described in relation to VAD. However, recent years have seen a great deal of research in this area and because of the implications of this research for the assessment of vitamin A status, it deserves to be given prominence at this point. Cross references are provided, especially in Chapters 9, 11, and 13.

The acute phase response is the term given to the phenomenon that occurs in response to inflammation, infection or trauma. Proteins in the circulation fall into two classes with regard to the APR. In direct response to APR some proteins are released by tissues and increase in concentration in the blood. These include C-reactive protein, serum amyloid, α1-antichymotrypsin, and α1-acid-glycoprotein. The release of pro-inflammatory circulating cytokines may be responsible. Other proteins, including many transport proteins, such as retinol-binding protein and consequently retinol, are reduced in concentration. The mechanisms responsible for this fall are not fully understood.

<table>
<thead>
<tr>
<th>Table 7.4: Increasing impairment of retinal rod function, illustrating the subclinical–clinical divide.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tests of retinal rod function response</strong></td>
</tr>
<tr>
<td><strong>Subclinical</strong></td>
</tr>
<tr>
<td>Dark adaptometry</td>
</tr>
<tr>
<td>Vision restoration</td>
</tr>
<tr>
<td>Pupillary contraction</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
</tr>
<tr>
<td>Night blindness</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 7.5: Progressive changes in conjunctiva and cornea, illustrating the subclinical–clinical divide.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test</strong></td>
</tr>
<tr>
<td><strong>Subclinical</strong></td>
</tr>
<tr>
<td>Conjunctival impression cytology</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
</tr>
<tr>
<td>Conjunctival xerosis (X1A)</td>
</tr>
<tr>
<td>Bitot’s spot (X1B)</td>
</tr>
<tr>
<td>Corneal xerosis (X2)</td>
</tr>
<tr>
<td>Keratomalacia (X3)</td>
</tr>
</tbody>
</table>
but leakage into extracellular space and the depression of synthesis in the liver are thought likely causes (Rosales, Ritter, Zolfaghari et al. 1996).

The fall in concentration is usually greatest in acute infections and levels return to normal during recovery (Figure 7.1). It is now clear that even subclinical infection can cause significant falls (Thurnham, McCabe, Northrop-Clewes 2003). Consequently, the fall in serum retinol levels, applying equally to RBP, the ratio of RBP to TTR and RDR and MRDR (see below), is widespread and needs to be taken into account at all times where appropriate. Thurnham, Mburu, Mwaniki et al. (2005) have recently reviewed the importance of the acute phase response for the assessment of micronutrient status in general. Figure 7.1 shows an explanatory scheme, based on field observations, of the effect of various degrees and stages of APR on plasma retinol. David Thurnham (2009) states that there have been problems with the use of antigens for assessment of APR. The most appropriate, AGP (α1-acid glycoprotein), has been in short supply and is relatively expensive. CRP (C-reactive protein) is often misinterpreted by setting the cut-off point at 10 mg/L (mean CRP is <1 mg/L in absence of inflammation). The correction of retinol data has not been taken up widely. A recent field study in Kenya confirmed the conclusions reached in 2003 (Thurnham, Mburu, Mwaniki et al. 2008).

Serum retinol

The level of retinol in serum is under homeostatic control over a wide range of body stores and reflects these stores only when they are very high or very low. Thus an isolated serum retinol value is not an accurate indicator of vitamin A status. Serum retinol is best used when a frequency distribution can provide useful information about the status of a population and about response to an intervention program (Gregory, Collins, Davies et al. 1995) (Figure 7.2).

**Figure 7.1:** Effects of different stages of subclinical infection on plasma retinol. Changes are unidirectional except for in convalescence, during which people might recover or relapse. (Adapted from Thurnham, McCabe, Northrop-Clewes 2003).  

---

**Figure 7.1:** Effects of different stages of subclinical infection on plasma retinol. Changes are unidirectional except for in convalescence, during which people might recover or relapse. (Adapted from Thurnham, McCabe, Northrop-Clewes 2003).
In the past it has usually been considered that a serum retinol level <0.35 μmol/L was “deficient” and a level of <0.70 μmol/L was seen as “low.” The Annecy Accords (Sommer, Davidson 2002) concluded that a serum retinol level of <0.70 μmol/L (20 μg/dL) at a prevalence rate of >15% should be a new prevalence criterion indicating significant VAD within a defined population. The thinking behind this change (from >5% at <0.35 μmol/L) took into account evidence that very low values are difficult to detect reproducibly and reliably in routine laboratory tests. Since the concentration has been doubled, more children will be found to have these higher levels, and in consequence the prevalence rate has been increased automatically from 5% to 15%.

Although the use of serum retinol may appear to be satisfactory in the absence of inflammation and infection (i.e. where the acute phase response is absent, see above and Chapters 9, 11, and 13), as may be assumed to be the case in most developed societies, doubts have to be raised. For example, serum retinol distribution data from residents of the United States taken from the National Health and Nutrition Examination Survey, 1988–1994, have recently been studied (Ballew, Bowman, Sowell 2001). Data from 16,058 participants aged 4–90 years were analyzed. In children serum retinol increased with age, BMI, serum lipids, and use of supplements. Serum retinol <1.05 μmol/L prevalence was 16.7–33.9% in children aged 4–8 years and 3.6–14.2% at 9–13 years. Significantly lower values occurred in non-Hispanic black and Mexican American children and women. In circumstances where low levels of serum retinol are common, it is difficult not to believe that minimal infections are influencing these data through the operation of the acute phase response (Stephensen, Gildengorin 2000). Subsequent analysis of these same data by the same group (Gillespie, Ballew, Bowman et al. 2004) for intra-individual variation in serum retinol caused them to conclude that “the actual population prevalence of inadequate vitamin A status may be 75% lower than the estimates previously reported.” This is an enormous difference and poses a serious challenge to acceptance of the currently recognized standards for serum retinol data.

Figure 7.3 shows additional mean retinol values in UK children surveyed in 1990, 1995 and 2000. As in the US (see Ballew, Bowman, Sowell 2001, above) there was a marked increase with
increasing age. This creates another problem in using serum retinol to assess vitamin A status.

It seems appropriate to raise another related problem at this point. It has been proposed that lutein may serve as a useful surrogate for retinol in plasma (Adelekan-Delana, Northrop-Clewes, Owa et al. 2003). It has been shown that lutein and some other carotenoids behave similarly to retinol in regard to the acute phase response. The problems with serum retinol mentioned above would seem to apply equally to lutein; as we know they do to RBP.

**Retinoids in serum in mothers and newborns**

Soderlund, Fex, and Nilsson-Ehle (2005) undertook the first measurements of the biologically active metabolite all-\textit{trans} retinoic acid and its isomer 13-\textit{cis} retinoic acid in newborns and their mothers, and in women in the first trimester of pregnancy. Newborns had significantly lower (1.0 \textmu mol/L) retinol concentrations than their mothers (1.7 \textmu mol/L). Serum all-\textit{trans} retinoic acid was also lower (3.4 nmol/L) in the newborns than in their mothers (5.8 nmol/L). Serum concentrations of 13-\textit{cis} retinoic acid were significantly lower (2.0 nmol/L) in the newborns than in their mothers (2.6 nmol/L). The serum concentrations of all-\textit{trans} retinoic acid and retinol did not correlate in any group. The authors conclude that retinol concentrations do not accurately reflect the concentrations of the biologically active derivative all-\textit{trans} retinoic acid.

**Serum retinol-binding protein (RBP)**

Several recent studies (Blaner, Piantedosi, Gamble 1999; Donnen, Dramaix, Zihindula et al. 1999; Almekinder, Manda, Kumwenda et al. 1999) have shown that measurement of serum RBP correlates very closely with serum retinol (see Chapter 3).

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

Using the immunodiagnostic kits referred to in Chapter 3, Gamble, Ramakrishnan, Palaox et al. (2001) confirmed the value of RBP as a surrogate for serum retinol. Criticisms of the latter concerning the APR, however, apply equally to RBP. In healthy people serum RBP was found to correlate well with serum retinol \( (r = 0.88) \). This remained so, even in the presence of HIV-1 infection, protein malnutrition, or APR (Baeten, Richardson, Bankson et al. 2004).

**RBP/TTR molar ratio**

This ratio has been introduced in an attempt to assess vitamin A status in the presence of inflammation (Rosales, Ross 1998). In the APR, RBP in plasma falls, as in VAD. On the other hand, transthyretin (TTR), to which retinol is also bound in plasma, falls in the APR but is unaffected by VAD. Studies in experimental animals and children with serious infections have indicated that a low molar ratio of RBP/TTR can distinguish VAD in the presence of infection. Recently, further work was carried out in malaria (Rosales, Topping, Smith et al. 1999). In HIV-1 infection the ratio is distorted, even in the absence of the APR (Baeten, Wener, Bankson et al. 2006).

Several more studies using this technique have been carried out recently. A cut-off value of less than or equal to 0.36 to indicate marginal vitamin A deficiency is recommended for young children by the work of Rosales, Chau, Haskell et al. (2002). For adults, Zago, Dupraz, Sarchi et al. (2002) proposed 0.37, for its high sensitivity, specificity and predictive value.

Recently (Sankaranarayanan, Suarez, Taren et al. 2005), it was shown that in deficient Nepalese women in late pregnancy the RBP:TTR index was a useful proxy for free holo-RBP concentration. Thirty percent were judged to be vitamin A deficient by an index of <0.36.

**Assessment of body reserves**

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

**Indirect assessment of liver stores**

In Chapter 5 it was mentioned that apo-RBP accumulates in the liver when retinol is in short supply. Once retinol becomes available, holo-RBP is released into the circulation. This phenomenon is the basis of the relative dose response tests. The magnitude of the retinol released from the liver when a test dose is given is proportional to the degree of prior depletion of the liver.

**Relative dose response (RDR)**

(Underwood 1990)

Very recently, Fujita, Brindle, Rocha et al (2009) have shown that replacing serum retinol with serum retinol-binding protein potentially allows more accurate assessment of body reserves of vitamin A than the usual employment of serum retinol in the RDR test.

A fasting serum retinol is measured \( (A_0) \). Vitamin A as 450–1000 μg retinyl ester in oily solution is given orally. Five hours after dosing, another serum retinol is measured \( (A_5) \). The RDR is calculated as follows:

\[
RDR = \frac{(A_5-A_0) \times 100}{A_5}
\]

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

**Modified relative dose response (MRDR)**

(Tanumihardjo, Koellner, Olson 1990)

This test employs a metabolite of vitamin A, 3,4-didehydroretinyl acetate (DR, also known as dehydroretinol, vitamin A2). DR binds to RBP and appears in the serum after a test dose is given if
liver reserves of vitamin A are low. A single oral dose of DR is given and only one blood test is required, 4–6 hours after the dose.

Serum concentrations of retinol (SR) and de-hydroretinol (SDR) are measured. The molar ratio is calculated in the following way: MRDR = SDR/SR. An MRDR value is considered to be abnormal when the above ratio is >0.06

Serum 30 day dose response (+S30DR) (Flores, Campos, Aranjo et al. 1984)

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

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

Disadvantages
Blood samples are required. In many populations this may be unacceptable for cultural reasons as well as in view of the risk of transmitting HIV, hepatitis etc. The single sample for MRDR raises less of a problem than the two samples required for the RDR.

Retinol-binding protein is among a number of proteins that take part in the acute phase reaction in infection and inflammation (see above). The release of RBP from the liver is repressed by a number of factors that are likely to be widely prevalent in the communities under study. These include acute and chronic infections and infestations and protein-energy malnutrition. This subject is considered in more detail later (see Chapter 10).

Other than in research projects, the means for estimating serum retinol on a routine basis are not always at hand. The compound DR, used in the MRDR test, is costly and is not readily available (WHO, 1996).

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

Deuterated-retinol-dilution technique

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

Recent work has vindicated Olson’s prediction (above, 1997) in regard to this technique. An oral dose of deuterated retinyl acetate was found to have a mean equilibrium time of 16–17 days, uninfluenced by the size of hepatic reserves (Wasawitsut 2002). Total vitamin A body reserves can be estimated according to principles of isotope dilution, a set of assumptions regarding retention of the labeled dose, the ratio of specific activities of plasma to liver vitamin A, and the irreversible loss of vitamin A over time.

The technique has been used to assess the vitamin A status of school children participating in a national sugar fortification program (Ribaya-Mercado, Solomons, Medrano et al. 2004). It has also been used to monitor dietary vitamin A intakes of elderly subjects of low vitamin A status (Ribaya-
Mercado, Solon, Fermin et al. 2004). In 31 elderly men and 31 elderly women who were marginally nourished it was shown that a sufficient dietary intake of vitamin A was associated with an adequate liver vitamin A concentration of 6.45 μg RAE/kg body weight. For a reference 76 kg man and 61 kg woman these values are approximately 500 and 400 μg RAE/day respectively.

Octa-deuterated and tetra-deuterated vitamin A have been given before and after dietary interventions respectively: in this way the impact on total body vitamin A stores of different dietary sources of provitamin A carotenoids have been studied.

Tang, Qin, Hao et al. (2002) studied whether the usual sample collection time of 11–26 days could be reduced to make field studies easier. They gave an oral dose of deuterium-labeled vitamin A to 58 Chinese children aged 11–12 years. It was found that results using 3 days’ isotope enrichment correlated well with 21 days, whereas 6 hours’ did not.

**Breast milk vitamin A concentration**

It has long been known that the concentration of vitamin A in the breast milk of undernourished mothers is low. The proposal to use this as an indicator of the vitamin A status of a community is relatively new and has been tested under varying conditions. It has the advantages of being non-invasive, readily acceptable and the sample is easy to collect. It is important to follow standardized methods of collection of the sample, and the method of expression of the concentration of vitamin A should be agreed upon. Results to date suggest that the borderline of deficiency in a population be set at <1.05 μmol/L.

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

Plasma and breast milk retinol were measured as indicators of vitamin A status during pregnancy. Acute phase proteins, where infections were present, were also measured. It was concluded that the measurement of retinol in breast milk was superior to the measurement of retinol in serum under these conditions (Semba, Kumwenda, Taha et al. 2000).

It was later shown that breast milk retinol concentrations are not affected by APR (Duncheck, Nussenblatt, Ricks et al. 2005).

**Histological indicator [conjunctival impression cytology (CIC)]**

In Chapter 6 attention was drawn to the major role played by vitamin A in cellular differentiation. The abnormal changes that occur when vitamin A is deficient have been best documented for the conjunctiva and to a lesser extent for the cornea. Basically there is a drying process, xerosis, which, in the conjunctiva, may ultimately lead to the development of a Bitot’s spot (X1B) (see Chapter 8). The earlier change that is visible to the naked eye, called conjunctival xerosis (X1A), is much less distinctive (see Chapter 8). This in turn is preceded by lesser degrees of change at the subclinical level.

With the introduction of the technique of CIC (Wittpenn, Tseng, Sommer 1986) and a modification termed impression cytology with transfer (ICT) (Luzeau, Carlier, Ellrodt et al. 1988) it has become possible to study these very early changes under a microscope. Details of the techniques that have been advocated are given in a training manual (Wittpenn, West, Keenum et al. 1988), in several original papers and also in the WHO 1996 report (WHO, 1996). In essence, a normal impression of conjunctival cells when stained will show one or more sheets of small regular epithelial cells and numerous mucin-secreting goblet
cells. In VAD the epithelial cells become flattened, enlarged and are reduced in number. The goblet cells are markedly reduced in number or absent (see Figures 7.4–7.9).

Figure 7.4: Normal conjunctival impression with abundant goblet cells, sheets of small epithelial cells, and mucin spots (periodic-acid Schiff (PAS) and Harris’s hematoxylin, x 160).

Figure 7.5: Higher magnification of normal conjunctiva, showing contrast between PAS-positive goblet cells and epithelial cells (PAS and Harris’s hematoxylin, x 400).

Figure 7.6: Abnormal conjunctival impression with complete loss of goblet cells and mucin spots, along with appearance of enlarged epithelial cells (x 100).

Figure 7.7: Higher power of abnormal, enlarged conjunctival cells (PAS and Harris’s hematoxylin, x 400).

Figure 7.8: PAS-positive mucin spots representing “impressions” of goblet cells on conjunctival surface (PAS and Harris’s hematoxylin, x 400).

Figure 7.9: Impression cytology from normal child showing transition from abundant normal epithelium (lower left) to abnormal epithelium (upper right). Specimen was graded as normal (x100).

Figures 7.4–7.9: Conjunctival impression cytology (Wittpenn, Tseng, Sommer, 1986).
Limitations

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

The presence of acute conjunctivitis or trachoma (Lietman, Dhital, Dean 1998) interferes with CIC. More recent experience suggests that conjunctival impression cytology with transfer (CICT) identical with ICT above is the preferable technique (Chowdhury, Kumar, Ganguly et al. 1996). These investigators reported that healing after vitamin A dosing takes about 70–110 days (Chowdhury, Kumar, Ganguly 1997). This may seem to be a long time for a minimal lesion such as early xerosis and keratinization of the conjunctiva, especially in comparison to the healing of more severe clinical lesions (see Chapter 8). In this regard it should be remembered that microscopic techniques are likely to be more precise than clinical observations. CIC is increasingly being used in clinical ophthalmology and new refinements are constantly being introduced (Thiel, Bossart, Bernauer 1997).

INDICATORS AT THE CLINICAL LEVEL

Impaired dark adaptation

As mentioned, the rod cells of the retina contain vitamin A in the form of 11-cis retinal bound to opsin to form rhodopsin (see Chapter 6). These cells are photosensitive under conditions of low levels of illumination (night or scotopic vision). Table 7.4 above shows the various stages of increasing deficiency at which different kinds of testing may be applied.

Night blindness

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

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

In young children, the major vulnerable group for VADD, direct observation and an interview have usually been employed. A standard set of interview questions for the child’s guardians has been developed (Sommer, Hussaini, Muhilal et al. 1982) and use is made of the fact that in endemic areas terms frequently occur in the local language, for example likening poor vision at dusk to “chicken eyes” (chickens have no rod cells in their retinas and are therefore night blind) or Bitot’s spots to “fish scales.” If the child is old enough to walk, it may be observed to stumble in a room at dusk, or it may fail to grasp a proffered toy or sweet (see also Chapter 8).

The recent development of sensitive tests such as those outlined below (as described more fully in Chapter 8) have led to the replacement, wherever possible, of this subjective and unreliable method.

Vision restoration time (VRT)

The ability of the bleached eye to recognize a letter under low levels of illumination has been measured in school-age children in Thailand.
(Udomkesmalee, Dhanamitta, Sirisinha et al. 1992). From this study, it appeared that zinc was a more important determinant of VRT than vitamin A (see also Chapter 11). The test is applicable only to older children with some degree of literacy. A vision restoration time of \( >180 \) seconds has been suggested as a cut-off for abnormal night vision, suggestive of VAD.

**Pupillary threshold**
This test is based on the fact that the weakest threshold of light visible in the dark-adapted state is approximately of the same intensity as that needed to cause pupillary contraction. The pupils of subjects who are night blind due to VAD fail to react or constrict normally in low illumination. Children as young as one year old have been studied successfully (Congdon, Sommer, Severns et al. 1995). Further testing in children with VAD in India (Sanchez, Congdon, Sommer et al. 1997) and of pregnant women in Nepal (Congdon, Dreyfuss, Christian et al. 1999) has been distinctly promising. The test has proved readily acceptable, is associated closely with serum retinol, and responds to vitamin A supplementation. It also has the advantages of being noninvasive, applicable to young children and sensitive at the subclinical stage of VAD. *(Figure 7.10).*

**Night vision threshold test (NVTT)**
This test of the lowest level of luminance at which the test object can be identified by the subject has recently been introduced. The apparatus is portable and early results are promising (see Chapter 8).

**Xerophthalmic fundus**
This abnormality in the appearance of the retinal fundus has usually been considered too rare to be used in the field for recording the presence of xerophthalmia. A recent reappraisal suggests it may have some practical value (see Chapter 8).

**ASSESSMENT OF DIETARY INTAKE OF VITAMIN A**

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

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

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

The original method ignored animal milk, but it has subsequently been shown that in some areas this can provide 5–18% of the RDA and should therefore be considered. Experience with five communities in the Philippines, five in Tanzania and five in Guatemala, using serum retinol 0.70 μmol/L as deficient, found correct classification as deficient in 73.3%, but a high false-positive rate of 42.9% (Sloan, Rosen, Paz et al. 1997).

Unfortunately, the uncertainty now prevailing as to the bioavailability and bioconversion factors for provitamin A carotenoids (see Chapter 5) overshadows these and all methods of assessment of dietary vitamin A intake. Until these matters are resolved, results of field work will have to be interpreted with caution.
8 Xerophthalmia

DEFINITIONS

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

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

All of these eye changes are included in the term xerophthalmia. In other words, xerophthalmia is synonymous with all of the clinical signs and symptoms that affect the eye in vitamin A deficiency. The various ocular appearances in xerophthalmia were classified by WHO in 1976 (WHO Expert Group 1976) and modified in 1982 (WHO Expert Group 1982). Criteria of a public health problem were also developed and were recently revised again. All of these recommendations are shown in the previous chapter (Tables 7.1; 7.2; 7.3) and should be referred to.

To a certain extent the ocular signs in Table 7.2 have been listed in an increasing order of severity. This means that retinal function tends to be impaired before xerosis affects first the conjunctiva and then the cornea. Liquefaction of the cornea, of increasing severity, is normally a very late stage. Corneal scars are not part of the active deficiency process. They may be considered to be stigmata of previous deficiency, providing there is circumstantial evidence suggestive of vitamin A deficiency as their cause. These characteristics are discussed later. The xerophthalmic fundus is a rarity and does not fit neatly into the classification (see later).

There are two other aspects of the subject of the definition of xerophthalmia that need to be made quite clear. Firstly, there are causes of xerophthalmia other than dietary vitamin A deficiency, the epidemic disease that primarily affects young children in developing countries and that is our main concern in this Manual. These cases arise sporadically and are caused by various defects in the utilization of vitamin A within the body. Most of these are quite uncommon. They are instances of secondary or endogenous VAD and are considered later in Chapter 14.

The other aspect that needs to be pointed out is that some of the eye changes may be caused by diseases that are not related to vitamin A deficiency. For example, night blindness is a symptom of several rare degenerative disorders of the retina. It is also a main feature of a not uncommon blinding disease called retinitis pigmentosa. For the most part these diseases do not respond to treatment with vitamin A (see Chapter 14).

Dryness of the conjunctiva and cornea is also a feature of several diffuse connective tissue disorders, one of which is Sjogren’s syndrome. The xerosis results from atrophy of the secretory epithelium of the lacrimal glands, and does not respond to vitamin A.
There are several facts that shed light on the way in which xerophthalmia has tended to be neglected over the years. For reasons that are not clear, xerophthalmia was usually not included among the classical vitamin deficiency diseases when these were first identified in the 19th and early 20th centuries. Beriberi, scurvy, pellagra and rickets were usually considered together and xerophthalmia was rarely included (Funk 1912). Even though fat-soluble A was the first vitamin identified (McCollum, Davis 1915) and was therefore given the first letter in the alphabet, xerophthalmia was not studied in detail at the time. Night blindness and Bitot’s spots tended to be described in isolation from the blinding changes of corneal xerophthalmia. It may be that scientists and general physicians did not feel competent to examine the eye and deal with eye disease. It is of interest that the only vitamin deficiency to have reached epidemic proportions and to persist at these levels today is xerophthalmia. This historical aspect of VADD has been recently discussed (McLaren 2004).

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

NIGHT BLINDNESS (XN)

It will be recalled that the subject of retinal dysfunction was considered previously in connection with indicators of VAD (see Chapter 7). As shown in Table 7.3, night blindness is the most extreme form of retinal dysfunction; sufficiently severe to cause subjective impairment of vision at night. As an indicator of VADD it has both advantages and disadvantages. On the positive side, tests are noninvasive, and some may be applied by investigators with no specialized ophthalmological training. In this category are questionnaires and observation of the performance of young children under conditions of low illumination (Table 8.1; WHO 1996).

Table 8.1: Scheme for the classification of night blindness by interview (WHO, 1996).

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

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

One study in Bangladesh (Hussain, Kvale, Odland 1995) compared over a hundred children aged 2–15 years and reported to be night blind by
their parents with a similar number of matched controls. Both groups received an eye examination, test of scotopic vision by a luxometer – a simple form of dark adaptometer – and measurement of serum retinol level. Although there was fairly close correlation between the two methods of diagnosing night blindness, the parents’ report appeared to be less sensitive.

The occurrence of names for night blindness in local languages suggests that this rather distinctive phenomenon occurs with some regularity in a community. The commonest terms used are “night eyes” and “chicken eyes”.

Little night blindness is detected in very young children because the effects of deficiency only become evident when the child tries to move around at dusk. From about 2 years onward rates of night blindness tend to rise, because of increased activity. Evidence has accumulated in recent years to show that school age children and pregnant and lactating women (Khan, Haque, Khan 1984; Katz, Khatry, West et al. 1995) are also vulnerable groups in which the measurement of rod vision may be a useful means of assessing vitamin A status in a community. These groups can cooperate in dark adaptometry, thus this is the method of choice.

Clinical dark adaptometers, designed for use in hospitals, are expensive and sensitive pieces of equipment. There are simpler and more robust forms of equipment that may be readily transported for use in field studies. Vitamin A deficiency results in delay in rod adaptation to conditions of illumination of low intensity, followed by reduction in threshold sensitivity. Figure 8.1 shows the curve that is obtained on testing a normal subject. Figure 8.2 illustrates how test curves rose progressively in a volunteer subject receiving a vitamin A deficient diet.

In practice, it is usually sufficient to allow both examiner and a group of subjects for testing to undergo dark adaptation for about 30 minutes

![Figure 8.1: Normal curve of dark adaptation showing cone-rod transition time, cone threshold, and final rod threshold (Hume, Krebs 1949). Log millilamberts are units of intensity of illumination.](image)
in a darkened room. At this point maximum possible rod adaptation will have occurred and the tests are carried out at the final rod threshold (see Figure 8.1).

Night blindness is frequently the most prevalent form of xerophthalmia, as might be expected. Although serum retinol of \( >0.70 \) μmol/L has customarily been considered to be “normal,” it was found that about 20% of children with night blindness and 10% with night blindness and Bitot’s spot had higher levels of serum retinol (see Table 8.2). As might be expected, impaired dark adaptation could be detected with serum retinol between 20 and 30 μg/dL.

Recent years have seen a surge of interest, especially in some of the countries of Southeast Asia, in the occurrence of xerophthalmia in women of child-bearing age. In particular, attention has focused on those who are, or have recently been, pregnant and may be lactating. Prevalence rates reported have been surprisingly high, causing health authorities to devote much more attention to this more recently recognized public health problem.

Accompanying this research activity has been a growing interest in the methodology of testing for night blindness; the salient feature of xerophthalmia in this group.

A large study in Nepal of the effects of vitamin A or β-carotene supplementation on maternal mortality was carried out during 1994–1997 (see Chapter 8). As part of this research it was found that 877 women had night blindness (as judged by weekly night blindness histories elicited throughout pregnancy). 9545 were negative, giving the high prevalence of 9.1% (Christian, West, Khatry et al. 2000). In the placebo group, mortality of night-blind women was 360/100,000 pregnancies. In comparison, the relative risk in the night-blind was 0.32 and in the non-night-blind 0.18. When

Figure 8.2: Curves of dark adaptation for one deprived subject at different stages of depletion (Hume, Krebs 1949). Log millilamberts are units of intensity of illumination, numbers on right are dates of tests.
infection-related causes of death were examined, it was shown that night-blind women were five times more likely to have died of infections than the non-night-blind.

**Pupillary threshold**

In Nepal the dark-adapted pupillary response was used to identify 298 pregnant and lactating women with night blindness (Congdon, Dreyfuss, Christian et al. 2000). In a placebo-controlled trial with vitamin A and β-carotene, women receiving vitamin A had better dark adaptation thresholds than women receiving placebo or β-carotene. Dark adaptation threshold was associated with serum retinol concentration in women receiving placebo or β-carotene, but not in those receiving vitamin A.

The same group (Christian, West, Khatry et al. 2001) carried out a stratified analysis by maternal night blindness status during pregnancy for 10,000 women. Mortality of infants of non-night-blind women was 63/1000 live births. Among those night-blind, infant mortality was relatively greater by 63% with placebo, 50% with β-carotene, and only 14% with vitamin A.

Recently, pregnant Nepalese women suffering from night blindness were studied during their response to small daily doses of various sources of vitamin A. These included amaranth leaves, carrots, goat liver, vitamin A fortified rice, or red palm oil. Pupillary threshold was used to assess vitamin A status. This test improved significantly in night-blind women of all groups. The improvement was greater in the group that received liver than in that receiving vitamin A fortified rice. Plasma retinol response was greater in the higher dose capsule and liver groups than in the vegetable groups. Only the group receiving vitamin A fortified rice failed to improve (Haskell, Pandey, Graham et al. 2005).

A study was recently carried out to validate different definitions of reported night blindness (Wedner, Ross, Congdon et al. 2004). No local words for night blindness were in use in this rural population of Tanzania. Participants were 461 children aged 24–71 months, 562 primary school children, and 191 pregnant or lactating women. In all, 152 subjects complained of night blindness and were group matched with 321 controls. Night blindness reports were validated by serum retinol and pupillary dark adaptation. All children and women who reported night blindness or had other signs of xerophthalmia were treated with vitamin A and were followed up 3–4 weeks later. Half of the control group were also treated and followed up. The overall prevalence of night blindness was 12.5%. At baseline, mean pupillary threshold and median serum retinol were not significantly different in cases and controls, either overall or by groups. More restricted case definitions significantly reduced the prevalence of reported night blindness to 5.5% (P<0.001), but there were no significant differences between cases and controls. After treatment dark adaptation improved significantly in cases in young chil-

---

**Table 8.2:** Association between xerophthalmia status and serum retinol (µg/dL). (From Sommer, West 1996).

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>Deficient (&lt;10)</th>
<th>Low (10–20)</th>
<th>Adequate (&gt;20)</th>
<th>Mean</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>XN (+), X1B (-)</td>
<td>27%</td>
<td>55%</td>
<td>18%</td>
<td>13.9</td>
<td>174</td>
</tr>
<tr>
<td>XN (-), X1B (+)</td>
<td>31%</td>
<td>57%</td>
<td>12%</td>
<td>13.4</td>
<td>51</td>
</tr>
<tr>
<td>XN (+), X1B (+)</td>
<td>38%</td>
<td>53%</td>
<td>9%</td>
<td>12.1</td>
<td>79</td>
</tr>
<tr>
<td>Neighborhood controls</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17.7</td>
<td>282</td>
</tr>
<tr>
<td>Random sample</td>
<td>8%</td>
<td>37%</td>
<td>55%</td>
<td>20.0</td>
<td>268</td>
</tr>
</tbody>
</table>

XN = Night blindness; X1B = Bitot’s spot; Controls = Neighborhood peers of same age and sex; random sample = peers from throughout the six villages studied
dren and primary schoolchildren, but only when restricted case definitions were used. It was concluded that night blindness reports were a poor indicator of vitamin A deficiency in this population.

Night vision threshold test
NVTT has also been advocated for use in the field. A detailed description of the apparatus has been published (Taren, Duncan, Greivenkamp et al. 2001). Using this test 1401 urban Nepalese pregnant women were examined (Taren, Duncan, Shrestha et al. 2004). Sixteen percent failed the test, but only 6.4% reported having night blindness. NVTT test failure was accompanied by lower serum retinol. Serum retinol was correlated with the NVTT scores (P<0.001).

CONJUNCTIVAL XEROSIS (X1A)

The term conjunctival xerosis could apply to any stage of xerotic change in the conjunctiva (Figure 8.3). This would range from abnormal impression cytology, through dryness of the conjunctiva, to keratinization and heaping up of material as in Bitot’s spot (X1B, see below). CIC is subclinical and was dealt with previously (see Chapter 7). In the Xerophthalmia Classification (see Table 7.1) X1A distinguishes dryness from Bitot’s spot. Before this scheme was agreed upon a great deal of attention was given to various conjunctival appearances, including thickening, wrinkling and pigmentation. It is now recognized that these changes and the degree to which they occur are not related directly to vitamin A deficiency. If the classification (see Table 7.1) and the criteria (Table 7.2) are compared it will be evident that X1A is not one of those appearances chosen for use in surveys. This is because it has repeatedly been found that, while advanced instances of X1A can be readily identified, minor changes are subject to great inter- and intra-observer variation. This makes X1A an unreliable indicator of VAD. Even so, it is often – wrongly – used in surveys, making interpretation of results very difficult. To obtain reproducible and comparable results it is of great importance that WHO guidelines be followed in matters like this (WHO Expert Group, 1982).

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

BITOT’S SPOT (X1B)

As was mentioned earlier, Bitot’s spot is the final part of the process of xerosis affecting the bulbar conjunctiva. The typical Bitot’s spot (see Figure 8.3 and Figure 8.4) occurs in the exposed part of the conjunctiva, the area between the lids normally exposed, called the interpalpebral fissure.

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

Bitot’s spots vary considerably in size and shape. The areas of conjunctiva affected may be multiple, but more usually there is a single spot to an eye. Some single spots are ovoid, others lin-
Figure 8.3: Bitot’s spot (X1B) on temporal aspect of bulbar conjunctiva in interpalpebral fissure. Bubbles of foam are clearly visible.

Figure 8.4: Bitot’s spot without foam and of a “cheesy” appearance. The nature of the material is not known to have any significance.
ear, and occasionally they take a roughly triangular form with the base close to the limbus, the junction between conjunctiva and cornea. None of these characteristics has any special clinical significance (McLaren 1962).

Broadly speaking, the appearance of Bitot’s spots has been likened either to 1) foam; or 2) cheese (see Figure 8.3 and 8.4). Gasforming bacteria may be responsible for the first appearance. There is no known significance to this difference in appearance.

Undoubtedly, the most significant way in which Bitot’s spots may be classified is as to whether they are 1) responsive to vitamin A treatment; or 2) unresponsive (Djunaedi, Sommer, Pandji et al. 1988). There are several characteristics that may be of assistance in any attempt to identify the nature of a Bitot’s spot (see Table 8.3).

It is usually not possible to determine the cause of an unresponsive Bitot’s spot. Some appear to be stigmata of earlier deficiency and may therefore in this sense resemble corneal scars (XS) that are considered to relate to earlier deficiency (see later). For others there is no evidence of either earlier or present deficiency and these may result from local trauma of some kind. This might be due to a combination of environmental factors, among which might be included ultraviolet exposure at high altitude, smoke-filled huts, or chronic eye infections, especially trachoma. This hypothesis is supported by the fact that Bitot’s spots form most readily on the most exposed part of the conjunctiva. Further evidence for a role of exposure in Bitot’s spot formation is reports of their presence on unusual aspects of the eyelids (McLaren 1980) (see Figure 8.5).

### CORNEAL XEROSIS (X2)

Many instances of conjunctival xerosis are accompanied by a superficial punctate keratopathy, which can be seen when examined with the slit-lamp microscope (Sommer, Emran, Tamba 1979).

<table>
<thead>
<tr>
<th>Table 8.3: Characteristics of vitamin A responsive and unresponsive Bitot’s spots (X1B).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Responsive</strong></td>
</tr>
<tr>
<td>• Subject usually a child of less than 6 years of age</td>
</tr>
<tr>
<td>• With the maximum vitamin A dosage, response usually evident within one week</td>
</tr>
<tr>
<td>• Usually accompanied by generalized conjunctival xerosis and night blindness</td>
</tr>
<tr>
<td>• Males more commonly affected than females</td>
</tr>
<tr>
<td><strong>Unresponsive</strong></td>
</tr>
<tr>
<td>• Commonly occur in children over 6 years and in otherwise healthy adults</td>
</tr>
<tr>
<td>• Usually a small, single spot</td>
</tr>
<tr>
<td>• No accompanying evidence of VAD</td>
</tr>
<tr>
<td>• Largely responsible for the apparent increase of X1B prevalence with increasing age</td>
</tr>
</tbody>
</table>

This suggests that the process of xerosis tends to spread from the conjunctiva to later involve the cornea. Clinically evident corneal xerosis (X2) in which the cornea has a distinct hazy appearance (see Figure 8.6) tends to last for a matter of only a day or two before advancing to deformation of the cornea known as keratomalacia (see below).

Up to the stage of corneal xerosis (X2) prompt treatment with large doses of vitamin A can result in full preservation of sight without any residual impairment. It is of paramount importance that all stages of xerophthalmia should receive the maximum treatment with vitamin A (see Chapter 15). In contrast to the lengthy full response to vitamin A therapy of abnormal conjunctival cytology mentioned earlier (see Chapter 7), clinical improvement has been reported to take place in 70% of corneal lesions (X2/X3) within four days, in 95% within one week, and the vast majority were entirely healed within two weeks (Sommer, West 1996:285). Histological and clinical healing are both subjective, and clearly are not strictly comparable.
Figure 8.5: A single Bitot’s spot associated with abnormal exposure of the conjunctiva due to ectropion associated with scar on cheek.
KERATOMALACIA (X3A, X3B)

In the 1982 WHO Xerophthalmia Classification, keratomalacia was divided into two stages according to the extent of the involvement of the cornea (see Table 7.1). Keratomalacia is characterized by softening of the corneal substance in addition to increasing xerosis of the epithelium (see Figure 8.7).

Corneal softening is due to a unique pathological process termed colliquative necrosis. The stroma becomes edematous. It is suspected that activation of collagenases and other enzymes may be responsible, but the precise pathogenesis is not known. Now that the clinical condition has become a comparative rarity, little effort has been made in recent years to try to understand the underlying mechanism. A Japanese research group (Toshino, Shiraishi, Zhang et al. 2005) has shown in the severely vitamin A deficient rat that as the cornea keratinizes there is an increase in keratinocyte transglutaminase (TG1, TG(K)) activity and the envelope proteins involucrin, loricrin, and keratin 10 begin to be expressed on the abnormal epithelial cells of the cornea.

Many years ago (Pillat 1929) cases of keratomalacia in adults in China were also described as having similar colliquative changes in the skin. This was termed “dermomalacia.” Figure 8.8 shows one eye and the surrounding skin of a five-month-old Palestinian refugee child who died shortly after admission to hospital in Amman, Jordan. There is advanced keratomalacia with almost the entire cornea affected and the central area about to prolapse. In addition, the skin has a markedly burnished appearance, which also affected much of the skin of the head and neck. The skin change is very reminiscent of that described earlier as dermomalacia. As a result of this process in the cornea there will always be some degree of residual damage and deformity.

In corneal ulceration there is usually only one ulcer per eye. The typical ulcer is infero-nasal in position, about 0–2mm in diameter and 0.25–0.5mm in thickness. In about 20% of cases both eyes are affected and the characteristics tend
Figure 8.7: Colliquative necrosis (keratomalacia) affecting the greater part of the cornea (X3B). The relative sparing of the superior aspect is typical. Plasma vitamin A, 4 µg/dL.

Figure 8.8: Entire cornea is undergoing liquefaction (X3B). Skin surrounding the eye shows hyperkeratinization suggestive of “dermalacia” (see text).
to be similar. Hypopyon (collection of sterile pus in the anterior chamber) is common and infection is frequent.

**CORNEAL SCAR (XS) – VITAMIN A RELATED**

Scarring of the cornea may result from a wide variety of diseases affecting the eye. Visual impairment is inevitable; its degree depends on the location and the density of the scar. Damage that is confined to the cornea may be overcome by surgery. This is not possible when internal structures are also involved, usually as a result of accompanying infection. Prevention is clearly infinitely preferable. Careful and detailed history taking and general physical examination, in addition to the eye examination, are of particular importance in this instance (see Figure 8.9). If, as a result of these, there is any residual doubt about the likely etiology of a corneal scar, then it should not be attributed to vitamin A deficiency. Table 8.4 gives the information to be elicited in order to come to a diagnosis.

**XEROPHTHALMIC FUNDUS (XF)**

This rare condition has been described mainly in school age children or young adults in Southeast Asia (Teng-Khoen-Hing 1959; Sommer, Sugana, Djunaedi et al. 1978). It appears to result from prolonged deficiency of vitamin A in which impairment of rod function is succeeded by structural damage to the retina (Figure 8.10).

Attention was recently drawn (McLaren 2004) to the potential value of XF in eye field surveys, especially in school-age children and adults. Figure 8.11 suggests that the lesion may not be as rare as has sometimes been suggested.

In view of the questions that are now being raised about the reliability of self-reporting of night blindness, especially in areas where the phenomenon is not so common as to have a name in the local language, it is surprising that examination for XF, which is easily detectable by ophthalmoscopic examination, has not been carried out. It was pointed out recently (McLaren 2004a) that this sign might have been used more frequently in

<table>
<thead>
<tr>
<th>Eye examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Location: typically nasal and inferior on the cornea if only a small part involved</td>
</tr>
<tr>
<td>• Often bilateral, not necessarily equal in extent</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Onset between about 2 months and 5 years</td>
</tr>
<tr>
<td>• Accompanied at that time by severe PEM, measles, severe diarrhea, respiratory infection</td>
</tr>
<tr>
<td>• Absence of trauma, or prolonged purulent discharge</td>
</tr>
</tbody>
</table>

Figure 8.9: Bilateral corneal scars (leucomata XS) in an anemic and generally malnourished infant. The inferior situation of the scars is typical.
the past when xerophthalmia was a much greater public health problem than at present. It should be employed currently when high prevalence rates are being reported in pregnant and lactating women and school-age children (see earlier) and when at the same time these high rates are being questioned by some.

Recently, a report from Malawi associated the whitening of the retina described in children with cerebral malaria with low vitamin A status and abnormal CIC (Lewallen, Taylor, Molyneux et al. 1998). Retinal changes and signs of raised intracranial pressure responsive to vitamin A were reported in a case of secondary VAD in the United States (Panozzo, Babighian, Bonora 1998).

In conclusion, there are several general points that should be noted. Signs of xerophthalmia are usually present in both eyes, but not necessarily to the same degree. Keratomalacia may proceed very rapidly, noticeably within a matter of some hours.

Figure 8.10: a) Numerous small yellowish-white lesions affecting most of this region of the retina. 24-year-old patient presented with night blindness and constricted visual fields. b) Two months after vitamin A treatment the lesions have largely disappeared (Sommer 1982).

Figure 8.11: Prevalence of xerophthalmic fundus (XF) (Sommer 1982).
rather than days. This is especially true in very young children. In this age group keratomalacia may be present without any evidence of xerosis in the conjunctiva or cornea. It follows that a diagnosis of vitamin A deficiency may be made in the absence of xerotic changes. When the cornea is involved, accompanying infection is probably the rule. Frequently this obscures the classical picture of changes due to xerophthalmia. Unless this is remembered, the result may be underdiagnosis of xerophthalmia.
9 Mortality and Morbidity, Especially in Relation to Infections

INTRODUCTION

It can be instructive, on occasion, to look back in time and speculate about the reasons why events in history took the path they did. In Chapter 8 it was pointed out that recognition of xerophthalmia as a vitamin deficiency disease lagged behind that of several other diseases of nutritional etiology. In the present context it is a cause for surprise that widespread acceptance of the significant impact of vitamin A on mortality and morbidity has come only in recent years (Semba 1999). Not long after the discovery of fat-soluble A the vitamin was dubbed “the anti-infective vitamin” (Green, Mellanby 1928). This was on the basis of the susceptibility of experimental animals fed on diets deficient in the vitamin to infections resulting in death and of the response of humans with infections to vitamin A.

Sommer and West (1996:pp19–61) reviewed hospital-based reports of children with xerophthalmia and severe PEM in whom mortality was significantly increased in the presence of eye signs accompanying general malnutrition. In some of these studies there was a fourfold (McLaren, Shirajian, Tchalian et al. 1965) or even greater mortality in children severely vitamin A deficient as compared with those equally generally malnourished but with normal eyes. The implications of these findings were not pursued further at that time. In explanation it should be pointed out that epidemiological and statistical methodologies had not advanced sufficiently in those times and there was still great reticence on the part of government and other agencies to recognize the magnitude and severity of VADD.

MORTALITY ASSOCIATED WITH NON-CORNEAL XEROPHTHALMIA

The significance of vitamin A deficiency in child survival in the community was first suggested by a study of Sommer, Hussaini, Tarwotjo et al. (1983) that followed a point-prevalence survey of vitamin A deficiency in Indonesia (see later). The results, presented in Table 9.1, show a significantly increased relative risk of death in those groups with evidence of xerophthalmia. The greatest risk is among those with both X1B and XN. It should be noted that only those with the milder, non-corneal signs of xerophthalmia were included.

It is probable that the mechanism of the greater mortality in those children who had clinical VADD was in some way related to impairment in their immune response to an accompanying infectious disease. Whilst it is relatively easy to

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

* Examined at six three-monthly intervals
ensure that all deaths are recorded in a study like this, it is much more difficult to obtain accurate information as to the cause or causes of death. In addition, in the circumstances that prevail in field studies it is usually not possible to make a definitive diagnosis of any accompanying infections. These matters are further discussed later when the relationship between vitamin A deficiency and morbidity is considered.

VITAMIN A INTERVENTION AND MORTALITY IN YOUNG CHILDREN

The first of these trials was carried out in Aceh, Indonesia (Sommer, Tarwotjo, Djunaedi et al. 1986). A large dose of vitamin A (200,000 IU) was given every 6 months. The initial report found a 34% reduction in mortality in the treated group. Alternative analyses suggested a reduction of at least 40% and possibly >50% (Tarwotjo, Sommer, West et al. 1987; West, Pokhrel, Katz et al. 1991). Figure 9.1 shows some of the results from this study.

As an indication of the magnitude of trials of this nature it should be noted that more than 150,000 preschool-age children took part in the eight trials described in outline below. All or some of them have formed the basis of several meta-analyses that have been made. The results of all the meta-analyses are in general agreement that improvement in the vitamin A status of deficient populations reduces significantly the overall preschool-age child mortality. Figure 9.2 is a summary of the results of one of these meta-analyses of the eight major mortality intervention trials (Beaton, Martorell, Aronson et al. 1993). The overall reduction of mortality from this was 23%.

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

It should also be noted that there are several reasons why the reported reductions in mortality may have underestimated the actual reductions. Some deaths may have been included that oc-

![Figure 9.1: Cumulative mortality, by sex, of preschool-age children in Aceh, Indonesia. From Sommer, Tarwotjo, Djunaedi et al. 1986.](image-url)
curred before the vitamin A intervention had a chance to act. In most studies children with xerophthalmia were identified, treated and excluded from the main study. In the absence of the intervention these children would be expected to be among the most vulnerable. Periodic large-dose vitamin A supplementation may not be the optimal means of improving vitamin A status in the long term. Consistent with this may be the highest reductions in mortality being reported from Bogor, Indonesia, and Tamil Nadu, India (see Table 9.2), where vitamin A was provided in a more physiological way. Finally, each of the eight studies reported what is termed an “intention-to-treat” analysis. In this it is assumed that a full intervention was received by each subject supposed to do so. It has been found (Tarwotjo, Sommer, West et al. 1987) that in most health interventions of this kind those who fail to participate, termed “noncompliers,” are usually those most in need of the intervention. Their mortality has been found to be greater than that of the control group (see Figure 9.3).

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

In a reassessment of most of these studies Sommer and West (1996:34–37) found that in children aged 6 months and older the reduction in mortality tended to increase in magnitude with age. The majority of deaths occurred in children <2 years of age. These authors also discuss the
Figure 9.3: Mortality in the Aceh trial among children in treatment villages who did and did not receive their intended vitamin A dose, compared with children in control villages. Mortality was higher among children who were assigned to receive vitamin A but did not get it than for any other group, including children in control villages (Tarwotjo, Sommer, West et al. 1987).
evidence for the effect on mortality of vitamin A dosing in children under the age of 6 months. Under the age of 3 months 100,000 IU appeared to have a detrimental effect, but 50,000 IU was beneficial. In infants over the age of 3 months the larger dose was effective. A single maternal dose of 300,000 IU has been shown to raise maternal stores, vitamin A levels in breast milk and the serum retinol of breastfed children, and to reduce mortality in infants by about 30% (de Francisco, Yasui, Chakraborty 1994).

Recently an analysis of the effect of Nepal’s vitamin A supplementation program on child mortality at age 12–59 months was made (Shyam, Kim, Retherford 2005). It was shown that 100% community-level vitamin A coverage since the child’s birth compared to no coverage reduced the odds of dying by slightly more than 50%.

CAUSE-SPECIFIC MORTALITY

This was studied in four of the eight interventions subject to meta-analysis and the results in Table 9.3 indicate that vitamin A intervention tends to make more of an impact on measles and diarrhea than it does on respiratory infections in this regard.

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

### CONTRIBUTION OF VAD TO CHILD MORTALITY

While subclinical vitamin A deficiency is much less fatal than overt xerophthalmia, it is very much more widespread (see Chapter 12). Beaton, Martorell, Aronson et al. 1993 (see Figure 9.4), were unable to find any association in the field trials that have been cited above between relative risk (RR) of mortality and prevalence of xerophthalmia.

This may have been due in part to differences in the reporting of xerophthalmia in the different studies (McLaren 1991), but it probably indicates that xerophthalmia does not make a major contri-

### Table 9.3: Cause-specific mortality, vitamin A supplementation community prevention trials
(Sommer, West 1996:41).

<table>
<thead>
<tr>
<th></th>
<th>Measles</th>
<th>Diarrhea</th>
<th>Respiratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamil Nadu Deaths (n) 7</td>
<td>12</td>
<td>16</td>
<td>33</td>
</tr>
<tr>
<td>RR=0.58</td>
<td>0.48</td>
<td>0.67</td>
<td>3</td>
</tr>
<tr>
<td>NNIPS Deaths (n) 3</td>
<td>12</td>
<td>39</td>
<td>62</td>
</tr>
<tr>
<td>RR=0.24</td>
<td>0.61</td>
<td>1.29/1.00B</td>
<td>27</td>
</tr>
<tr>
<td>Jumla Deaths (n) 3</td>
<td>4</td>
<td>94</td>
<td>129</td>
</tr>
<tr>
<td>RR=0.67</td>
<td>0.65</td>
<td>0.95</td>
<td>17</td>
</tr>
<tr>
<td>Ghana VAST Deaths (n) 61</td>
<td>72</td>
<td>69</td>
<td>111</td>
</tr>
<tr>
<td>RR=0.82</td>
<td>0.66</td>
<td>1.00</td>
<td>45</td>
</tr>
</tbody>
</table>

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**A** RR (relative risk): Cause-specific mortality rate of vitamin A group divided by rate in control group

**B** Originally published results RR=1.29; reanalysis as an associated cause that recognizes other underlying causes RR≠1.00

**C** For details see Table 9.2
The largest contribution is most likely to be attributable to subclinical vitamin A deficiency.

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

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

It has been estimated (Humphrey, West, Sommer 1992) that worldwide vitamin A deficiency may be responsible for as many as 1.3 to 2.5 million deaths annually. In addition, PEM is considered to be a contributory cause of infant and child deaths in nearly 50% of cases (Pelletier, Frongillo, Schroeder et al. 1995). Recently much attention has been given to the importance in infections of other micronutrient deficiencies, especially of zinc (see Chapter 11).

A recent account from Mozambique (Aguayo, Kahn, Ismael et al. 2005) of planning for a new vitamin A supplementation program for children under the age of five, as a short- to medium-term strategy to assist in the reduction of a high under-5 mortality rate, is of interest. Low coverage (46%) soon emerged as a critical problem.

By way of contrast, examination of the results of a long-standing vitamin A supplementation program in Nepal with excellent coverage showed that in this way inequalities in child mortality by social class could be removed (Fiedler 2000; Bishai, Kumar, Waters et al. 2005) (see also Chapter 15). In Zimbabwe Malaba, Iliff, Nathoo et al. (2005) studied a group of HIV-negative mothers.

Figure 9.4: Relative risk of mortality and prevalence of xerophthalmia. No relationship could be established (Beaton, Martorell, Aronson et al. 1993).

...
and their young infants. The effect of postpartum maternal and neonatal vitamin A supplementation on infant mortality up to 12 months was studied. No effect was shown in these mothers who were shown to have adequate vitamin A status.

Benn, Fisker, Diness et al. (2006) have drawn attention to the adverse effect on mortality in girls in some programs where vaccines and VAS are combined. This important and complex issue is dealt with in Chapter 15 in a separate section (see p. 170).

MEASLES MORTALITY AND VITAMIN A TREATMENT

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

Measles generally takes a more serious form in the generally undernourished child, resulting in more frequent and more serious complications and a much higher death rate than in the well-nourished. This was shown in the community prevention trials referred to already (see Table 9.3).

Table 9.4 shows that in hospital the majority of deaths occur in children under the age of 2 years. Dramatic reduction in mortality rates in hospitalized cases of measles has been repeatedly shown in those where treatment included vitamin A supplementation (Figure 9.5).

In 2002 the UN General Assembly Special Session on Children adopted a goal to reduce deaths due to measles by half by the end of 2005, compared with 1999 estimates. This goal was achieved; mortality due to measles was reduced by 60% from an estimated 873,000 deaths in 1999 to 345,000 in 2005. Nearly 7.5 million deaths were prevented, with supplemental immunization activities (including vitamin A supplementation) and improved routine immunization accounting for 2.3 million of these prevented deaths (Wolffson, Strebel, Gacic-Dobo et al. 2007). The latest data (BMJ 2009) show that measles deaths worldwide fell by more than 78% between 2000 and 2008 but in India the drop was only 46%. In

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Vitamin A</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children</td>
<td>Deaths</td>
</tr>
<tr>
<td>&lt;6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6–12</td>
<td>53</td>
<td>1</td>
</tr>
<tr>
<td>13–23</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>0–23</td>
<td>76</td>
<td>2</td>
</tr>
<tr>
<td>≥24</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 9.4: Measles mortality – Cape Town Trial (Hussey, Klein 1990).
Africa the figure was 92%. “Delayed implementation of large-scale vaccination campaigns in India, the country with the majority of measles deaths, is largely accountable for this lack of progress,” a spokesperson for Measles Initiative said. Experts fear resurgence in measles mortality if current strategies are not maintained.

**VITAMIN A SUPPLEMENTATION AND MATERNAL MORTALITY**

The preliminary results of the first trial of this kind caused a considerable stir when they were reported at the XVIII IVACG Meeting in Cairo (West, Khatry, Katz et al. 1997). The definitive paper was published nearly 18 months later (West, Katz, Khatry et al. 1999) together with an editorial (Olsen 1999). An area of Nepal was chosen for the study where VADD is known to be common in pregnant women as well as in young children. Well over 40,000 women participated and were observed over 3½ years. Approximately equal numbers in three matched groups received vitamin A (7,000 μg RE on a weekly basis), β-carotene (7,000 μg RE on a weekly basis), or a placebo.

Maternal deaths from any cause during pregnancy or within 12 weeks of delivery were the end point. The preventive effects, expressed as relative risks, were 0.60 (40% reduction) for vitamin A and 0.51 (49% reduction) for β-carotene (see Table 9.5).

It is suggested that the antioxidant effect of β-carotene might have had some relationship to the higher reduction rate in comparison with vitamin A. There was neither reduction in fetal or infant mortality through until 6 months of age nor any impact on neonatal birth weight.

A preliminary report at the XIX IVACG Meeting 1999 (Hakimi, Dibley, Suryono et al. 1999) on a trial to show the effects of vitamin A and zinc supplements on maternal postpartum infections showed that there was significant benefit

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Figure 9.5: Measles case-fatality rates among hospitalized patients randomized to receive high-dose vitamin A (cod liver oil in the London trial) compared with those of their controls. Vitamin A supplementation reduced mortality by 50% in all three trials (Sommer, West 1996:53).
in groups receiving vitamin A but zinc gave no improvement. The results of two other large trials of a similar nature to that of the trial in Nepal have been reported (Palmer and Stewart 2009).

In Bangladesh, where maternal mortality is lower (than in Nepal) and maternal vitamin A status better, there was no evidence of an impact (Klemm, Labrique, Christian et al. 2008). A third trial from Ghana (Kirkwood, Hurt, Amenga-Etego et al. 2010), where maternal mortality and vitamin A status were similar to those in the Bangladesh trial, found no significant impacts on pregnancy-related mortality and pregnancy-associated morbidities. The combined results of these trials confirm that, while potentially important in high-mortality, deficient settings, a global policy for maternal VAS is not warranted.

| Table 9.5: Impact of supplementation on mortality related to pregnancy up to 12 weeks postpartum (West, Katz, Khatry et al. 1999). |
|----------------------------------|---------|---------|---------|---------|
| Number of pregnancies*          | 7,241   | 7,747   | 7,201   | 14,948  |
| Number of deaths                | 51      | 33      | 26      | 59      |
| Mortality (per 100,00 pregnancies) | 704      | 426      | 361      | 395      |
| Relative risk (95% CI)          | 1.0     | 0.60    | 0.51    | 0.56    |
|                                 | (0.37–0.97) | (0.30–0.86) | (0.37–0.84) |
| P value                         | <0.04   | <0.01   | <0.005  |

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

VITAMIN A SUPPLEMENTATION AND INFECTIOUS MORBIDITY

Mortality and morbidity are related but clearly quite distinct. All disease states carry some element of morbidity. In a medical context the word relates in an unspecific way to illness. Some instances of morbidity may result in death, but that aspect is normally dealt with separately. Fatal diseases have, until recently, been given more attention than the much more numerous non-fatal ones. This may be partly because the latter have been considered to be of only secondary importance. It may also be because they are much more difficult to document. A fatality is a discrete, one-off event; a morbidity or disability is quite vague. Morbidity may be acute or chronic, mild or severe.

The relationship of morbidity due to VADD and infection is complicated by several further points. There is an apparent synergistic relationship between impaired vitamin A status and infections; each seems to increase the risk of the other (see also Chapter 13). Other nutritional deficiencies, especially PEM of mild degree, are often also present and this makes interpretation of results difficult. Episodes of infectious diseases vary greatly from case to case and may also differ to a considerable extent from one location to another. For many reasons their recognition in different studies may be based upon very different criteria. Finally, although the phenomenon has been noted for many years, it is only relatively recently that research workers have given due regard to the fact that the acute-phase reaction associated with infections also causes a depression of serum retinol. This casts doubt on the value of serum retinol as an indicator of vitamin A status under such circumstances. This subject will be considered in more detail in Chapter 13.
Vitamin A deficiency has been induced in experimental animals for many decades. The uniform experience has been that this nutritional deficiency, more than any other, is associated from a relatively early stage with the development of infections, especially those affecting epithelial tissues. Most prominent have been those of the upper and lower respiratory tracts, parts of the gut, and the genito-urinary tract (Moore 1957). These infections often became apparent early on in the deficiency, long before the changes of xerophthalmia occurred (McLaren 1959).

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

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

Intervention trials cast some light on this last point. Vitamin A supplementation does not appear to have an effect on mild diarrhea (usually defined as 3 or 4 stools/day). As was noted earlier, it does reduce diarrhea-related deaths. As expected then, it also reduces the incidence of severe diarrhea and the benefit is proportional to the number of stools/day. This evidence supports the suggestion made long ago (see Chapter 13) that in many instances diarrhea is an integral part of VADD.

Recently the application of more appropriate and powerful statistical methods to a large community trial of the effect of vitamin A supplementation on diarrhea in young children in Brazil revealed much enhancement of beneficial effects (Andreozzi, Bailey, Nobre et al. 2006).

Zinc supplementation has been shown to have a marked effect in diarrheal disease in undernourished children (see Chapter 11). In a trial in which vitamin A was also given it was shown that groups receiving zinc had better outcomes in every way measured compared with those receiving vitamin A or those serving as controls. Parameters measured were stool output, cumulative stool weight, body weight gain, and rapidity of clinical recovery (Khatun, Malek, Black et al. 2001).

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

Animal studies, clinical and autopsy studies in children, and observational studies in human populations all show an association between vitamin A deficiency and respiratory infections. Respiratory disease prevalence increased in a linear fashion with increasing severity of xerophthalmia in a large hospital study (see Figure 9.6).

However, meta-analysis from mortality and morbidity intervention trials showed in general a lack of any impact of vitamin A supplementation on acute lower respiratory tract infections (ALRIs). There was some indication that there might be reduction in the severity, if not the duration, of respiratory symptoms. It has been pointed out that increase in cough in the supplemented group might be a favorable response (Herrera, Fawzi, Nestel 1996). Strength of the cough reflex could be beneficial in removing infective material. These conclusions have been supported in general by subsequent studies from other countries. Treatment with vitamin A of respiratory syncytial virus (RSV) infection had no
significant benefit (Dowell, Papic, Bresee et al. 1996).

In a meta-analysis of all adequate studies that were available at the time (Vitamin A and Pneumonia Working Group 1995) no major reduction occurred in the mortality or morbidity associated with respiratory infections as a result of vitamin A supplementation. A meta-analysis of randomized controlled trials of vitamin A supplementation for both diarrheal disease and respiratory infections concluded that high-dose vitamin A supplementation should not be recommended routinely for all preschool-age children (Grotto, Mimouni, Gadlevich et al. 2003). A more recent meta-analysis (Brown, Roberts 2004) involved 5 studies with acceptable criteria and included a total of 1,067 intervention and 1,110 control children. Main outcome measurements were: time to normalization of fever; respiratory rate and oxygen dependence; time to discharge; and mortality. There were no significant differences for any measurement. Another study (Rodriguez, Hamer, Rivera et al. 2005) failed to find improvement with vitamin A supplementation, but better results were associated with higher initial serum retinol concentrations. Although this was not investigated it might have related to the acute phase response (APR) (see Chapter 7).

A study was undertaken in Tanzania (Fawzi, Mbise, Spiegelman et al. 2000) to determine the effect of vitamin A supplementation on the risk of young children with pneumonia developing diarrhea and acute respiratory infection. Supplementation resulted in less risk of severe watery diarrhea, but higher risk of cough and elevated respiratory rate. The latter was confined to children who were seronegative for HIV.

In southern India, Coles, Kanungo, Rammathullah et al. (2001) studied pneumococcal nasopharyngeal colonization in young infants. In their experience, Streptococcus pneumoniae is the most frequent bacterial cause of morbidity and mortality in this group. Among 464 newborns studied, samples collected at ages 2, 4, and 6 months were positive in 54%, 64.1%, and 70.2% respectively. The presence of maternal night blindness increased the risk of colonization three-fold. It was considered that the high rate of pneumococcal carriage in early infancy might explain the high risk for pneumonia.

Figure 9.6: The prevalence of respiratory disease among Indonesian children presenting to the Cicendo Eye Hospital increased with the severity of their xerophthalmia (P<0.01 for linear trend) (Sommer 1982).
An interesting hypothesis has been put forward by Sempertegui (2001). Based on impairment in lung function as measured by FEV1 (forced expiratory volume in one second) and FVC (forced vital capacity) in children with evidence of vitamin A deficiency it is proposed that this might predispose them to respiratory infections.

It will not be possible to make real progress in this area until the precise diagnosis of respiratory diseases is clarified. What they call a “descriptive necropsy study” has been reported by Chintu, Mudenda, Lucas et al. (2002) from Lusaka University Teaching Hospital, Zambia. Autopsies were performed on 137 boys (93 HIV-1 positive) and 127 girls (87 HIV-1 positive) aged 1 month to 15 years. The four most common respiratory disease diagnoses were acute pyogenic pneumonia (39.1%), Pneumocystis carinii pneumonia (27.5%), tuberculosis (18.8%), and cytomegalovirus infection (20.2%). HIV-1 positivity predisposed to the occurrence of P. carinii pneumonia and cytomegalovirus infections. Eighty-eight percent of P. carinii infections were in cases younger than 12 months. Detailed pathology is bound to vary widely from place to place and would seem to render invalid blanket statements being made at present. The same may apply for diarrheal diseases, and the nutritional status, including but not confined to vitamin A, will be important.

Measles

The evidence for the association between vitamin A deficiency and measles, in its various aspects (see earlier and Chapter 13), is stronger than for any other infectious disease. Diagnosis probably has a firmer basis, as a single organism is responsible and the clinical picture is usually typical. Measles is the only specific disease for which there is firm evidence that vitamin A status influences morbidity and mortality (Sommer, West 1996:62–98).

Hospital-based studies in South Africa demonstrated that both the complications (see Table 9.6) and outcome (see Table 9.7) in measles are significantly improved by vitamin A supplementation.

Outcome during the prolonged recovery after discharge home is also markedly improved by a further dose of vitamin A (see Table 9.8).

Both measles and vitamin A deficiency are known to impair immune response and this combination helps to explain the serious nature of the disease in most developing countries. With the widespread take-up of measles immunization as part of the WHO Expanded Program on Immunization (EPI) there is already evidence of a marked fall in corneal blindness associated with measles (Foster, Yorston 1992). The combination of vitamin A supplementation with measles immunization is discussed later (see Chapter 15).

Some work suggests that a single dose of 200,000 IU (210 mmol) retinol does not enhance

**Table 9.6: Measles complications – Cape Town vitamin A controlled treatment trial (Hussey, Klein 1990).**

<table>
<thead>
<tr>
<th>Complication</th>
<th>Vitamin A N=92</th>
<th>Controls N=97</th>
<th>Relative Risk Vitamin A : Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia ≥10 days</td>
<td>12</td>
<td>29</td>
<td>0.44 (0.24, 0.80)</td>
</tr>
<tr>
<td>Diarrhea ≤10 days</td>
<td>8</td>
<td>21</td>
<td>0.40 (0.19, 0.86)</td>
</tr>
<tr>
<td>Post-measles croup</td>
<td>13</td>
<td>27</td>
<td>0.51 (0.28, 0.92)</td>
</tr>
<tr>
<td>Requiring airway intervention</td>
<td>3</td>
<td>9</td>
<td>0.35 (0.10, 1.26)</td>
</tr>
<tr>
<td>Herpes stomatitis</td>
<td>2</td>
<td>9</td>
<td>0.23 (0.05, 1.06)</td>
</tr>
<tr>
<td>Intensive care</td>
<td>4</td>
<td>11</td>
<td>0.38 (0.13, 1.16)</td>
</tr>
<tr>
<td>Hospital days</td>
<td>10.54</td>
<td>15.24</td>
<td>P&lt;0.004</td>
</tr>
</tbody>
</table>
the immune response nor is it effective in reducing measles complications (Rosales, Kjolhede, Goodman 1996) and doubling the dose may be required. Where access to healthcare and immunization is good and where VADD is mild to moderate, vitamin A supplementation may have no effect on general morbidity (Ramakrishnan, Latham, Abel et al. 1995). High-dose treatment (one dose of 200,000 IU vitamin A) did not improve morbidity in hospitalized, malnourished children in Congo, while daily low dosage (5,000 IU) reduced the incidence of diarrheal disease but had no effect on acute lower respiratory infections (Donnen, Dramaix, Brasseur et al. 1998). In one study in Indonesia (Humphrey, Agoestina, Wu et al. 1998) neonatal vitamin A supplementation reduced infant mortality and the prevalence of severe respiratory infection among young infants.

The most serious nonfatal complication of measles is blindness. A recent review (Semba, Bloem 2004) calculates that measles affects about 30 million children/year, of whom about 1 million die. It is suggested by these authors that measles is now the single leading cause of blindness among children in low income countries; about 15,000–60,000/year.

Table 9.7: Hospital outcome – Durban measles vitamin A controlled treatment trial (Coutsoudis, Broughton, Coovadia 1991; Coutsoudis, Kiepiela, Coovadia et al. 1992).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Vitamin A (N = 29)</th>
<th>Placebo (N = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (days) – pneumonia</td>
<td>3.8 ± 0.4</td>
<td>5.7 ± 0.8</td>
</tr>
<tr>
<td>Duration (days) – diarrhea</td>
<td>3.2 ± 0.7</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>Duration (days) – fever</td>
<td>3.6 ± 0.3</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>Clinical recovery in &lt; days</td>
<td>28 (96%)</td>
<td>20 (65%)</td>
</tr>
<tr>
<td>IMS on day 8*</td>
<td>0.24 ± 0.15</td>
<td>1.37 ± 0.40</td>
</tr>
</tbody>
</table>

* Integrated morbidity score

Table 9.8: Post-hospital outcome – Durban measles vitamin A controlled treatment trial (Coutsoudis, Broughton, Coovadia 1991; Coutsoudis, Kiepiela, Coovadia et al. 1992).

<table>
<thead>
<tr>
<th></th>
<th>6 Weeks</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin A (N = 24)</td>
<td>Placebo (N = 24)</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>1.29 ± 0.17</td>
<td>0.90 ± 0.14</td>
</tr>
<tr>
<td>Diarrhea episodes</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Score/episode – diarrhea</td>
<td>2.17 ± 0.31</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>URI* episodes</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Score/episode – URI</td>
<td>1.71 ± 0.28</td>
<td>2.66 ± 0.17</td>
</tr>
<tr>
<td>Pneumonia episodes</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Score/episode – pneumonia</td>
<td>4.40 ± 0.98</td>
<td>6.67 ± 0.67</td>
</tr>
<tr>
<td>Chest x-ray score ≥3</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>IMS **</td>
<td>2.21 ± 0.45</td>
<td>5.74 ± 1.17</td>
</tr>
</tbody>
</table>

* Upper respiratory infections
** Integrated morbidity score
Although up-to-date data are not available in suitable form to settle this important issue it seems more likely that xerophthalmia is still the major single cause of childhood blindness, although greatly reduced in recent years. In the 1990s it was estimated that there were worldwide about 300,000 young children blind from xerophthalmia; ten times that number had clinical xerophthalmia (i.e. night blindness and Bitot’s spot) (see Chapter 1). It is not possible to know whether that number has changed since. The world’s population is still rapidly growing; data collection is of better quality and wider in coverage; on the other hand many national and part-national control programs have been started in this period.

About two decades ago there was general agreement that about 5–10 million cases of xerophthalmia (all stages) were occurring annually. Of these about 10% (0.5–1 million) led to blindness. About half of those affected would not survive, leaving a minimum of about 250,000 cases of blindness – very much greater than the range suggested for measles (see also Chapters 12 and 13).

A recent study in the slums of Kolkata (Calcutta), India gives an impression of the very unsatisfactory situation facing many children in the ever growing shanty towns throughout the developing world (Ray, Mallik, Munsi et al. 2004). Twenty clusters, each of 250 children under 5 years old who had a history of measles in the past year, were studied. Only 19.7% had been immunized. Only 16.9% had received vitamin A before measles and 8.6% afterwards.

A closer look has been taken at the results of the use of vitamin A supplementation in the treatment of measles (Souza, Souza 2002). In a meta-analysis there was no significant reduction in mortality in the vitamin A group when all studies were pooled. There was a 64% reduction with two doses of 200,000 IU each. Two doses of water-dispersible vitamin A were associated with an 81% reduction (only 48% with oil). A single dose of 200,000 IU was not effective. These effects were greater in children under the age of 2 years.

A new hypothesis has been put forward to explain the greater beneficial effect of vitamin A in measles, as compared with other infections (Enwonwu, Phillips 2004). The hypothesis suggests that measles, more than any other childhood infection, causes hyporetinemia and marked hypercortisolemia. The latter promotes a shift in lymphocyte profile from TH1 to TH2 and influences hepatic acute phase protein response through enhancement of the activity of interleukin 6. It also antagonizes effects of retinoids at the cellular and transcriptional levels. Glucocorticoids decrease expression of receptors for retinoic acid. It is therefore suggested that increased tissue levels of cortisol could explain the well-documented benefits of vitamin A in acute infections, especially measles.

In infants under the age of 9 months with measles the presence of pneumonia on admission and low vitamin A status (by conjunctival impression cytology) were independently associated with mortality (overall 16%) (Courtright, Fine, Broadhead et al. 2002).

HIV/AIDS

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

It was at first reported that mothers with HIV infection have an increased chance of passing on the infection to their offspring if their serum retinol is low (Semba, Miotti, Chipangwai et al. 1994). However, this has not been subsequently supported by three controlled trials of vitamin A supplementation of HIV-positive pregnant women, and an extensive review more recently (Wiysonge, Shey, Sterne et al. 2005). No significant differences were found in mother-to-child-
transmission between control and supplemented groups (Humphrey 2000; Blaner, Gamble, Burger et al. 1997). One study was unable to show any beneficial effects of vitamin A supplementation on pregnancy outcomes and T-cell counts in HIV-infected women, although these occurred with multivitamins (Fawzi, Msamanga, Spiegelman et al. 1998). Maternal vitamin A deficiency during HIV infection is reported to predispose to growth failure (Semba, Miotti, Chipangwri et al. 1997) and to increased infant mortality (Semba, Miotti, Chipangwri et al. 1995). Vitamin A supplementation of HIV-infected infants significantly reduced morbidity (Coutsoudis, Bobat, Coovadia et al. 1994) and reduced mortality among both HIV-infected and non-infected malnourished children (Fawzi, Mbise, Hertzmark et al. 1999).

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

Some earlier conclusions about an association between low serum retinol levels and certain aspects of HIV infection need to be reconsidered in the light of what was said earlier about the influence of the acute phase response (APR) (see Chapter 7).

Kennedy, Kuhn, and Stein (2000) undertook an exhaustive review of vitamin A and HIV infection and came to certain conclusions. In-vitro studies have provided evidence for the role that retinoic acid (RA) plays in suppressing HIV-1 viral expression. However, supplementation trials have not provided consistent evidence of therapeutic benefits of vitamin A. Randomized placebo-controlled trials conducted on adults have not been shown to confer benefit as far as vertical transmission, immune status, and disease progression are concerned. An exhaustive meta-analysis of vitamin A supplementation and mother-child transmission of HIV infection has shown that earlier hopes were not confirmed (Shey, Brocklehurst, Sterne 2002). However, evidence from supplementation trials conducted among HIV-infected children suggests some potentially beneficial uses of vitamin A during HIV-1 infection. For example, there was a reduction in the occurrence of diarrhea-related morbidity and mortality.

A disturbing report from Tanzania (Fawzi, Msamanga, Hunter et al. 2002) found that among 1,078 HIV-infected pregnant women vitamin A alone versus supplementation with vitamins B, C and E increased the rate of HIV transmission. Multivitamin supplementation (vitamins B, C and E) reduced child mortality and HIV-transmission. Further research to clarify the potential clinical application of vitamin A supplementation is still warranted.

Three studies have investigated genital shedding of viral cells with differing results. No relation was found between genital viral burden in women infected with HIV and vitamin A status (French, Cohen, Gange et al. 2002). In women infected with both HIV and HSV (herpes simplex virus) vitamin A supplementation did not influence genital HSV shedding (Baeten, McClelland, Corey et al. 2004). In a trial with vitamin A and/or multivitamins B-complex, C and E, significantly more women had detectable levels of HIV-1 in cervicovaginal lavage compared with the multivitamin group. The results raise concern about the use of vitamin A supplementation in these circumstances.

Mastitis, or increased mammary permeability, in an HIV-infected mother appears to increase the risk of mother-to-child-transmission (MTCT) of HIV through breast milk. Vitamin A reduces the prevalence of bacterial mastitis, and vitamin A deficiency in HIV-infected women is associated with increased risk of MTCT. Vitamin A supplementation, surprisingly, increased the risk of MTCT (Dorosko 2005).

In Uganda, (Melikian, Mmiro, Ndugwa et
al. 2001) it was found in infants infected with HIV that serum retinol and carotenoids were both predictive of growth retardation, but only carotenoids were related to risk of death. It would seem that the acute phase response was operative in these sick children. Carotenoids and retinol might have been affected differently. Carotenoids are much more indicative of recent dietary intake, which would have been very low near death.

As in other contexts attention has been turned in recent years to supplementation with multimicronutrients. In particular three studies have been carried out by members of the Department of Nutrition of the Harvard School of Public Health. Villamor, Msamanga, Spiegelman et al. (2002) studied HIV-infected women during pregnancy. Multivitamin supplementation, not including vitamin A, significantly improved weight gain during the 3rd trimester. Multivitamins, including vitamin A, improved weight gain during the second trimester. Fawzi, Msamanga, Wei et al. (2003) gave women with HIV supplementation during pregnancy and lactation. Children of women who received multivitamins (not including vitamin A) had lower risk of diarrhea and higher CD4+ count. Those receiving vitamin A had children with a lower risk of pneumonia, but diarrhea and CD4+ cell count were unaffected. Fawzi, Msamanga, Spiegelman et al. (2004) monitored progression of disease and mortality. Multivitamin supplements (without vitamin A) delayed the progression of HIV and resulted in higher CD4+ and CD8+ counts and lowered viral loads. Adding vitamin A to the multivitamin regimen reduced the benefit with regard to some of the end points. Vitamin A alone was no better than placebo. Miller, Stoltzfus, Iliff et al. (2005) reported that it appears that while postpartum vitamin A supplementation did not affect mortality it reduced severe morbidity. Neonatal and maternal vitamin A supplementation had a beneficial effect on anemia in infants of both HIV positive and negative mothers and should be considered for inclusion in programs to prevent infantile anemia.

Recently a report has been published on the effect of periodic vitamin A supplementation on mortality and morbidity of HIV-infected children in Uganda (Semba, Ndugwa, Perry et al. 2005). 181 HIV-infected children were enrolled at 6 months and randomized to receive vitamin A supplementation, 60 mg retinol equivalent, or placebo every 3 months from age 15 to 36 months. Mortality and some morbidity features were significantly reduced in the vitamin A supplemented group.

Another long-awaited contribution to this complex area of field research on vitamin A and HIV infection is a massive trial in Zimbabwe with a total of over 14,000 mother-infant pairs (Humphrey, Iliff, Marinda et al. 2006). It is accompanied by a critical editorial by Fawzi (2006). From these papers and earlier evidence cited above the conclusions section of the paper may be quoted as reflecting a consensus view at present. “Targeted vitamin A supplementation of HIV-positive children prolongs their survival. However, postpartum maternal and neonatal vitamin A supplementation may hasten progression to death in breastfed children who are PCR (polymerase chain reaction) negative at 6 weeks. These findings raise concern about universal maternal or neonatal vitamin A supplementation in HIV-endemic areas.”

Malaria
In an early study no relation was found between vitamin A status and malarial infection (Binka, Ross, Morris et al. 1995). A later study in Papua New Guinea (Shankar, Genton, Semba et al. 1999) found a substantially lower incidence of clinical episodes of Plasmodium falciparum malaria (P=0.001), parasite density (P=0.09), and prevalence of spleen enlargement (P=0.01) in children attending health facilities. No such effects were found in Plasmodium vivax infection. The role of iron and zinc supplementation in malaria is considered in Chapter 11.

Shankar (2000) in a comprehensive review pointed out that malaria had been on the increase for the past three decades. Morbid episodes stand at about 200 million and deaths at about 203 million/year. Malnutrition increases both mortality
and morbidity. Supplementation with vitamin A or zinc can substantially reduce clinical attacks. Although iron supplementation may aggravate some aspects of malarial infection, the author argues that these low-cost measures should be added to current methods of malaria prevention and treatment.

Dreyfuss and Stoltzfus (2000) demonstrated the interrelationships between hookworm infestation, malaria, and vitamin A deficiency, iron deficiency and anemia in pregnant Nepalese women. Hookworm infestation was the strongest predictor of iron status. Low serum retinol was most strongly associated with mild anemia. \( P. \) vivax malaria and hookworm infestation were strongest predictors of moderate to severe anemia. The need for planners to take account of all these adverse factors is stressed.

In Mozambique, children hospitalized with severe malaria, in addition to routine treatment, received either vitamin A or a placebo and were followed for six weeks after discharge (Varandas, Julien, Gomes et al. 2001). Deaths in the two groups were 7 and 13% respectively. Although the difference was not statistically significant, routine supplementation with vitamin A would seem justified under these circumstances.

Some rather puzzling results have recently been published of the effects of vitamin A and anti-malaria treatment on erythropoietin production in severely anemic and vitamin A deficient children in Zanzibar (Cusick, Tielsch, Ramsan et al. 2005). Vitamin A decreased erythropoietin production, inflammation was reduced rapidly, and iron was mobilized from stores and new erythrocytes were produced.

Vitamin A supplementation may reduce placental parasitemia, believed to play a part in the adverse effects of malaria infection in pregnancy (Cox, Staalsoe, Arthur et al. 2005).

Increasingly, resistance of \( P. \) falciparum to antimalarial drugs is posing a serious public health problem. Two recent papers from the same research group in Austria suggest that vitamin A in vitro inhibits schizont maturation in the presence of artemisinin (Thriemer, Wernsdorfer, Rojanawatsirivet et al. 2005) and desbutyl-benflumetol (Samal, Rojanawatsirivet, Wernsdorfer et al. 2005).

**Tuberculosis**

Pulmonary tuberculosis has long been known to be a common and serious infection throughout the developing world. It is on the increase there and in the developed world, especially in the dangerous form that is multi-drug resistant.

A considerable body of both animal experimental and clinical human research on the relationship between vitamin A deficiency and the spread and progress of the disease had been carried out throughout the latter part of the 20th century. This has been well summarized in the chapter on nutrition and tuberculosis by Whalen and Semba (2001). Unfortunately this topic had escaped our attention when the first two editions of the Manual were written. More recent research is summarized below.

In childhood tuberculosis it has been shown that sCD30 (a putative marker of type-2 cytokine-producing cells) is greatly increased. Malnutrition made the levels even higher and vitamin A therapy caused them to modulate over time.

Pulmonary tuberculosis and HIV-infection frequently occur together; especially in malnourished patients. Perhaps not surprisingly, this concurrence leads to even greater impairment of vitamin A status. Vitamin A supplementation, as judged by raised serum retinol levels, appears to be of benefit in both diseases (Mugusi, Rusizoka, Habib et al. 2003).

A double-blind, placebo-controlled trial of patients with pulmonary tuberculosis was carried out in Indonesia (Karyadi, West, Schultink et al. 1997). The study group of newly diagnosed patients received both vitamin A 1,500 RE, as retinyl acetate, and 15 mg zinc, as zinc sulfate, daily for 6 months. In this group sputum conversion and resolution of X-ray lesion area occurred earlier.

Several studies have shown that patients with active tuberculosis have lowered serum retinol levels. In error the conclusion has frequently been drawn that this is indicative of subnormal
vitamin A status. The explanation, relating to the acute phase response (APR) to infection and inflammation, is given in Chapter 7.

Interestingly, a recent study (Ramachandran, Santha, Garg et al. 2004) of serum retinol levels in sputum-positive pulmonary tuberculosis patients in comparison with household contacts and healthy ‘normals’ demonstrated this point. Before treatment serum retinol levels were significantly lower in tuberculosis patients. After anti-tuberculous treatment, to which vitamin A supplementation was not added, serum retinol levels returned to normal.

In Indonesia a double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis (Karyadi, West, Schultink et al. 1997) was carried out. After antituberculosis treatment plasma zinc concentrations were not altered. Plasma retinol concentrations were significantly higher in the micronutrient group than in the placebo group after 6 months (P<0.05), as were sputum conversion (P<0.05) and resolution of X-ray lesion area (P<0.01). Deficiencies of micronutrients, including vitamin A, in adult patients with pulmonary tuberculosis are associated with wasting and higher HIV load (van Lettow, Harris, Kumwenda et al. 2004).

**Other infections**

**Urinary tract**

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

**Otitis media**

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

**Meningococcal disease**

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

**REMARKS**

Despite a great deal of research there is still much we do not yet know about the precise contribution that VAD makes to morbidity in infectious diseases. This will probably remain so until we are in possession of more accurate methods of defining the various manifestations of morbidity and until some existing discrepancies have been resolved. These latter have been discussed by Kirkwood (1996). Epidemiological studies, described here, have indicated that vitamin A supplementation reduces child mortality and severe morbidity and that most of the reduction is due to the effect on diarrhea and measles. These studies have not shown a similar effect on acute lower respiratory tract infections (ALRIs). No explanation exists at present for this difference, especially in view of the evidence that supplementation with zinc benefits both diarrheal and respiratory infections (see Chapter 11). Observational studies and animal experiments tend to show a beneficial effect of supplementation on lung disease. Moreover, in clinical trials on measles much of the impact of vitamin A on mortality and morbidity has been related to reduction in incidence and severity of associated pneumonia. Notwithstanding, all are agreed that undernourished children treated for pulmonary infections should be given supplemental vitamin A to improve their status.
10 Other Effects of VAD

In the first edition of the Manual it was suggested that much of the evidence for the importance of VAD in this chapter was based on work in experimental animals and lacked firm evidence as far as human disease was concerned. In recent years, there has been a steady increase in our knowledge of the role of vitamin A, especially in the form of retinoic acid (see Chapter 6) in many systems of the body. We can with ever increasing confidence use the term vitamin A deficiency disorders (VADD) to cover all possible consequences of the lack of dietary intake of vitamin A.

ANEMIA

It has long been known that deficiencies of iron and vitamin A tend to exist together in population groups. This might be expected to occur because deficiencies in natural diets are likely to affect more than a single nutrient. Controlled trials have been carried out in several countries in which vitamin A supplementation has brought about a significant increase in hemoglobin level (Sommer, West 1996:150–62). The best results were obtained when daily ingestion of vitamin A in amounts close to recommended levels of intake was used.

Fortification of sugar with vitamin A was used in Guatemala to study the effect on iron status over a prolonged period (Sommer, West 1996:150–62). Table 10.1 shows the mean changes sampled at 6-monthly intervals over a 2-year period. It was concluded that initially enhanced iron mobilization led to lower iron stores. This probably triggered increased efficiency of iron absorption, which would gradually restore iron stores. These in turn would make iron available for hemopoiesis, at a level greater than present before sugar fortification began.

An entirely different mechanism of vitamin A-iron interaction is suggested by the work of Layrisse, Garcia-Casal, Solano et al. (2000). They measured double-labeled iron absorption in nearly 200 healthy adults. The object was to determine whether there was any interaction between vitamin A or β-carotene and inhibitors of iron absorption. The presence of vitamin A increased iron absorption threefold from rice,

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

* P ≤ 0.05

Table 10.1: Change in serum retinol and iron status in preschool children following sugar fortification with vitamin A in Guatemala (Mejia, Arroyave 1982).
2.4-fold from wheat, and 1.8-fold from corn. β-carotene increased iron absorption almost threefold from the three cereals tested. It is suggested that vitamin A and β-carotene may form a complex with iron reducing the binding to phytate or polyphenols.

It has been suggested that vitamin A may improve anemia by increasing the synthesis of erythropoietin, a hormone produced in the kidney that stimulates red cell production. Semba, Kumwenda, Taha et al. (2001) could not confirm this in anemic pregnant women. However, recently Zimmermann, Biebinger, Rohner et al. (2006) found that vitamin A supplementation in children with poor vitamin A and iron status increased erythropoietin levels as iron was mobilized from existing stores.

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

The prevalence of anemia associated with vitamin A deficiency has not been estimated but is likely to be quite high, as both deficiencies are common. Especially vulnerable are young children and women in the reproductive age period. In the Pacific region, Lloyd-Puryear, Humphrey and West (1989) showed that the prevalence of subclinical vitamin A deficiency, as evidenced by abnormal conjunctival impression cytology (CIC), was inversely proportional to the hemoglobin level (see Figure 10.1).

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

Animal work showed that a fall in hemoglo-

\[ \text{Figure 10.1: Relationship between hemoglobin level and prevalence of vitamin A deficiency assessed by conjunctival impression cytology (CIC) in children 3–6 years of age in Truk, Micronesia (Lloyd-Puryear, Mahoney, Humphrey et al. 1991).} \]
bin due to feeding low-iron diets was accompanied by a lowering of plasma retinol, but by an increase of hepatic retinyl esters. Low iron status seems to interfere with hepatic release of vitamin A (Rosales, Jang, Pinero et al. 2000). In animals it was also shown that marginal vitamin A intake during lactation caused iron levels in milk to fall by depressing the action of iron transporters in mammary glands (Kelleher, Lonnerdal 2005).

A recent large human study in Zimbabwe (Miller, Stoltzfus, Iliff et al. 2006) raises some questions. Nearly 1,600 infants were allocated, with their mothers, to one of four treatment groups – vitamin A to mothers and infants; vitamin A to mothers and placebo to infants; placebo to mothers and vitamin A to infants; placebo to both. Over 8–14 months no effect of vitamin A on hemoglobin or anemia was observed. It is possible that in this, as in a similar study (van den Broek, White, Flowers et al. 2006), initial vitamin A levels were mostly within the normal range.

Since 2000, with the discovery of a peptide hormone produced by the liver, later named hepcidin, it has become clear that iron hemostasis is maintained through meticulous regulation of circulating hepcidin levels. Many factors may influence this process, of which vitamin A status is one. Details of the mechanisms involved are the subject of intense current research (Wrighting, Andrews 2006).

**GROWTH**

The first clearly evident response of a young animal to dietary restriction of any kind is a decrease in the velocity of growth. Young animals, and children, have relatively higher nutritional requirements partly because they need nutrients for growth as well as maintenance. Vitamin A is no exception (see Chapter 6), and in fact the growing organism appears to be especially sensitive to VAD.

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

Another difficulty is the multiplicity of ways in which growth is customarily assessed. Skeletal measurements, such as height and head circumference, cannot decrease during undernutrition but undergo a decrease in the velocity of their rate of increase. There is no absolute loss. Height/age is usually employed to indicate “stunting.”

On the other hand, measurements of soft tissues like muscle and fat, including body weight, skinfold thickness and muscle circumference measurements, may involve absolute loss of tissue. Weight/height indicates “wasting,” a more acute state of growth retardation. Some of these measurements are subject to quite large observer errors. While body weight can be measured with considerable accuracy, there has to be some doubt about its interpretation, especially in children. Body composition is known to vary considerably in malnutrition; fat and muscle decrease but water increases proportionately. Consequently weight may not accurately reflect healthy body mass. The relationship between anthropometric status and mortality is mentioned in Chapter 9.

Children with corneal xerophthalmia have consistently been observed to be stunted. This has perhaps best been documented in the countrywide survey in Indonesia carried out in 1978–79 (Sommer 1982) (see Figure 10.2). The degree of vitamin A deficiency was proportional to the degree of retardation of growth. There is also evidence of frequent wasting in xerophthalmic children. In this more acutely deficient state, mortality may make interpretation difficult.

It appears that periodic large-dose vitamin A supplementation has a significant impact on growth in children with xerophthalmia, but not on those with evidence of subclinical deficiency. However, three studies in which preformed vitamin A was consumed in adequate amounts
regularly showed slight improvement in linear growth (Sommer, West 1996:163–88).

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

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

In Sudan Sedgh, Herrera, Nestel et al. (2000) studied more than 8000 initially stunted children aged 6–72 months. Their intake of vitamin A over a 24-hour period and their height and weight were measured. The observations were repeated at 6-month intervals for 18 months of follow up. Children who were initially in the highest quin-

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Figure 10.2: Children with corneal disease (X2/X3) were shorter than children who had Bitot’s spots (X1B) (P<0.01); children with X1B were shorter than their matched controls (P<0.01). There were no significant differences in height between randomly sampled children without xerophthalmia (normal random sample) and matched controls (Sommer 1982).
tile grew on average 13 mm more than those in the lowest quintile. Carotenoid intake was proportional to improvement in height. Dietary effects on growth appeared to be strongest among very young children and upon those who had been most malnourished. Other factors, including breastfeeding and socioeconomic status, were also important.

In recent years, a great deal of evidence has been obtained that suggests that impaired growth in utero and during the first year or two of life is strongly associated with the development in later life of such chronic degenerative diseases as coronary heart disease, hypertension, central obesity, and diabetes type II (Barker 1998). While the association seems definite, the mechanisms are unclear. Work from Brazil (Rondo, Abbott, Tomkins 2001) found a significant direct relationship between several growth measurements at birth and serum retinol in cord blood. Several other studies have not confirmed such a relationship.

It has become clear that supplementation with vitamin A alone will not bring about optimal improvement in growth. Other micronutrients must also be taken into account, just as they are in relationship to control of infections (see Chapter 11).

A review from Mexico concluded that zinc had the greatest effect on children’s growth, followed to a lesser extent by vitamin A and iron. These beneficial effects were confined to children with severe deficiency (Rivera, Hotz, Gonzalez-Cossio et al. 2003).

The same group (Rivera, Gonzalez-Cossio, Flores et al. 2001) found that feeding a formula containing 1.5 RDA of 13 vitamins and 6 essential elements to infants daily for an average period of just over 12 months resulted in significantly greater increase than in those receiving placebo. The main lesson from this unusual study is perhaps that the majority of young children in developing countries are stunted because they are undernourished with regard to many nutrients.

An important contribution to our understanding of the complex interaction of infection, diet and seasonal growth response was made recently in a study of Indonesian preschool-age children (Hadi, Dibley, West 2004). 1,405 children aged 6–48 months were given high dose vitamin A every 4 months. A significant effect of vitamin A supplementation on linear growth occurred at all seasons in children with a low burden of respiratory infections (<21 days with respiratory illness). In each season, the highest effect was found in children with low burden of respiratory infection and low vitamin A intake (<400 RAE/d). There was no benefit for linear growth from vitamin A supplementation in children with both high burden of respiratory infection and high vitamin A intake, regardless of season. Under these conditions, it is evident that vitamin supplementation did play an important part in countering seasonal growth retardation.

Research in Gambia and India points to the possible part that impairment of gut integrity may play (Thurnham, Northrop-Clewes, McCullough et al. 2000). These workers showed progressive deterioration of integrity of the gut mucosa after introduction of weaning food, which often introduces bacterial contamination. A dual sugar permeability test was used. Impairment was least from April–June, when vitamin A intake was maximum, due mainly to this being the mango season. Evidence to support this association was obtained by the finding that measurements of the acute phase protein alpha-1-antichymotrypsin (ACT) were lowest between April and June. This coincided with the lowest prevalence of subclinical infection, best gut integrity, and most rapid growth. More such studies would be very valuable.

It is appropriate to end this section with reference to the wider picture of the importance of general malnutrition in child health and survival. A recent study (Pelletier, Frongillo 2003) has quantified the effects of changes in general malnutrition, as measured by child weight-for-age, on changes in child survival in 59 developing countries. They concluded that “continued reduction in mortality will require improved targeting of selective interventions and general nutritional improvement to the most marginal populations.” Vitamin A supplementation is an important part
of this program, but, as recognized above, only a part.

There is one aspect of growth and development that has received little attention: a possible effect of vitamin A deficiency on brain growth and intellectual development. In a trial of vitamin A and folic acid during pregnancy in Indonesia the results were negative (Schmidt, Muslimatun, West et al. 2004), but it surely merits more thorough research.

**IMMUNE RESPONSE**

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

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

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

It has been proposed (Ross, 1996) that there are broadly speaking two hypotheses to explain the protective action of vitamin A against infection (see Table 10.2).

The epithelial cell linings of organs and tissues are generally considered to have a defensive function. They have been considered as providing a first line of defense in resistance to infection taking place. Ross argues that this is a misconception and that they primarily have an offensive role. On the other hand the immunologic response is a defensive response against infection once it has taken place. The beneficial effect of vitamin A supplementation has on childhood diarrhea may be determined by the regulatory effect of supplementation has on the mucosal immune response in the gut (Long, Santos, Garcia et al. 2006).

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

<table>
<thead>
<tr>
<th>Epithelial barrier hypothesis</th>
<th>Immunologic response hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>The basic reaction is OFFENSIVE</td>
<td>The basic reaction is DEFENSIVE</td>
</tr>
<tr>
<td>This protects against invasion by infection</td>
<td>This enhances the body’s defense against pathogens</td>
</tr>
<tr>
<td>Structural integrity is of greatest importance</td>
<td>Functional integrity is most important, as is cell differentiation</td>
</tr>
<tr>
<td>Consequently resistance to establishment of infection will be reduced by VAD</td>
<td>Consequently resistance to proliferation of infection will be reduced by VAD</td>
</tr>
<tr>
<td>Major effect of vitamin A intervention will be decreased incidence of infection</td>
<td>Major effect of vitamin A intervention will be decreased duration and/or severity of infection</td>
</tr>
</tbody>
</table>
Most of the evidence from both animal and human studies suggests that the various aspects of humoral immunity are little affected by vitamin A deficiency. Cell-mediated immunity, however, has been shown to be markedly impaired. The production and maturation of lymphocytes are reduced by lack of vitamin A. In a study in Indonesia it was found that the ratio of T-cells bearing CD4+ and CD8+ antigens was lower in the peripheral blood lymphocytes of xerophthalmic children compared with non-xerophthalmic controls (Semba, Muhillal, Ward et al. 1993). After vitamin A supplementation the proportion of CD4+ to CD8+ T-cells and the percentage of naive CD4+ T-lymphocytes increased.

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

Table 10.3: Main immune response elements that may be regulated by retinoids. (After Semba 1998).

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

As in other aspects of vitamin A activity, attention is beginning to be focused on the effect of deficiency of other micronutrients as well (see Chapter 11). Wieringa, Dijkhuizen, and van der Ven-Jongekrijg et al. (2003) measured cytokine production in healthy Indonesian infants. A strong positive correlation was found between production of interferon-γ, interleukin-12, and interleukin-6. In contrast, interleukin-18 was negatively correlated to interleukin-12 and interleukin-6. Interferon-γ was significantly lower in vitamin A deficient infants than in controls. Vitamin A deficient infants had significantly higher neopterin levels, reflecting increased macrophage activity. In zinc deficient infants there was a tendency towards a lower level of production of all cytokines.

In intervention studies, six groups of infants received either iron (10 mg/day), zinc (10 mg/day), iron and zinc (10 mg/day each), β-carotene (2.4 mg/day), zinc and β-carotene (10 and 2.4 mg/day respectively), or placebo. Iron supplementation enhanced type 1 immune responses. Zinc and β-carotene had immunomodulatory effects that are opposite to those of iron.

Early studies with provitamin A carotenoids showed results similar to vitamin A, but more recent work using non-provitamin carotenoids, such as lycopene and lutein, showed these to be very active in both cell-mediated and humoral immunity (Chew, Park 2004).

In mice, Stephensen, Jiang, and Freytag (2004) recently demonstrated that vitamin A deficiency impairs both T-helper type 1 (Th1) and type 2 (Th2)-mediated immune responses. At the time of initial antigen exposure, VAD enhances the development of IL-10-producing Th2 or T regulatory cells, and diminishes the development of Th1 memory cells.

Finally, in the last few years several controversial papers from the research group based in Denmark that has been investigating immunization programs in Guinea-Bissau in West Africa for many years have appeared (Benn, Lisse, Balé et al. 2000; Kristensen, Aaby, Jensen et al. 2000). These have been criticized and commented upon.
by others (e.g., Fine 2000; Folb 2001). The first issue relates to the claim they make that some vaccines, particularly measles and BCG, have a more beneficial effect on child survival than do others, such as diphtheria, tetanus, pertussis, or polio. At present agreement has not been reached and immunization programs should certainly continue.

A second issue directly involves the use of vitamin A supplementation together with immunization (Benn, Balé, George et al. 2002; Benn, Balé, Sommerfeldt et al. 2003, with comments by Stephensen 2003; McLaren 2004). The hypothesis put forward here is, in essence, that the effect of VAS depends on the routine vaccines with which it is distributed. Vitamin A, for many years, has been known to exert such adjuvant activity (Dresser 1963). This important issue is pursued further in Chapter 15 when the prophylaxis and management of infectious diseases in the control of VADD is considered.

A critical review of the known effects of vitamin A supplementation on immune responses and correlation with clinical outcomes (Villamor, Fawzi 2005) shows how many uncertainties remain in this field.

NORMAL AND DISEASED SKIN

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

In recent years, numerous retinoids have been synthesized for use in the treatment of a variety of dermatological disorders (see Chapter 14).

THYROID FUNCTION

Until recently, this area of possible impairment of function resulting from vitamin A deficiency has not been explored adequately. It has been shown that in goitrous children the severity of vitamin A deficiency is proportional to thyroid volume and concentrations of TSH and thyroglobulin. Vitamin A deficiency severity was also a strong predictor of higher concentrations of total T4. Supplementation with vitamin A reversed these abnormalities (Zimmermann, Wegmuller, Zeder et al. 2004).

REPRODUCTIVE SYSTEMS

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

There is growing evidence that vitamin A, in some form, is required for every stage in the reproductive process in the female. This is in addition to the implication of both deficiency and excess of vitamin A during the organogenetic period in the production of malformations of the fetus discussed elsewhere (see Chapters 6 and 14).

BONE

Vitamin A deficiency in cattle has a marked effect on the modeling of bone. Most notably this has led to compression of the optic nerve in the optic foramen, leading to blindness. Nothing comparable has been reported in man. However, there is increasing evidence that in man, over a period of many years, the ingestion of a higher than average consumption of vitamin A may be associated with bone abnormalities in the elderly (see Chapter 14).
11 Interaction of Vitamin A and Other Micronutrients

INTRODUCTION

Both during and for many years after what was called The Vitamin Era (McLaren 2004a), it was customary to think of each vitamin as having one particular function, at least so far as human health and nutrition was concerned. Thus iron prevented the most common form of anemia, vitamin D cured rickets, vitamin C scurvy, and vitamin A blindness. Although it was understood that people on poor diets usually had a variety of deficiencies, it was easy to lose sight of this fact when these micronutrients alone were seen to be doing such a good job at curing their associated diseases.

More recently these severe states of deficiency have been largely brought under control, only to reveal much more widespread marginal, but important, states of deficiency underneath, as it were. Perhaps not surprisingly, such marginal deficiencies very frequently coexist, and it is logical that multiple deficiencies demand a multiple approach. Hence the need to devote several sections of this book to the interaction between, and joint action of vitamin A and other micronutrients.

In theory, the task has barely begun. The number of computations and permutations of the actions and interactions of more than 40 micronutrients is astronomical. It would take courage for any scientist to say that most could be ruled out as not significant for public health nutrition. Little is known about the majority, even at the experimental level. Human trials have already been carried out in which diets were supplemented with precise amounts of as many as 13 micronutrients at a time. Multiple supplementation has not always resulted in enhanced effects over those of single nutrients. Sometimes these effects have even been adverse. Even though a start has been made in this field, there is a great deal that we do not know (McLaren 2008).

VITAMIN A AND IRON

Despite the need for a more detailed understanding of the interrelation between vitamin A and iron, it has been concluded that the prevention of VAD should also be considered when iron supplementation is given in the control of nutritional anemia (IV ACG, 1998).

The previous chapter looked at the effects of vitamin A supplementation on anemia and iron status. In the present context, most studies relate to the results of joint vitamin A and iron supplementation.

Muslimatun, Schultink, West et al. (2001) found that weekly supplementation of pregnant women with iron and vitamin A improved hemoglobin concentration; serum ferritin fell, however, and it was concluded that this resulted from improved iron utilization.

In Bangladesh it was shown that adding vitamin A to the weekly supplementation of iron and folic acid given to nonpregnant, anemic teenage girls significantly improved the response (Ahmed, Khan, Akhtauzzaman 2005; Ahmed, Khan, Jackson 2001).

When the modified relative dose response test (MRDR) was used to assess vitamin A status in a small group of pregnant Indonesian women (Tanumihardjo 2002) the MRDR was depressed by supplementation with both iron and vitamin A, but iron status was improved.

In a particularly interesting recent study mental and psychomotor development was tested in Indonesian infants whose mothers had been supplemented with vitamin A and iron during pregnancy (Schmidt, Muslimatun, West et
The subjects were considered to be moderately deficient in vitamin A and iron at the beginning of the test, and in this case, no significant improvement resulted from supplementation. It is clear that this important and complex subject requires much more thorough investigation.

An interesting approach to the investigation into the cause(s) of anemia was adopted recently in northeastern Thai schoolchildren (Thurlow, Winichagoon, Green et al. 2005) which may be beneficially replicated elsewhere. In over 500 schoolchildren aged 6–12.9 years a battery of serum tests were carried out. Importantly, those with the elevated C-reactive protein concentrations indicative of infection were excluded. The prevalence of anemia was 31%. Hemoglobinopathies, retinol concentration <0.70 μmol/L and age were the major predictors of hemoglobin concentration. Only 16% of the HbAA and HbAE children were iron deficient. The contribution of iron deficiency to anemia was low.

VITAMIN A AND ZINC

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

A number of recent studies have demonstrated that supplementation with zinc has a beneficial effect in diarrheal and some other infectious diseases (Bhutta, Black, Brown et al., 1999; Shankar, 1999) very similar to that of vitamin A. The only trial to date of both vitamin A and zinc supplementation in diarrheal disease and respiratory tract infections comes from Mexico (Long, Montoya, Hertzmark et al. 2006).

Where vitamin A supplementation was given alone there was an increase in diarrheal disease and cough with fever, whereas zinc given alone had no effect. There was no report on combined therapy. This subject in which the demonstrated beneficial role of vitamin A supplementation is being ignored is discussed further in Chapter 15.

The whole area of multi-micronutrient supplementation tends to become overcomplicated by the use of supplementation with varying combinations of the three micronutrients: vitamin A, iron and zinc. Lymphocyte responsiveness has been enhanced with vitamin A and zinc (Kramer, Udomkesmalee, Dhanamitta et al. 1993). Growth in height and weight was improved by vitamin A but not by zinc (Smith, Makdani, Hegar et al. 1999). An interesting report claimed a better hematologic response in anemic women when vitamin A and zinc were added to iron than when iron was given alone or with vitamin A (Kolsteren, Rahman, Hildebrand et al. 1999). More recently Muñoz, Rosado, López et al. (2000) have shown that supplementation with iron and zinc improved serum retinol levels, and it seems likely that there will be many more studies like these in the near future.

A trial of vitamin A and zinc in undernourished children with persistent diarrhea was undertaken in Bangladesh (Khatun, Malek, Black et al., 2001). Those children receiving zinc had lower stool output, lower cumulative stool weight, greater weight gain, and more rapid clinical recovery. Vitamin A supplementation, however, gave no benefit.

A similar study in slum children in Bangladesh (Rahman, Vermund, Wahed et al. 2001) gave rather different results. Combined vitamin A and zinc supplementation was more effective in reducing persistent diarrhea and dysentery than either zinc or vitamin A alone. In the case of respiratory infection, zinc given alone was found to significantly increase the risk. This was not entirely unexpected, as zinc is known to enhance microbial growth, function and virulence. It also inhibits some aspects of cellular immunity and facilitates pulmonary tissue injury. Combined supplementation with vitamin A and zinc significantly reduced respiratory tract infections.
Also in Bangladesh, Mahanlabis, Labiri, Paul et al. (2004) found that in boys (but not girls) treated with zinc, recovery rates from severe acute lower respiratory infection were significantly higher than for those receiving vitamin A, which had no effect when compared with a placebo.

Further studies in Bangladesh by the same group failed, perhaps not surprisingly, to demonstrate an increase in growth after short term supplementation with vitamin A and zinc (Rahman, Tofail, Wahed et al. 2002), but found that dual supplementation improved vitamin A status whilst vitamin A given alone did not (Rahman, Wahed, Fuchs et al. 2002). This study may have implications for control programs. In the National Vitamin A Week campaign 4–6 months previously, it had been found that 38% of child recipients were still vitamin A deficient.

Children with diarrhea and respiratory infections received combinations of vitamin A and/or zinc or a placebo (Rahman, Vermund, Wahed et al. 2001). Combined zinc and vitamin A supplementation was more effective in reducing persistent diarrhea and dysentery than either vitamin A or zinc alone. Zinc given alone increased respiratory illness, but the addition of vitamin A reduced this adverse effect.

More evidence for the beneficial effects of combining vitamin A and zinc supplementation comes from Nepal, Indonesia, and India. In the first, acute diarrhea in children responded to dosing with zinc, and the result was not influenced by concomitant vitamin A administration (Arne, Krisna, Rajiv et al. 2002). In the second, a case-control study of tuberculosis, combined supplementation improved the effects of treatment after 2 months and resulted in earlier sputum smear conversion (Karyadi, West, Schultink et al. 2002). In the third, in about 2,500 New Delhi slum children aged 6–30 months, the incidence of pneumonia was significantly reduced only in the group that received zinc supplementation (Bhandari, Bahl, Taneja et al. 2002).

A small study in Kolkota (Calcutta) India (Mahanlabis, Wahed, Khaled, 2002) showed no beneficial effect on measles accompanied by pneumonia when zinc was added to vitamin A supplementation.

Dijkhuizen, Wieringa, West et al. (2004) supplemented pregnant women with iron and folic acid, in combination with zinc, and/or β-carotene or neither, and the mother-infant pairs were followed for 6 months after delivery. Supplementation with β-carotene and zinc significantly improved serum retinol levels of both mothers and infants up to 6 months postpartum, and reduced the prevalence of vitamin A deficiency in infants by >30%. Breast milk retinol concentrations only increased in women who had received zinc.

In children given oral cholera vaccine supplementation with zinc, improved seroconversion to vibriocidal antibody was observed. This was not the case with vitamin A (Albert, Qadri, Wahed et al. 2003).

In undernourished children in northeast Brazil it was found that improvement in intestinal permeability, as measured by the lactulose/mannitol ratio, occurred only in those receiving both vitamin A and zinc supplementation (Chen, Soares, Lima et al. 2003).

A recent study of immunoregulatory cytokines in Indonesian infants deficient in vitamin A and zinc (Wieringa, Dijkhuisen, West et al. 2004) showed that vitamin A deficiency impaired primarily Th1 (cell-mediated) response and zinc deficiency mainly Th2 (humoral) response.

VITAMIN A, IRON, AND ZINC SUPPLEMENTATION

Amongst nonpregnant women with iron deficiency anemia who received combined supplementation of iron, vitamin A and zinc (Kolsteren, Rahman, Hildebrand et al. 1999), only in the group receiving zinc and vitamin A as well as iron did hemoglobin levels rise more than with iron alone.

The effect of combinations of iron and zinc supplementation on indicators of vitamin A status was studied in a group of preschool children (Munoz, Rosado, Lopez et al. 2000). After
6 months, improvements were observed in all groups, but with certain differences: zinc was associated with higher plasma retinol and transthyretin, but not RBP; iron was associated with increased plasma retinol, RBP, and transthyretin whereas supplementation with zinc and iron was associated with increased plasma retinol, but not RBP or transthyretin.

The effect of zinc and vitamin A was studied in young children who were receiving iron therapy for anemia and who also had diarrhea (Alarcon, Kolsteren, Prada et al. 2004). Adding zinc to iron therapy increased hemoglobin response, improved iron indices, and had a positive effect on diarrhea. Vitamin A produced no additional effect.

VITAMIN A AND IODINE

VADD and IDD (iodine deficiency disorders) frequently occur concurrently in developing countries. In rats deficient in both of these micronutrients it was shown that vitamin A supplementation, independent of iodine repletion, reduced thyroid hyperstimulation and gland size (Bieberger, Arnold, Langhans et al. 2007). The mechanism was probably through the effects of vitamin A on pituitary TSH-β gene expression.

VITAMIN A, RIBOFLAVIN AND OTHER MICRONUTRIENTS

There is some anecdotal evidence that riboflavin plays a part in both retinal rod function and in anemia. Inclusion of riboflavin in the supplementation of deficient pregnant women improved the response of anemia in China (Ma, Schouten, Zhang et al. 2008) and of dark adaptation, measured by pupillary threshold (see Chapter 8) in Nepal (Graham, Haskell, Pandey et al. 2007) in the presence of iron and folic acid supplementation.

OTHER MULTI-MICRONUTRIENT COMBINATIONS

Selenium is an essential trace element, required in µg amounts daily, and both deficiency and excess can cause disease. Baeten, Mostad, Hughes et al. (2001) found that 11% of women examined in Kenya were deficient (serum selenium <85 µg/L). Those deficient in selenium were found to be more than three times more prone to genital mucosal shedding of HIV-1-infected cells. VAD was also found to increase shedding.

Great interest is centered around the possible relationship between low birth weight and disease in later life. In Nepal, Christian, Khatry, Katz et al. (2003) studied a large group of mothers and infants and found that antenatal supplementation with folic acid and iron modestly reduced the risk of low birth weight. Additional multi-micronutrients brought no additional benefit. All groups received vitamin A, which served as a placebo.

The same group (Christian, West, Katz et al. 2005) found that a multi-micronutrient mix, consisting of iron, folic acid, and vitamin A as well as 11 other micronutrients, may reduce risk of some obstetric complications. However, the risk of obstructed and prolonged labor, possibly from increased birth size, may rise.

Also from the same group (Katz, Christian, Dominici et al. 2006) comes a statistical comment that could have important implications for the analysis and interpretation of data from this kind of study. The importance of examining whether there is a constant or variable treatment effect across the distribution of birth weight is demonstrated. Further results from this group indicate that maternal supplementation with folic acid or folic acid, iron and zinc reduced the risk of kidney dysfunction and, to some extent, metabolic syndrome among children at 6-8 years of age (Stewart, Christian, Schulze et al. 2009).

A study from Mexico (Moriaty-Craig, Ramakrishnan, Neufeld et al. 2004) raises an important question. In nonpregnant women they found
that iron supplements given alone improved hemoglobin status significantly more than did a multi-micronutrient mix containing iron at the same level. Micronutrient interactions might have had an adverse effect on iron absorption.

An unusual, but interesting, example of the fortification of salt with multi-micronutrients comes from Switzerland (Zimmermann, Wegmueller, Zeder et al. 2004). Microcapsules of salt were fortified with 30 μg iodine, 2 mg iron, and 60 μg vitamin A/g salt and given to goitrous school children. Status indices improved significantly over a 10 month period of supplementation in those receiving the mixture of vitamin A, iodine and iron. A similar study took place in Thailand in which a seasoning powder was supplemented (Winichagoon, McKenzie, Chavasit et al. 2006).

Recently the results of the largest multi-micronutrients supplementation trial so far were published. Infants aged 6–12 months received different combinations of nutrients in four countries: South Africa, Vietnam, Peru and Indonesia. The results of each country study were published separately and were remarkably similar. For simplicity, the pooled data are referred to here (Smuts, Lombard, Benade et al. 2005). Overall the best results were obtained in the group which received 11 micronutrients daily with supplements given in the form of foodlets, which have been defined as “a crushable hybrid between a tablet and a water miscible food.” This group produced greater gain in weight, but not height, than the placebo group. Anemia also improved in this group, but the results were not clear-cut. Although other similar studies are planned, with larger supplements, it is questionable whether daily dosing can, or even should, be sustained on a long term basis. Fortification may be a more sustainable approach.

It is likely that multi-micronutrient deficiencies are common in vulnerable communities, as recently shown in Nepal (Jiang, Christian, Khatry et al. 2005), and therefore many more studies, in a number of variations, may be expected in the near future. When the Johns Hopkins Group pooled the results of its two trials of antenatal micronutrient supplementation in Nepal (Christian, Khatry, Katz et al. 2003; Osrin, Vaidya, Shrestha et al. 2005), they showed that birthweight was significantly higher in the supplemented groups, while neonatal mortality was also significantly higher. Subsequent correspondence in the literature left most issues unresolved. Such unexpected results posed difficult questions.

In a broader context an NIH panel in the US is advising against multi-micronutrient supplements for treating various chronic diseases, and research discourages the taking of antioxidant vitamins for the prevention of preeclampsia (Jeyabalan, Caritis 2006). More research is needed to investigate the long-term consequences of low micronutrient intakes. Hooper, Ashton, Harvey et al. (2009) very recently have developed a new methodology for assessing potential biomarkers of the status of a number of micronutrients.

Much attention has been given recently to the significance for an organism’s age of the length of the telomere part of the gene structure of cells. Xu, Parks, De Roo et al. (2009) found that on average the relative telomere length of leukocyte DNA was 5.1% longer among daily multivitamin users than non-users.
12 Global Occurrence

PREVALENCE OF VADD

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

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

In recent years, the Micronutrients Unit of the WHO’s Department of Nutrition for Health and Development in Geneva, Switzerland, with its worldwide contacts, has taken on the role of collecting and disseminating data. The Unit claims that its data file, the Vitamin and Mineral Nutrition Information System (VMNIS), formerly known as the The Micronutrient Deficiency Information System (MDIS), is continuously updated. The previous two editions of this book, published in 1997 and 2001 respectively, used the compilation of data from WHO published in 1995. The latest publication, WHO (2009), which is used here, contains data obtained between 1995 and 2005. For more detailed information on the subject of prevalence the original should be consulted. For our purposes several key tables and figures have been reproduced here and the authors are grateful to WHO for their permission to do so.

There is a bewildering array of indicators to choose from in order to assess the vitamin A status of populations at risk of VAD across the world. It should be recognized that these data do not come from some massive study that has been planned from the center. It is a collection of data that have been produced by various organizations, using various methods, frequently not following guidelines that WHO has recommended in the past (WHO Expert Group 1976, WHO Expert Group 1982). These inadequacies are dealt with in the next section. They are not apparent from a reading of WHO (2009) but they should be addressed in future.

VAD AND BLINDNESS

The magnitude of the effects of VAD on the eye was touched on in Chapter 1. Chapter 8 was devoted to descriptions of various manifestations of xerophthalmia. Chapter 7 gave accounts of how some of the eye signs are used in the assessment of vitamin A status. Chapter 13 will include accounts as to how the eye signs have been used to increase our understanding of the epidemiology of VADD.

It has to be recognized in the present context of global occurrence of VAD that no account whatsoever is being taken of its contribution to global blindness. No data have been collected for a decade or more to indicate whether or not it is on the decrease. All data on childhood blindness, or sometimes corneal blindness, relating to prevention are merged and consequently xerophthalmia is not being recorded. Moreover, in a clinical sense xerophthalmia does not appear in the latest data published by WHO (2009). In all the maps and tables of this publication there is no mention
Figure 12.1: Night blindness as a public health problem by country 1995–2005: Preschool-age children.
Countries and areas with survey data and regression-based estimates.
Figure 12.2: Biochemical vitamin A deficiency (retinol) as a public health problem by country 1995–2005: Preschool-age children.
Countries and areas with survey data and regression-based estimates
Figure 12.3: Night blindness as a public health problem by country 1995–2005: Pregnant women. Countries and areas with survey data and regression-based estimates.
Category of public health significance (prevalence of serum retinol <0.70 µmol/l)
None (<2%)
Mild (≥2% – <10%)
Moderate (≥10% – <20%)
Severe (≥20%)
GDP ≥ US$15,000 (countries assumed to be free of vitamin A deficiency of public health significance)
No data

Figure 12.4: Biochemical vitamin A deficiency (retinol) as a public health problem by country 1995–2005: Pregnant women. Countries and areas with survey data and regression-based estimates.
Table 12.1: Country estimates of the prevalence of serum retinol <70 μmol/L in preschool-age children and pregnant women 1995–2005 showing the proportion as well as the number of individuals. (Adapted from WHO).

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* GDP ≥ US$ 15000
Table 12.1: Country estimates of the prevalence of serum retinol <70 μmol/L in preschool-age children and pregnant women 1995–2005 showing the proportion as well as the number of individuals. (Adapted from WHO).

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Table 12.1: Country estimates of the prevalence of serum retinol <70 μmol/L in preschool-age children and pregnant women 1995–2005 showing the proportion as well as the number of individuals. (Adapted from WHO).

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<td>Spain</td>
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<tr>
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<td>Severe</td>
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<tr>
<td>Tonga</td>
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<td>Trinidad and Tobago</td>
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</table>

* GDP  ≥ US$ 15000
Table 12.1: Country estimates of the prevalence of serum retinol <70 μmol/L in preschool-age children and pregnant women 1995–2005 showing the proportion as well as the number of individuals. (Adapted from WHO).

<table>
<thead>
<tr>
<th>Preschool-age children</th>
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<th>Pregnant women</th>
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<td></td>
<td>Country</td>
<td>%</td>
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<td>Public health problem</td>
<td>%</td>
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<td>*</td>
<td></td>
</tr>
<tr>
<td>United Kingdom of Great Britain and Northern Ireland</td>
<td>*</td>
<td></td>
<td>No public health problem assumed</td>
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<td>984</td>
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<td>35.8</td>
<td>610</td>
<td>Severe</td>
<td>20.0</td>
<td>75</td>
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</table>

* GDP ≥ US$ 15000
of xerophthalmia. There is yet another mistake that has persisted for two decades or so: in this period eye disorders attributable to VAD (i.e. that part of VADD affecting the eye) have not been divided, as they were in the past, into those causing blindness and into those that do not.

The result is not only that the world of micro-nutrient deficiency prevention is more than ever misled and bemused by this latest authoritative report on the subject, but also the world of blindness prevention will now have nothing to guide it on xerophthalmia. Already recent (WHO) publications on the subject fail to address vitamin A related childhood blindness in a uniform way. It may just be grouped with other causes of “childhood blindness”, or its identity may be lost in “corneal blindness in young children”. In effect, nutritional blindness, once generally recognized as the most common cause of blindness in young children worldwide, has ceased to exist. The possible implications for the work of the many preventive eye care programs are serious.

The close association between measles keratitis and xerophthalmia has been noted in other chapters, especially 9 and 13. The latter, in particular, draws attention to the frequent concurrence, especially in parts of Africa, of measles keratitis, xerophthalmia, herpes simplex, and the use of harmful local eye medicine. Measles and xerophthalmia both have a high mortality in malnourished children. In recent years mortality from measles has been reduced dramatically by the Expanded Program on Immunization (EPI) (see Chapter 15). Between 1999 and 2005 it decreased from an estimated 873,000 deaths to 345,000 deaths (Wolfson, Strebel, Gacic-Dobo et al. 2007). Unfortunately, there is no record as to what part accompanying VAS might have been playing in EPI and National Death Index (NDI) (see Chapter 15).

It has been suggested (Semba, Bloem 2004) that measles is now the leading cause of childhood blindness in low income countries, accounting for an incidence of 15,000–60,000. This range will be falling with continuing success with EPI. When numerous large field surveys of the eye signs of xerophthalmia were being made, it was found that about 10% of these led to blindness, but about 20% of those blinded were dead within a year. Xerophthalmia in young children is now estimated to have a prevalence of about 4.4 million (see Table 1.3). Reduction for deaths would still leave xerophthalmia blindness many times greater than the highest estimate for measles (McLaren 2004).

**VITAMIN A IN GLOBAL FOOD SUPPLIES**

It will be recalled from Chapter 3 and Chapter 4 that the major underlying cause of VADD is the inadequacy of some basic diets in vitamin A sources. Peoples of developing countries are especially dependent on provitamin A carotenoid sources. In recent years more thorough investigation of the vitamin A activity of these vegetable and fruit sources has shown that they are of considerably less activity than previously thought. Some of these recent estimates have been more pessimistic than others. Even when the more liberal estimates have been made there is still about a 55% overestimate globally. As a consequence of recent estimates it has become evident that many people, especially young children, living in poverty are unable to meet their vitamin A requirements through consumption of fruit and vegetables alone. Some preformed vitamin A sources appear essential. These estimates also explained the occurrence for many years of signs of xerophthalmia in the presence of apparently rich provitamin A carotenoid sources. These points are made evident in Figure 12.5.

The reader’s attention should be given to certain facts about the data that lie behind this important figure. The basic data come from FAO food intake sheets that date back as far as 1979–1981. Not only have they not been kept updated since, but the originals have not been given the pride of place which they deserve. It is no excuse to say that they are not precise enough to be applied in any way; earlier in the chapter other data in this field have been shown to be similar in this respect.
Table 12.3: Preliminary assessment of vitamin A status of a population. (After Sommer, 1995).

| 1. Interviews, by structured questionnaire, with central and provincial public health officials, clinicians, nutritionists, community health workers, directors and staff of hospitals, feeding and rehabilitation centres, and schools for the blind. |
| 2. Chart reviews at hospitals, clinics, institutions where children suspected to suffer from corneal destruction, or where coded charts do not exist, records from malnutrition, infectious diseases, pediatric and ophthalmic services. |
| 4. Search for old, healed disease in schools for the blind etc. |
| 5. Collect existing data on child rearing, including breast and other feeding and hygiene practices. |

However, it has to be questioned to what extent they may be applied to current situations.

METHODOLOGY

It is evident that careful attention should be given to the methods used for data collection on the prevalence of VADD. In a country or area where there has been no previous study it is advisable to employ a preliminary assessment, along the lines of that advised by WHO (WHO Expert Group, 1982). Table 12.3 indicates the kind of background data that should be sought in the first instance.

This can be done in a simple way that is economical in terms of personnel and resources. It will be observed that most of these data relate to the detection of a problem of xerophthalmia. If the health concern is at the subclinical level of

Figure 12.5: Vitamin A activity in the regional and world food supplies as provitamin A carotenoids with their percent of total (based on a 12:1 β-carotene:retinol conversion ratio, white segments) and as preformed retinyl esters (black segments). Stippled bar represents the previous estimate of total vitamin A using a 6:1 conversion ratio, reflecting a 55% overestimate globally. (West, Darnton-Hill 2008).
VAD then an assessment may be made using the socioeconomic and ecological indicators recommended by WHO (1996). Only if the results are highly suggestive of the presence of a significant problem should a full point prevalence survey be embarked upon. This is much more expensive and time-consuming and must be carried out meticulously if the results are to be relied upon (Sommer 1995).

**TRENDS**

A comprehensive document was published recently (Mason, Rivers, Helwig 2005) detailing trends in malnutrition in developing regions: vitamin A deficiency, anemia, iodine deficiency, and child underweight. With the ever increasing emphasis on multi-micronutrients it is especially useful to have these data available together. This Manual concentrates on data for vitamin A deficiency. The maps that appeared in its second edition are reproduced again here as the data from which they were constructed dates from October 2000, and the data behind the new documents is not more recent. A new feature is the provision of information on trends in the prevalence of both xerophthalmia and vitamin A deficiency for each region over the decade 1990–2000. Figures 12.6 and 12.7 summarize this information and indicate that, with the exception of xerophthalmia in India, trends have followed a steady downward course. Annex 1 of the WHO document should be consulted for data from individual countries and for individual years, where available.

Estimates for cases of xerophthalmia (non-blinding and blinding) have fluctuated greatly over the years, influenced in opposite directions mainly by better reporting and by an increasing number of control measures. Most recently, the prevalence figure for young children stands at 4.4 million (West 2002).

It is not known how many of those cases are for blinding xerophthalmia. The simplified procedure where all forms of xerophthalmia are grouped together and the recommended level of 1.0% is accepted as the cut-off point for “xerophthalmia” (see Chapter 1) has had the unfortunate consequence that no figures are now available for blindness due to vitamin A deficiency. Experience suggests that the ratio between nonblinding and blinding xerophthalmia is approximately 10:1. This suggests that somewhat less than half a million cases exist worldwide, although the usual fatality rate of about 20% in the first year (McLaren 2004a) will reduce this.

It has been customary in the past to estimate that approximately 10% of all blindness in young children (about 30 million) could be attributed to xerophthalmia. In the absence of current figures the present position is unknown.

Two other groups are also especially susceptible to vitamin A deficiency, namely pregnant and lactating women and school-age children. Provisional estimates suggest that each year there are nearly 20 million pregnant women with low vitamin A status (serum or breast milk retinol <1.05 μmol/L), of whom 7 million are deficient (<0.70 μmol/L). Six million are night blind (West, McLaren 2003).

Singh and West (2004) examined various sources of data to calculate the prevalences of VAD (<0.70 μmol/L) and xerophthalmia. The prevalence of VAD in children aged 5–15 years in southeastern Asia was 23.4% or 83 million (an estimated 127 million worldwide, see above). Xerophthalmia (Bitot’s spots and night blindness) was present in 10.9% – a very high percentage compared with the estimate of 4.4 million worldwide given above. Potentially blinding corneal xerophthalmia was negligible in this age group.

It is very important to recognize that all data of a biochemical nature that is based on the estimation of retinol in serum – especially such data from developing countries – is subject to the effect of the acute phase response that depresses serum retinol. Although efforts are being made to correct for this effect in data analysis (see Thurnham, McCabe, Northrop-Clewes et al. 2003 and Chapter 6), standardization and general acceptance are likely to be some time coming.

Figure 12.7: Trends in the prevalence of vitamin A deficiency (VAD) <0.70 µmol/L in children, 1990–2000 (Mason, Rivers, Helwig 2005).
COUNTRY SITUATIONS

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

The situation is of course fluid, and improvement or deterioration may occur over quite a short period of time. For example, there is good evidence that the situation has improved considerably in three of the major countries where VADD were highly endemic only two decades or so ago (see Figure 12.8).

A National Nutrition Survey carried out by the Food and Nutrition Research Institute in the Philippines in 1993 showed that the prevalence of clinical VAD among children aged 6 months to 6 years was 0.4% for XN and 0.2% for X1B, and for the total population the prevalence of XN was 0.8% and of X1B 0.3%, suggesting that VAD may no longer be a clinical public health problem. However, serum retinol <0.35 μmol/L occurs in more than 5% of children aged 6 months to 6 years in 11 out of 15 regions. Low serum retinol (<0.70 μmol/L) occurs in 24.9%. These results suggest that the Philippines should have been categorized at the time as having a severe subclinical VAD public health problem (Solon, personal communication). More recent data (Solon, 2002) for 1993 and 1998 for pre-school children (Figure 12.9) and pregnant and lactating women (Figure 12.10) in the Philippines shows continuing evidence of a VADD problem. Rare details like this suggest the kind of problems that may be encountered in this field.

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

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

Figure 12.8: Trends in xerophthalmia in children up to six years (ACC/SCN Consultative Group, 1994).
Figure 12.9: Prevalence of subclinical vitamin A deficiency among pre-school children (1993 and 1998) from the Philippines (Solon 2002).

Figure 12.10: Prevalence of subclinical vitamin A deficiency among pregnant and lactating women from the Philippines (Solon 2002).
In China in 1999–2000, 8,681 children aged 0–6 years were examined for vitamin status (Jingxiong, Toschke, von Kries et al. 2006), whereby 12.2% were discovered to have VAD (serum retinol <0.7 μmol/L) and 0.5% to have serum retinol <0.35 μmol/L. No correction was made for the presence of APR and no clinical disease was reported. Serum levels were lowest in minority groups and in the less developed west of the country. A prophylaxis program is planned.

A public health problem has long been suspected in certain other countries, but no data were available. This seems to be the situation in Laos (Malyavin, Bouphany, Arouny et al. 1996) and in Afghanistan.
13 Epidemiology

INTRODUCTION

Epidemiology may be defined as the study of the distribution and determinants of disease in a population. In simple terms various parts of the Manual may be considered to have dealt in turn with the answers to the how, what, where and how much of VADD.

The present chapter sets out to give some answers to the question “Why do VADD occur?” A clear understanding of these matters is vital before appropriate measures can be taken for the control of VADD. It is logical, therefore, that this chapter on epidemiology should precede Chapter 15 on “What can be done?” to control VADD.

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

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

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

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

AGE

Evidence from every source agrees that preschool-age children form the most vulnerable group (Sommer 1982). This applies to corneal xerophthalmia in hospitals (see Figure 13.1) and noncorneal xerophthalmia in field studies (see Figure 13.2). From the data taken together it will be evident that the more serious lesions peak at an earlier age than the less serious.

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

Keratomalacia in the infant usually proceeds rapidly without any tendency for xerosis of the conjunctiva to develop. From some parts of India there have been recent reports of increase in the occurrence of infant keratomalacia. This may be related to the increasing tendency for women of low socioeconomic status to go out to work and to leave their infants at home without adequate attention (Rahmathullah, Raj, Darling et al. 1994).
Figure 13.1: Age distribution of consecutive cases with corneal xerophthalmia (X2 or X3) presenting to the Cicendo Eye Hospital (n=162), Bandung, Indonesia, and to the Lahan Eye Hospital (n=295), Nepal (Sommer, West 1996:337).

Figure 13.2: Age distribution of mild xerophthalmia in selected countries in Asia and Africa: Nepal, Zambia, India and Indonesia (showing data from both countrywide surveys in 1978–79 and 1992) (Sommer, West 1996:338).
School age is another period of increased susceptibility, usually involving the milder degrees of xerophthalmia (night blindness or Bitot’s spots) (Khan, Haque, Khan 1984). This may be due to increased requirements for growth, especially during the adolescent growth spurt (Ahmed, Hasan, Kabir 1997).

**PHYSIOLOGICAL STATUS**

It has long been recognized that the pregnant and lactating woman is especially vulnerable. Recently some very high rates have been reported especially from the Indian subcontinent (Katz, Khatry, West et al. 1995). In this study from Nepal 16.2% of women reported XN at some time during the pregnancy that produced the child they were breastfeeding at the time of the interview. XN was reported by 8.1% at the time of the interview. The chances of XN in the current pregnancy were six times greater for those who reported XN in their previous pregnancy than for those who did not.

Only mild xerophthalmia is usually seen and the mother’s health may not be permanently affected. However, low vitamin A content of breast milk will contribute to the increased susceptibility of the infant. The proposal that breast milk retinol be used as a biological indicator of vitamin A status in the community was discussed in Chapter 7.

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

As part of a large study of the effects of vitamin A and β-carotene supplementation in pregnancy in Nepal (see Chapter 9), Christian, West, Khatry et al. (2000a) recorded the impact of weekly supplementation on the prevalence of pregnancy and postpartum illness symptoms among 15,832 women. No impact of either supplement was observed up to 28 weeks of gestation on morbidity rates. After 28 weeks symptoms of nausea, faintness and night blindness were reduced with vitamin A, but not with β-carotene. Vitamin A was associated with shortened labor by 1.5 hours in nulliparous, and 50 minutes in multiparous women. Both interventions were associated with reduced postpartum prevalence of at least 4 loose stools and night blindness. β-carotene was associated with reduced symptoms of high fever postpartum, vitamin A with reduced number of days of illness symptoms over the last 12 weeks of pregnancy. Vitamin A or β-carotene reduced selected illness symptoms during late pregnancy, at the time of birth, and during 6 months postpartum.

Vitamin A deficiency, as assessed by serum retinol (see Chapter 11) and impaired dark adaptation, is not infrequently accompanied by iron deficiency anemia during pregnancy (Radhika, Bhaskaram, Balakrishna et al. 2002; van den Broek, Letsky 2000).

**DIET**

Regular ingestion of the customary dietaries around the world, both now and in the past, has provided for vitamin A requirements to be met. It has only been when consumption was limited, perhaps at certain seasons of shortage or when certain food items were omitted altogether, that problems have arisen. Young children have to rely on others to feed them, and pregnant and lactating women are sometimes especially targeted for food taboos.

Staple foods, mostly cereals but including some starchy roots, provide the bulk of a diet. They generally contain small concentrations of carotenoids but because they are eaten in bulk the amount supplied may be considerable. Rice is the main exception to this rule. It contains no carotenoids in the form in which it is consumed (see Chapter 15). Furthermore, mothers often consider it to be a complete diet for the young child. Infants find it easy to eat and satisfying.
“Rice-dependent” communities, where rice and little else forms the daily diet, have proved to be especially prone to suffer from VADD. It is ironical that this situation occurs mainly in the moist tropics, where carotene is readily available on every hand. This is truly an instance of nutritional “poverty in the midst of plenty” (McLaren 1962).

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

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

**BREASTFEEDING**

There is abundant evidence that breastfeeding is highly protective against xerophthalmia (Sommer, West 1996: 343–345 and Figure 13.3). This may be partly due to the regular supply of preformed vitamin A in the milk. Another impor-

![Figure 13.3](https://example.com/image.png)

*Figure 13.3:* Association between percent of children breastfeeding and mild xerophthalmia (XN, X1B), by age. Open bar, non-xerophthalmic children; black bar, cases. A, Indonesia, B, Nepal, C, Bangladesh, D, Malawi (Sommer, West 1996).
tant factor is the lower rate of infections as compared with artificially fed children.

A study in south India (Ramakrishnan, Martorell, Latham et al. 1999) showed that while non-breastfed children met only 60% of the Indian RDA, those breastfed met 90% during the second year of life. The WHO and UNICEF recommend that all infants be exclusively breastfed for at least 6 months and that they continue to be breastfed up to two years or beyond with the addition of adequate complementary foods from about 6 months of age.

The composition of the weaning diet is obviously of great importance. Infrequent consumption of dark green leaves or yellow fruits was associated with fourfold to sixfold increase in the risk (odds ratio) of xerophthalmia in one study (see Figure 13.4).

CULTURAL FACTORS

The customs practiced by a community are usually deeply embedded within the fabric of the society. They are not as open to understanding by outsiders as are less complicated characteristics like others considered here. Groups under study often speak a language not readily understood by the investigators, even if of the same country. For outsiders, and this is what scientists investigating a community inevitably are, understanding of the worldview of others is always limited.

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

Figure 13.4: Relative risk (case-control odds ratio) (±95% CI) of mild xerophthalmia (vitamin A deficiency) by type of food reportedly consumed by children daily or every other day during their first twelve months of weaning (Mele, West, Kusdiono et al. 1991). Not eating dark green leaves, for example, increases the risk about sixfold.
ucts of their primary work (medical missionary and colonial government medical officer respectively), and from long and intimate contact with the people concerned.

**INFECTIOUS DISEASES**

As was noted earlier, when the topic of the impact on infections of vitamin A deficiency was considered (see Chapter 9), the relationship is complex. Infections also predispose to the development of vitamin A deficiency, as will be considered here.

The acute phase response (APR) needs to be looked at in the present context. The account given in Chapter 7 may be consulted as an introduction to the subject. Thurnham, McCabe, Northrop-Clewes (2003) carried out a meta-analysis from the results of which Figure 7.1 was constructed. This suggests approximate levels of effect of subclinical infection on plasma retinol, and consequently on vitamin A status.

An important implication of these observations is that care must be exercised in the interpretation of serum retinol and RBP levels in relation to the assessment of vitamin A status (see Chapter 7).

As knowledge of this phenomenon has spread in recent years studies have been carried out that increasingly underline the practical importance of taking it into account in studies in communities in developing countries. Stephensen and Gildengorin (2000) reinterpreted the NHANES III data for serum retinol in the United States. Other similar large data bases will presumably also be affected in a similar manner.

A recent study of preschool children in Zambia, correcting for the presence of infection by measuring C-reactive protein (CRP) concentrations, raised the mean plasma retinol concentrations by approximately 10% overall. The retinol concentrations of those children with no infection, as defined by a normal CRP, were 13–20% lower than the UK mean for preschool children (1.0 μmol/L) (Northrop-Clewes, Mwela, Kankasa et al. 2005).

Two studies conducted on infants, one on healthy infants in Germany (Abraham, Mueller, Grueters et al. 2003), and another in Indonesia on infants with infections (Wieringa, Dijkhuizen, West 2002) have provided new evidence. The first showed that in this age group APR is triggered at very low inflammatory levels and can even occur in apparently healthy infants. A high-sensitivity assay was used and the high prevalence of positive results is attributed to this. It was concluded in the Indonesian study that the prevalence of vitamin A deficiency was ‘overestimated’ by >16%.

In patients with HIV infection low serum retinol levels are considered to be mainly attributable to the infection itself and the associated APR, rather than an indication of low vitamin A status (Baeten, McClelland, Richardson et al. 2002).

The situation appears to be even more complicated in pregnancy (Christian, West, Khatry et al. 1998a). Physiological changes that take place include hemodilution and an increase in acute phase proteins. Both of these changes lead to reduction in serum retinol concentration (Dijkhuizen, Wieringa, West 2005). Recently the same group has studied other micronutrients for effects of the acute phase response (Jiang, Xu, Christian, et al. 2005). The situation is complex. Results differ according to the kind of test used. Nutrients that, like serum retinol, are decreased include β-carotene and lutein (both P<0.0001).

Clearly much further research is required before standards can be set for use in realistically assessing vitamin A status of populations. Maqsood, Dancheck, Gamble et al. (2004) found in a study of preschool-age children in the Marshall Islands that removing children with evidence of raised acute phase proteins introduced sample bias for several variables.

In a group of Venezuelan children aged 4–7 years with subclinical vitamin A deficiency, as measured by conjunctival impression cytology, circulating cytokines IL-10, IL-4, IFN-γ, and IL-2 were measured. Only IL-10 was found to be diminished (Leal, Castejon, Romero et al. 2004).
Diarrheal diseases
There is strong evidence from both clinical and field studies that diarrheal disease is closely associated with xerophthalmia and with impaired vitamin A status.

Considerable interest was aroused by the report from Peru (Alvarez, Salazar-Lindo, Kohatsu et al. 1995) that diarrheal disease, especially that due to rotavirus infection and with accompanying high fever, may lead to as much as a tenfold increase in the urinary excretion of vitamin A (see also Chapter 14). Alvarez and colleagues reported a similar urinary loss of vitamin A in adults with sepsis and pneumonia in the United States and have extended their studies to Bangladesh (Mitra, Alvarez, Stephensen 1998). In shigellosis urinary retinol loss was proportional to the severity of the disease, and impaired tubular reabsorption of low molecular weight proteins like RBP appeared to be the cause.

When *Sight and Life* published a monograph to mark the centenary of the first description of a large outbreak of xerophthalmia (Mori 1904), the nature of diarrhea accompanying xerophthalmia was discussed (McLaren 2004). The weight of evidence seems to support the view that it is primarily a symptom and sign of the vitamin A deficiency state itself.

Perhaps the most significant recent development that supports the suggestion that diarrhea is part of VAD is the work of Mayo-Wilson and colleagues (2011). Evidence was obtained to show that vitamin A deficiency induces impairment of the gut integrity.

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

It is well known that vitamin A supplementation often improves hemoglobin concentration. An additional response was observed if deworming (for *Ascaris lumbricoides* and *Trichuris trichuria*) took place at the same time (Tanumihardjo, Permaesih, Muhiilal 2004).

Respiratory diseases
As was the case for diarrheal diseases, for respiratory infections there is also good evidence from field and hospital studies that they worsen vitamin A status at all levels. Several possible mechanisms may be at work including loss in urine and impaired absorption of both vitamin A and carotenoids (Sommer, West 1996:191–220).

Measles and associated conditions
The effect of vitamin A deficiency on the course of measles infection was discussed earlier (see Chapters 9 and 12). Decades ago measles was recognized to carry a high mortality risk in children in Africa with severe PEM, and involvement of the eye was occasionally noted. It took many years to provide the evidence that was necessary to bring about general acceptance of the fact that vitamin A deficiency is frequently responsible for the blindness that sometimes followed an attack of measles. There is evidence from other parts of the world, as well as from several regions of Africa, that about one quarter to one half of all cases of corneal blindness in young children are associated with measles (Sommer, West 1996:191–220).

It is now recognized that often there is not only the combination of VAD and measles but that other factors may also play a part. Sometimes the initial eye involvement is treated by the application of traditional eye medicines that usually serve to increase the damage. Ulceration in the periphery of the cornea, especially in otherwise quiet eyes, is then characteristic (Lewallen, Courtright 1995). Severe involvement of the cornea may be due to superadded infection with the herpes simplex virus (see Table 13.1). In this series...
of cases of corneal ulceration treated in hospital there is a higher proportion of cases following measles infection in the group with xerophthalmia as a specific cause than in those in which herpes simplex or traditional eye medicine was the cause. This is perhaps not surprising as severe measles tends to precipitate nutritional deficiency.

This combination has been reported from several parts of Africa but is not known to have the same importance elsewhere. Malaria has also been reported to be a precipitating factor in some places (Yorston, Foster 1992; Genton, Al-Yaman, Semba et al. 1994). This merits investigation elsewhere in view of the widespread occurrence of drug-resistant malaria.

**HIV / AIDS**

Only in recent years has the association between HIV infection and vitamin A status begun to be investigated (see Chapter 9). There is no evidence yet as to whether infection impairs vitamin A status. It is likely that a serious disease like AIDS would have such an effect; patients with AIDS infection, rather than just HIV positive, are usually markedly undernourished.

<table>
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<th><strong>PROTEIN-ENERGY MALNUTRITION (PEM)</strong></th>
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Impaired growth usually accompanies xerophthalmia and tends to increase *pari passu* with the degree of vitamin A deficiency (see Chapter 10). Severe degrees of PEM, evidenced clinically as marasmus, marasmic-kwashiorkor, or kwashiorkor, are not necessarily associated with eye signs of xerophthalmia, although serum retinol is usually depressed. Corneal xerophthalmia on the other hand is usually accompanied by severe PEM in some form (McLaren, Shirajian, Tchalian et al. 1965).

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

| **Table 13.1:** Corneal ulceration within one month of measles infection by cause and age as a percentage of all ulcers in Tanzanian children (Foster, Sommer, 1987). |
|--------------------------------------------------|--|---|---|---|---|---|---|
| **Age (years)** | **Xerophthalmia** | | | | | | |
| | **N** | **n** | **%** | | | **N** | **n** | **%** | | **N** | **n** | **%** |
| <2 | 16 | 10 | 62 | 24 | 3 | 12 | 8 | 1 | 12 |
| 2–4 | 12 | 11 | 92 | 10 | 3 | 30 | 5 | 5 | 100 |
| 5–10 | 6 | 3 | 50 | 13 | 4 | 31 | 5 | 2 | 40 |
| **Total** | 34 | 24 | 71 | 47 | 10 | 21 | 18 | 8 | 44 |

N=total number of children seen with corneal ulceration due to a specific cause; n = number of children seen with corneal ulceration due to a specific cause which developed within one month of measles infection.
Three eminent nutrition scientists (Schuftan, Ramalingaswami, Levinson 1998) pointed out that PEM was largely neglected and excessive attention was being given to multi-micronutrient (MMN) deficiencies. In more recent years the emphasis has grown even stronger. It should not be forgotten, however, that MMN is almost invariably accompanied by PEM of some degree; usually wasting. Control of both states should be concurrent.

SEASON

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

Among communities that subsist in semi-desertic terrain, as in the deserts of Rajasthan (Desai, Desai, Desai 1992), a single season with a lack of monsoon rainfall can spell nutritional disaster (Figure 13.5).

Frequently superimposed upon the seasonal pattern described above there is the added effect of seasonal outbreaks of infectious diseases (Oomen, McLaren, Escapini 1964; McLaren, 1969).
Shirajian, Tchalian et al. 1965). The most important of these is the group of diarrheal diseases, gastroenteritis, often known locally as “summer diarrhea” because of its peak season. VAD is usually accompanied by PEM and emerges towards the end of the diarrhea season, as the effects of prolonged and repeated attacks of diarrhea take their toll. Respiratory infections tend to peak in the winter, which in some developing countries with a continental climate or at high altitude may be bitterly cold.

The most precise documentation of seasonal patterns and VADD was made by Sinha, who resided for two years in the West Bengal village area of Ichag (see Figure 13.6 and 13.7).

**SOCIOECONOMIC STATUS**

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

**LOCATION**

Sommer and West (1996:335–354) summarize a large body of evidence that shows that VADD tend to cluster at a variety of levels. This phenomenon applies to provinces, districts, subdistricts, villages and households (IVACG 1996a). Figure 13.8 shows this clustering in regions in Bangladesh.

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

As for any disease, the occurrence of clusters may be seen as the probable presence at one time and place of a combination in some degree of intensity of several of the risk factors considered here. There might also be other variables that are as yet unrecognized.

![Figure 13.6: Seasonal prevalence of vitamin A deficiency signs, Ichag, 1971–73 (Sinha, Bang, 1976).](image-url)
Figure 13.7: Seasonal prevalence of vitamin A deficiency (night blindness and/or Bitot's spot) according to sex, Ichag, 1971–73 (Sinha, Bang, 1973).

Figure 13.8: Regional clustering of VAD by severity and district in Bangladesh (Cohen, Rahman, Mitra et al., 1987).
Katz, Zeger, West et al. (1993) have studied clustering of xerophthalmia within households and villages in Malawi, Zambia, Indonesia and Nepal. The magnitude of clustering varied between countries and in all places studied it was much greater within households than within villages. Infectious diseases appeared not to explain much of the clustering. Other household factors related to diet and nutrition are suspected to be more important. Identification of clustering can considerably influence the implementation of an intervention. In Nepal it was estimated that measures to prevent xerophthalmia were 7–34 times more efficient in high risk, highly populated areas than in remoter communities (West, Pokhrel, Khatry et al. 1992).

SEX

Most of the evidence from animals and humans suggests that males are more susceptible to VAD than females. In healthy human adults plasma retinol and RBP are both about 20% higher in males, but the significance of this is unclear (Pilch 1987; Smith, Goodman 1971). Night blindness and Bitot’s spot are almost uniformly reported to be from 1.2 to 10 times more common in males (Paton, McLaren 1960; ten Doesschate 1968; Solon, Popkin, Fernandez et al. 1978). These selected samples were supported by the country-wide survey in Indonesia (Sommer 1982) with about twice as many Bitot’s spots in males as in females (Figure 13.9).

Interestingly, no evident sex difference was found in the detailed longitudinal study of Sinha and Bang (1973) (see Figure 13.7). Prevalence of VAD was extremely high in this area. Not only did it reach an astonishing peak of 15–16% in both sexes at the beginning of the rainy season; it never fell to a rate below that of the WHO criteria (1% and 0.5%).

Male preponderance was again found in the largest hospital series ever reported (Oomen

![Figure 13.9](https://example.com/figure.png)

**Figure 13.9:** The prevalence of Bitot’s spots increased significantly (P>0.01) at 1, 2, and 3 years of age. Bitot’s spots were more common among boys (12.5/1000) than among girls (7.2/1000) (P<0.001) (Sommer, 1982).
Figure 13.10: Age distribution of 6,300 cases of xerophthalmia in the Yap Eye Hospital at Jogjakarta, Indonesia, from 1935 to 1954. The male preponderance throughout increases with age (Oomen, 1961).
1961) from the eye hospital of Dr Yap Kie Tiong in Jogjakarta, Indonesia (Figure 13.10). Among the 6,300 cases the male:female ratio varied with age. It was 1.4:1.0 in the preschool-age period, and 6.0:1.0 at around 10 years of age.

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

THE VADD CYCLE

The flow diagram (Figure 13.11) has been devised to give some impression of the kind of combinations of risk factors that conspire together at different stages of the life cycle to predispose to the development and persistence of a VADD problem in a community.

Figure 13.11: The Vitamin A Deficiency Disorders (VADD) cycle.
INTRODUCTION

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

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

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

Vitamin A is one of the vitamins that can lead to disease as the result not only of deficient but also of excessive intake. The latter is usually known as hypervitaminosis A and may result from prophylactic or therapeutic use of vitamin A. This will be discussed later, in Chapter 15. It is more appropriate to deal with some aspects of an excessive intake of retinol or other retinoids here.

Many retinoids have been synthesized for use in various diseases, especially those in which epithelial cell differentiation has been disturbed. Some attention will be paid to this topic here.

Finally, in recent years, many of the carotenoids, irrespective of whether provitamins or non-provitamins, have been shown to have the potential to play a protective role in a number of common diseases. This is poorly understood at present. There is growing evidence that habitual consumption of diets that are rich sources of these carotenoids is associated with low incidences of some of these diseases.

SECONDARY VAD

In most instances, the degree of vitamin A deficiency is not severe. As little attention is given to VAD or any other nutritional deficiency in general medicine, it is usually not until some clinical manifestation, like night blindness or conjunctival xerosis, occurs that deficiency is suspected. Consequently, it is really not known how common deficiency is in these conditions, especially at the subclinical level. The various causes of malabsorption are by no means uncommon and most cases of secondary VAD have this basis.

A rare group of disorders affecting the transport of lipids in plasma includes two related conditions called abetalipoproteinemia and hypobetalipoproteinemia (see Table 14.1). Retinal degeneration is a constant feature of these conditions. Patients have usually been treated with vitamin A and vitamin E for prolonged periods. Recent long-term assessment of 13 patients who had been treated for an average of 11.7 years showed that visual impairment persisted even in those receiving treatment soon after diagnosis (Chowers, Banin, Merin et al. 2001).

On the other hand, only single cases have so far been reported of the enzyme defect, the transport abnormality, the genetic problem in twins, and the mutations in plasma retinol-binding protein referred to in Table 14.1. However, there are
indications that genetic predisposition to VAD may be of more importance than has been generally recognized hitherto (Ward, MacGowan, Hornby et al. 2000). Larger doses than normal of vitamin A are required to overcome the metabolic problems present, and where there is impaired fat absorption a water-miscible preparation should be given by mouth and perhaps initially intramuscularly. It is often not appreciated that the usual form of oily vitamin A given intramuscularly stays in the muscle and is not released into the circulation to any extent (McLaren 1969).

Recently, a second report was published of a loss-of-function mutation in CMO1 (see Chapter 5), the enzyme that catalyzes the first step in the conversion of dietary provitamin A carotenoid to vitamin A in the small intestine. The patient had hypercarotenemia and hypovitaminosis A (Lindqvist, Sharvill, Sharvill et al. 2007).

A recent study of 138 patients with cystic fibrosis showed that serum retinol was inversely correlated with C-reactive protein (Greer, Buntain, Lewindon et al. 2004). As these patients have respiratory and other infections almost constantly, the diagnosis of vitamin A deficiency is problematic in relation to serum retinol and APR (see Chapter 7). Routine vitamin A supplementation would seem to be necessary.

### HYPERVITAMINOSIS A

#### Acute toxicity

Vitamin A toxicity may be either acute or chronic. Acute toxicity usually follows a large single dose of vitamin A and is recognized by symptoms suggesting an acute rise in intracranial pressure – nausea, vomiting, and headache. Providing that no further vitamin A is given, symp-
toms rapidly subside and there are no permanent ill effects (see Chapter 15). The subjects have usually been young children in a supplementa-
tion program or Arctic explorers short of food who have consumed the liver of polar bear, seal, or their own sled dogs.

**Chronic toxicity**

This is uncommon but may be very difficult to diagnose. This is partly because the physician may neglect to take a careful dietary history and routine clinical laboratories are not able to measure serum retinol. Perhaps even more important is the fact that the symptomatology of hypervitaminosis A may be very varied, affecting a number of different systems and sometimes mimicking other diseases. Common symptoms are headache, vomiting, diplopia, alopecia, dryness of mucus membranes, desquamation, bone and joint pain, liver damage including cirrhosis, hemorrhages into the skin, and coma. If the condition is not correctly diagnosed and further excessive vitamin A intake prevented, death can result.

A review of both acute and chronic toxic effects of vitamin A has been published recently (Penniston, Tanumihardjo 2006). Much of what is said here has been said before but, importantly, the authors place new emphasis on the possibility of harmful effects resulting from high and/or prolonged dosing with vitamin A in VAD-prevention programs in developing countries.

High intake of vitamin A by whatever means results in the adverse effects of exposure to toxic metabolites of vitamin A such as retinyl palmitate, 13-cis-retinoic acid, and 13-cis-4-oxo-retinoic acid (Hartmann, Brors, Bock et al. 2005). The phototoxicity and photomutagenicity of these compounds is well-known (Yan, Xia, Cherng et al. 2005).

**Vitamin A and bone**

It has long been known that both deficiency and toxicity of vitamin A can damage bone in experimental animals (see Chapter 6). Three recent animal experiments contribute to this topic. In the rat it was shown that osteopenia caused by bilateral ovariectomy, which resembled the bone changes seen in postmenopausal women, was intensified by daily dosing with vitamin A (Pytlik, Cegiela, Folwarczna et al. 2004). The same group (Sliwinski, Janiec, Pytlik et al. 2004) found that the preventive action of alendronate on osteopenic changes was lessened by retinol. On a rather different note, Uchiyama, Sumida and Yamaguchi (2004) showed that dosing aged rats daily with β-cryptoxanthin had an anabolic effect on bone components in femoral tissues, both *in vivo* and *in vitro*.

In elderly human populations, several large surveys of vitamin A intake and serum retinol and occurrence of various fractures (Michaelsson, Lithell, VESSBY et al. 2003; Lim, Harnack, Lazovich et al. 2004; Barker, McCloskey, Saha et al. 2005) have not provided consistent results.

A meta-analysis of vitamin A intake and bone (Crandall 2004) showed an association between dietary and supplemental vitamin A and hip and other bone fractures. Adverse effects seemed to occur when the intake was about twice the current recommendation for adult females. This latter point is consistent with the data in Table 6.2 which show serum retinol levels rising constantly with age.

A further important contribution to our understanding of the problem was recently provided by analysis of data of the NHANES I follow-up study in the United States (Opotowsky, Bilezikian 2004). Increased risk of hip fracture was shown to be associated with both low and high serum retinol concentrations, as evidenced by the U-shaped curve of association. This would be consistent with the animal studies.

No firm conclusions can be drawn at present and larger and better studies are needed. Meanwhile, several observations come to mind. The bone lesions of hypervitaminosis A in experimental animals do not resemble the osteoporosis associated with fractures in elderly humans. Serum retinol is not by itself a valid indicator of vitamin A status. Moreover, many elderly people will have raised acute phase proteins and lowered serum retinol from infections and inflammation.
Many other factors, including lack of vitamin D, have not hitherto been taken into account.

Governments in some countries advise elderly people not to consume more than one helping of liver per week – advice also given to pregnant women. The average vitamin A intake of most elderly people in developed countries tends to be higher and excessive supplementation is clearly to be guarded against. In the context of food fortification the issue of the risk of hypervitaminosis A has been thoughtfully reviewed recently (see also Chapter 15) (Kraemer, Waelti, de Pee et al. 2008).

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

In humans the evidence for the involvement of vitamin A deficiency is slender, amounting mainly to isolated case reports. In striking contrast, there are numerous reports of high incidences (>20%) of spontaneous abortions and birth defects in the fetuses of women ingesting therapeutic doses of 13-cis RA and other retinoids during the first trimester of pregnancy (Ross 1999).

The most controversial area has been the possible teratogenic effect of excess vitamin A intake during early pregnancy. The present consensus is that in communities where VAD is known to be present, vitamin A supplementation throughout pregnancy should not exceed 10,000 IU per day (see also Chapter 15). Where the diet supplies the RDA and more, even this level may be excessive. Nevertheless, a recent pan-European study found no association between high vitamin A intake in early pregnancy and major malformations (Mastroiacovo, Mazzone, Addis et al. 1999).

One hundred and twenty infants exposed to more than 50,000 IU daily showed no malformations. Another very large study from Europe (Czeizel, Rockenbauer 1998) reported on the population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities for 1980–94. This included 35,727 normal infants and 20,830 infants with congenital abnormalities of all kinds. The mothers of 3,399 (9.5%) of the normal infants received vitamin A supplements, as did 1,642 (7.9%) of those giving birth to infants with malformations. The difference was highly significant (P<0.001). It was suggested that this analysis considered low or moderate doses of vitamin A to be protective and not teratogenic. An additional analysis, not pursued by the authors, would be to group the infants according to whether or not their mothers received vitamin A and to compare the numbers of those with malformations in each group. Of 5,041 receiving vitamin A, 1,642 (32.5%) had malformations; of 51,516 not receiving vitamin A, 19,188 (37.2%) had malformations. This difference is also highly significant at the P<0.001 level and also suggests that vitamin A might be protective. All of the evidence available to date is reassuring with regard to the safety of the use of vitamin A supplements according to generally accepted practice.

Fraunfelder, Fraunfelder and Edwards (2001) reported on their experience of treating various resistant skin disorders using isotretinoin, 13-cis-retinoic acid (Accutane/Roaccutane), over a period of 20 years. Thirty-eight different signs or symptoms of ocular abnormalities probably attributable to isotretinoin usage have been reported. Those classified as “certainly” attributable to this cause include corneal opacities, decreased dark adaptation, decreased vision, myopia, and teratogenic ocular abnormalities.

Recent evidence for the involvement of vitamin A in various congenital malformations has been published. A population-based case control study in the United States (Botto, Loffredo, Scanlon et al. 2001) showed that levels of maternal dietary intake of vitamin A or carotenoids were not related to cardiac outflow tract defects. However, compared with an average intake from retinol supplements of less than 10,000 IU daily, an intake of 10,000 IU or more was associated with a ninefold increase in relative risk of transposition of the great arteries, but not of
outflow tract defects with normally related arteries.

The toxicity of vitamin A during pregnancy was assessed in the macaque or cynomolgus monkey (Hendrickx, Peterson, Hartmann et al. 2000). Extrapolation to humans using a safety factor of 10 yielded safe levels of vitamin A during human pregnancy in the range of approximately 25,000 to 37,000 IU/day – well above the current generally recommended safe level of up to 10,000 IU/day.

Recently a large international team of researchers has observed two unrelated consanguineous families with malformation syndromes with some features in common (Pasutto, Sticht, Hammersen et al. 2007). Pathogenic homozygous mutations were identified in STRA6. This is a member of a large group of “stimulated by retinoic acid genes that encode novel transmembrane proteins, transcription factors, and secreted signaling molecules or proteins of largely unknown function” (see Chapter 6).

NON-NUTRITIONAL DISEASES RESPONSIVE TO VITAMIN A AND/OR CAROTENOIDS

Bronchopulmonary dysplasia (BPD)

This disease is the leading cause of chronic lung disease in infancy and affects particularly very low birth weight infants. In the United States, of the 3.9 million or so births per year, approximately 55,000 newborns weigh less than 1,500 g at birth. These are classified as very low birth weight (VLBW). About 49,000 survive hospitalization and about 24% of these suffer from BPD. The histological changes in the lungs that accompany this condition are similar to those described in children dying with xerophthalmia. Most of these infants have serum retinol and RBP in the deficient range. They are also born with low liver reserves. Several trials have shown benefits from dosing with vitamin A. The usual regime is 5,000 IU of water-miscible vitamin A intramuscularly three times a week for four weeks (Shenai 1999). The precise requirement of vitamin A, the optimal mode and the duration of its administration need further investigation.

A recent meta-analysis (Darlow, Graham 2000) confirmed the value of vitamin A supplementation, especially in terms of reduced oxygen requirement and mortality rate. In view of the very small size of these infants, it was recommended that intravenous vitamin A be considered. Another study urged the need for prolonged nutritional support until catch-up growth has been achieved (Atkinson 2001).

In a large study of 120 infants with BPD it was demonstrated that high-dose or once-per-week regimes did not give better results than the standard dose, which should continue to be given (Ambalavanan, Wu-Tzong, Tyson et al. 2003). Recently, a large study of vitamin A supplementation for extremely low birth weight (ELBW) infants with an outcome of 579 (88%) surviving infants at 18 to 22 months was reported (Ambalavanan, Tyson, Kennedy et al. 2005). BPD was reduced without increasing mortality or neurodevelopmental impairment.

The results of a recent study of ELBW infants, in whom C-reactive protein and the RBP/TTR molar ratio (see Chapter 7) were measured, suggest that inflammation rather than vitamin A deficiency might be the cause of low serum retinol in these cases (Ambalavanan, Ross, Carlo 2005). This may prove to be yet another example of misdiagnosis arising from incorrect use of serum retinol.

Emphysema

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

EYE DISEASES

Retinitis pigmentosa and some other retinopathies
In addition to VAD, several other diseases affecting the retina may also have night blindness as part of their symptomatology. Almost all of these are rare genetic disorders. Retinitis pigmentosa (RP) affects about one in 4,000 worldwide and eventually leads to total blindness. There are many different genetic forms of the disease. Over many years claims have been made for improved vision with vitamin A and other treatments, but the only large, fully controlled trial with vitamin A over a prolonged period was carried out in the United States (Berson, Rosner, Sandberg et al. 1993). It was claimed that patients receiving vitamin A tended to experience a slowing in the rate of deterioration of their electroretinogram compared with those not receiving vitamin A. There was no improvement in vision. There was considerable controversy about these findings at the time. The publicity the report received has, unfortunately, meant that many patients have started self-medication with vitamin A in the absence of any proven treatment. The equivocal nature of the results may have been due at least in part to the mixed nature of RP cases included in the trial. Recent evidence suggests that some forms of RP may be more likely to respond than others (Fariss, Zong-Yi, Millam 2000).

Bassen-Kornzweig Syndrome and Sorsby Fundus Dystrophy are other rare diseases in which vitamin A has brought about some improvement in rod function (Berson 1999). This whole complex subject was discussed at greater length recently (McLaren 2000).

At the time of writing, 32 genes have been shown to be associated with retinitis pigmentosa. Recently, the results of treatment of a subgroup of the large study reported above have given considerable encouragement to the addition of docosahexaenoic acid (DHA) to previously instituted vitamin A treatment (Berson, Rosner, Sandberg et al. 2004). The course of the disease was slowed.

Age-related macular degeneration (AMD)
It was mentioned in Chapter 2 that carotenoids commonly accumulate in various tissues and certain carotenoids appear to have a predilection for certain tissues. β-carotene accumulates preferentially in the human ovary but the significance of this is unclear. In primates there is a specific uptake from the diet of two non-provitamin A carotenoids, lutein and zeaxanthin, by the retina and other ocular tissues including the lens. The greatest uptake is by the macula lutea or yellow spot which contains the site of maximal visual acuity, the fovea centralis, where there are only cones and no rods (Harnois, Samson, Malenfaut et al. 1989; Demmig-Adams, Gilmore, Adams III 1996). Zeaxanthin and meso-zeaxanthin (presumably formed from lutein) predominate in the fovea, and lutein predominates in the peripheral retina (Bone, Landrum 2005).

Age-related macular degeneration (AMD) is a disease, or group of diseases, that causes a devastating loss of vision in the elderly. It is the commonest cause of blindness in the 75 years plus age group. The cause is unknown but intensive research, both epidemiological and clinical, is now underway, in which it is hypothesized that diet plays a part.

Lutein and zeaxanthin absorb short (blue) light. These are the highest energy photons across the visual spectrum to reach the retina. The carotenoids may serve to filter these, as well as to exert their known antioxidant properties. A recent article (Schalch 2004) explains how, according to one hypothesis, the level of concentration of these pigments in the macular region might influence the ability of cones there to override the more sluggish signals from rods in a zone of vision known as the “twilight” zone or zone of mesopic vision.

It has been shown that the macular pigment
optical density (MPOD) is directly related to the dietary intake of lutein and zeaxanthin and even more strongly to serum concentrations (Mares, LaRowe, Snodderly et al. 2006).

Lutein and zeaxanthin are present in the diet mainly in various dark green, leafy vegetables. One study found that eating spinach and collard greens five or more times a week significantly reduced the risk of the wet form of macular degeneration (Landrum, Bone 2001). Another study (Van Leeuwen, Boekhoorn, Vingerling et al. 2005) found a benefit from a high dietary intake of β-carotene, vitamins C and E and zinc.

Recent animal research has contributed possible clues to our understanding of AMD. Mice lacking the component collagen XVIII/endostatin in the retinal pigment epithelium (RPE) develop massive deposits under the RPE, abnormal RPE and age-related macular degeneration, as in the human disease AMD. Progressive loss of visual function is due to decreased retinal rhodopsin content (Marneros, Keene, Hansen et al. 2004).

Gollapalli and Rando (2004) found that RPE65 acts as a chaperone for all-trans-retinyl esters in the retina. Some forms of retinal and macular degeneration in man are caused by the accumulation of vitamin A based retinotoxic products. The identification of RPE65 as the visual cycle target for retinoic acid makes the development of useful drugs to treat retinal and macular degeneration feasible. However, there is currently a conflict over the possible benefit (Owsley, McGwin, Jackson et al. 2006) or harm (Radu, Han, Bui et al. 2005) of a substance accumulation in the retina. Many pharmaceutical companies are marketing lutein and other carotenoid supplements for AMD (Stringham, Hammond 2005).

In a population-based cohort study it was found that a high level of intake of lutein and zeaxanthin was accompanied by up to a 65 percent reduced risk of neo-vascular AMD over up to a 10-year period of observation. There were 3,654 participants at the start and 2,454 of those were re-examined at 10 years (Tan, Wang, Flood et al. 2008).

Persons with intermediate risk of age-related macular degeneration or advanced age-related macular degeneration in one eye are recommended to take the formulation proven in the Age-Related Eye Disease Study (AREDS) to prevent the development of advanced AMD by 25%. The formulation consists of vitamins C, E, β-carotene and zinc (Coleman, Chew 2007). Another recent successful preventive approach in AMD involved the taking of folic acid, vitamin B_{6} and vitamin B_{12} for an average of 7.3 years (Christen, Glynn, Chew et al. 2009). The subjects in this placebo-controlled trial were women with a high risk of ischemic coronary heart disease; 2,607 control and 2,598 treatment. The supplements reduced the risk of new early AMD by 34%. This study was embedded in a larger trial of various vitamin supplements for the prevention of IHD. Combination of these three B vitamins is known to lower serum homocysteine, an amino acid that is known to be raised in both IHD and AMD, but not shown to prevent IHD. These are promising results in early AMD prevention and progression.

It has recently been suggested that there might be a causal relationship between the increased occurrence of AMD and obesity (Johnson 2005). It is suggested that in this condition increased oxidative stress, changes in lipoprotein profile and increased inflammation might result in increased destruction and decreased circulatory delivery to the macula of lutein and zeaxanthin.

**Age-related cataract**

Cataracts, which consist of a gradual opacification of the lens of the eye leading eventually to blindness, have hitherto only been capable of treatment by removal of the damaged lens. On the basis that oxidative damage may be responsible, a possible association of dietary intake of antioxidant nutrients, such as carotenoids, and vitamins C and E, is being studied. As with AMD, there is no conclusive evidence at present (Taylor 1999). A recent study of monozygotic and dizygotic twins in the UK showed that almost 50% of the variation in severity of nuclear cataracts could be explained by heredity, about 38% by age and only 14% by environment (Hammond, Snieder,
Spector et al. 2000). In other parts of the world, where nutritional deficiencies and other environmental factors are very different, results might also be very different. However, a recent study in India found no evidence of slowing cataract progression with the use of antioxidants (Gritz, Srinivasan, Smith et al. 2006).

In a recent study, spectral fundus reflectance was used in a group of 276 subjects between 18 and 75 years of age to assess lens optical density. Lutein and zeaxanthin are the only carotenoids found in the human lens. The results suggested that the presence of these pigments may retard aging in the lens and, consequently, cataract formation (Berendschot, Broekmans, Klopping-Ketelaars et al. 2002).

The US Food and Drug Administration (FDA) has recently claimed in its evidence-based review system for health that “no credible evidence exists for a health claim about the intake of lutein or zeaxanthin (or both) and the risk of age-related macular degenerations or cataracts” (Trumbo, Ellwood 2006).

DERMATOLOGICAL PHARMACEUTICAL USE OF SYNTHETIC RETINOIDS

Natural and synthetic retinoids influence epithelial cell proliferation and differentiation and are increasingly being used in dermatological practice to treat hyperkeratotic disorders. Etretinate and acitretin are very effective in psoriasis. 13-cis-retinoic acid and retinoyl-β-glucuronide suppress sebum production and are used in acne and acne-related disorders (Figure 14.1). Tretinoin has a beneficial effect in some cases of actinic keratosis, caused by chronic exposure to sunlight. At present only three retinoids are approved for oral administration in many countries: isotretinoin, etretinate, and acitretin. Their teratogenic effects were discussed above.

Erythropoietic porphyria is an uncommon disorder of porphyrin metabolism in which severe dermatosis may affect areas of the skin exposed to sunlight. The regular ingestion of β-carotene leads to hypercarotenosis in which the blood level is high and deposition occurs in the skin. This was shown to be protective in this disease and the recommended dose is about 180mg per day (Moshell, Bjornson 1977).

CANCER CHEMOPREVENTION AND TREATMENT

Many studies in experimental animals have shown the ability of both natural and synthetic retinoids to reduce carcinogenesis in organs such as the breast, skin, liver, colon, prostate, lung and other sites. Natural retinoids are too toxic for prolonged use in man, but some synthetic retinoids are well tolerated and have the potential for prophylactic use. In recent years, attention in this field has turned more to the potential of some carotenoids to act as agents in chemoprevention of cancer and some other conditions (see below).
Retinoids have proved to be particularly effective in the treatment of one form of leukemia, called acute promyelocytic leukemia (APL). In this disease chromosome 15 is abnormal, with a translocation that affects the gene for RAR-α (Norum 1994). The best results have been obtained with a regime that consists of all-trans retinoic acid/arsenic trioxide-based therapy that is applied as soon as possible after diagnosis (Hu, Liu, Wu et al. 2009). In addition, selected RXR ligands, known as rexinoids, because they are involved in the control of a variety of metabolic processes, are showing promise in cancer therapy (Nahoum, Perez, Germain et al. 2007).

As with vitamin A (see above), the types of cancer that appear to be most closely related to carotenoid intake are those of epithelial cell origin – lung, head and neck, esophagus and stomach, colorectal, breast, cervix, and prostate cancers. β-carotene has been studied the most, but in the case of prostate cancer lycopene appears to be most promising (Obermueller-Jevic, Olano-Martin, Corbacho et al. 2003). Trials of β-carotene supplementation in high-risk groups like asbestos workers and cigarette smokers have given unexpected results showing either negative or even adverse effects of supplementation. It is generally concluded that supplementation with single nutrients, especially in large doses, is not advisable. Increased intake of fruit and vegetables provides a variety of antioxidant and other beneficial substances (Orfanos, Braun-Falco, Farber et al. 1981).

From several recent studies the provitamin A carotenoid cryptoxanthin appears to have a protective effect against the development of certain epithelial cancers. These include lung cancer (Yuan, Stram, Arakawa et al. 2003) and cervical dysplasia (Goodman, Kiviat, McDuffie 1998).

Cellular retinol-binding protein 1 (CRBP-1) mediates retinol storage, and CRBP-1 down-regulation chronically compromises retinoic acid receptor (RAR) activity. This leads to loss of cell differentiation and progression of some human tumors (Farias, Ong, Ghyselinck et al. 2005).

The β-Carotene and Retinol Efficacy Trial (CARET) referred to briefly above (see Omenn et al. 1996) tested the effect of daily β-carotene (30 mg) and retinyl palmitate (25,000 IU) on the incidence of lung cancer, other cancers, and death in 18,314 participants. The participants were at a high risk of lung cancer because of a history of smoking or asbestos exposure. The trial was stopped in 1996 because of a significant increase in lung cancer, death and cardiovascular disease mortality. A six-year follow-up without further intervention has shown that adverse effects have persisted but are no longer statistically significant. Analyses suggest the excess risk of lung cancer was restricted primarily to females, and cardiovascular disease mortality primarily to females and former smokers (Goodman, Thornquist, Balmes et al. 2004). This subject has recently been extensively reviewed (Sommerburg, Langhans, Salerno et al. 2005).

Animal experiments suggest that lycopene may be protective against smoke-induced lung lesions by means of its inhibiting effect on the most abundant insulin growth promoting (IGF) binding protein in human serum. This protein, IGFBP-3, is known to be growth inhibitory and apoptosis-inducing (Wang 2005).

CAROTENOIDs AND CHRONIC DISEASEs

There is a great deal of evidence to show that serum levels of β-carotene are lower than usual in many disease states. It has usually been assumed that this indication of what might be called low “β-carotene status” is an adverse effect and, if corrected, should lead to some health benefit. Surprise has been expressed that β-carotene supplementation under some circumstances, such as in cigarette smokers with lung cancer, has been related to deterioration rather than benefit (The α-Tocopherol, β-Carotene Cancer Prevention Study Group 1994; Omenn, Goodman, Thornquist et al. 1996). The hypothesis has recently been put forward (Jandacek 2000) that low β-carotene reflects the result of disease, serves as an indicator
of the presence of disease, and is not causally related. This might explain some of the anomalous results obtained, but much more work needs to be done.

Modest increase in β-cryptoxanthin dietary intake, equivalent to a single glass of freshly squeezed orange juice daily, has been associated with a reduced risk of developing polyarthritis (Pattison, Symmons, Lunt et al. 2005).

On the other hand it has been suggested, on the basis of work in experimental animals, that abnormal oxidation products of β-carotene might be responsible for the adverse effects of large doses mentioned above. It is noteworthy that the concurrent use of other antioxidants such as vitamins C and E mitigated the formation of β-carotene oxidation products and lung damage in experimental animals (Kim, Chongviriyaphan, Liu et al. 2006).

Until recently it has always been assumed that hypercarotenosis had no harmful effects, causing only high blood levels and pigmentation of the skin. It seems that there is still a lot to learn about the safety as well as the benefits of carotenoid ingestion.

**CARDIOVASCULAR DISEASE**

The results of numerous epidemiological studies involving β-carotene and other micro-nutrients in relation to the occurrence of ischemic heart disease are conflicting (Gaziano, Sesso 2005; Kabagambe, Furtado, Baylin et al. 2005; Gasparetto, Malinverno, Culacciati et al. 2005). A possible mechanism — if there is an effect — is reduced oxidation of low density lipoproteins, which seem to play a part in atherogenesis. Carotenoids other than β-carotene may be effective or the intervention time may have been too short for improvement to have been seen. A great deal more research is required in this and other related areas touched on here. Lycopene and its richest source in the average diet, tomato, have shown some promise in supplementation trials. However, it might be possible that lycopene is a biomarker for tomato consumption and other carotenoids, such as phytoene and phytofluene or oxidized metabolites of lycopene, might be bioactive (Petr, Erdman 2005).

**OTHER SYSTEMS**

Because retinoid-sensitive nuclear receptors have been found in all types of cell examined, it is likely that retinoids, especially in the form of retinoic acid, are capable of influencing any tissue. Experimental research reports have provided initial evidence for tissues and diseases that include the following.

β-carotene and vitamin A metabolism appears to be abnormal in patients with abnormal thyroid function (Aktuna, Buchinger, Langsteger et al. 1993). See Chapter 11 for effects of vitamin A supplementation on subjects with iodine deficiency.

A recent review (Berdanier 2003) on the role of vitamin A in diabetes mellitus provides convincing evidence for the need for research to be focused on this problem. Recently it has been shown experimentally that serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. A synthetic retinoid, fenretinide, increases urinary excretion of RBP4, normalizes serum values and improves insulin resistance and glucose intolerance (Yang, Graham, Mody et al. 2005). Human studies confirm some of these findings (Graham, Yang, Bluher et al. 2006).

Sudden infant death syndrome (SIDS) occurs worldwide and occurs in 1.5:1000 live births in the United States. Peak incidence is between the second and fourth month of life. The exact cause is unknown but various factors are considered to be of importance. A study in Scandinavia found a significant association between increased risk of SIDS and infants not being given vitamin A during the first year of life (Alm, Wennergren, Norvenius et al. 2003).

Sickle cell disease is a chronic hemolytic anemia occurring almost exclusively in African-
origin populations. It has been shown that a high proportion have vitamin A deficiency. In comparison with people with normal vitamin A status, these have lower BMI, Hb and Hct, and ten times as many hospitalizations, increased pain and fever episodes (Schall, Zemel, Kawchak 2004). Routine vitamin A supplementation appears warranted.

The role of vitamin A in mammalian reproduction and embryonic development has been reviewed (Clagett-Dame, DeLuca 2002). There is strong evidence that all-trans retinoic acid generated \textit{in situ} is the functional form.

It has been shown that mild vitamin A deficiency during fetal life can significantly reduce nephron number in the kidney (Burrow, 2000). Retinoids have an essential role in the branching growth of the ureteric bud in early fetal life. Many retinoid target genes involved in kidney development have been identified.
15 Control

INTRODUCTION

At the World Summit for Children in 1990 and at the International Conference on Nutrition in 1992, the goal agreed upon was to “virtually eliminate vitamin A deficiency and all its consequences including blindness by the year 2000.” 1995 passed without the mid-decade goal of “ensuring that at least 80% of children under 24 months of age receive adequate vitamin A through a combination of strategies” being met. The first edition of this book noted that “With very little time remaining the goal for the year 2000 is most unlikely to be reached in its entirety.” In Chapter 12 the latest figures from WHO are given on global prevalence of VADD and commented upon.

None of the goals of the past has been fully met, and while blinding xerophthalmia has ceased to be the major public health problem in young children that it once was, it is no longer being monitored (see Chapter 12). Low vitamin A status remains at about the same level and is now recognized to be a common problem in some women and school-age children.

Several distinct types of intervention have, however, been designed to control VADD, and these will be briefly introduced here before they are considered at greater length later.

Research into multi-micronutrient deficiency disease states, a concept which emerged in the early years of the third millennium, began only recently (see Chapter 11). It will be some years before the control of such complex deficiency disease states can become part of a chapter like the present one.

Treatment

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

Prophylaxis

Periodic high-dose vitamin A distribution or supplementation in the community may be viewed as a prophylactic extension of treatment in a hospital. The form of vitamin A is usually the same – capsules. The aim here is prevention. The measure, like treatment of the established case, should be superseded or supplemented by something of longer-term effect.

Control of infections

In recent years, the close two-way association between VADD and infectious diseases has become well recognized (see Chapters 9 and 13). This has led to the view that the combination of vitamin A supplementation with immunization has distinct theoretical and practical advantages. In recent years, this combined approach has been tested and found not to have important adverse effects. The promotion of National Immunization Days (NID) by many countries has provided
a novel opportunity for vitamin A supplementation of young children on a large scale. This and other aspects of the subject are discussed further below.

**Food fortification**

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

**Dietary interventions**

Dietary interventions of various kinds would seem to be the logical approach to the problem in most circumstances. For those who approach the problem for the first time it is usually a surprise and a shock to learn that the vast majority of young children who go blind and die from vitamin A deficiency do so surrounded by readily available sources of the vitamin. There are indeed “many slips between cup and lip”: problems in getting the vitamin out of the food and into the child. Recent evidence that the bioavailability of provitamin A carotenoids from fruit and vegetables is usually less than previously thought (Chapter 5) has complicated attempts to control VAD by means of dietary improvement. As a consequence, greater emphasis is being placed on the value of promoting the much more readily bioavailable preformed vitamin A sources wherever this is feasible.

**New plants**

Recently plant breeding and genetic modification have been applied to the development of a number of high-carotene foods. The genetic modification of rice to provide β-carotene (Golden Rice) has attracted worldwide attention and requires careful consideration in relation to the control of VADD.

**Disaster relief**

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

**GENERAL CONSIDERATIONS**

A report by the Administrative Committee on Coordination, Subcommittee on Nutrition (ACC/SCN Consultative Group 1994), *Controlling Vitamin A Deficiency*, set out results of a comparative evaluation of different interventions, drawing on 46 individual evaluations. Twenty-five were of supplementation interventions (vitamin A dosing), thirteen of dietary modification, four of fortification, two of public health, and two of breastfeeding. Supplementation is easiest to implement and to evaluate. It is difficult to make valid comparisons between the different types of intervention. In addition to this, it should be realized that some studies have been carried out under carefully controlled research conditions while others have been of a more programmatic nature. What goes on in strictly routine programs is very unlikely to be reported in the scientific literature.

In practice, choices will need to be made at the local level between the various control measures on offer that have been outlined above. Whenever possible, this choice should be made after the situation has been assessed in some way (see Table 12.2). A baseline may then be established and the efficacy of measures introduced evaluated to some extent. Treatment of cases and prophylaxis for vulnerable groups can be instituted almost right away. Other measures take much longer to be implemented fully. However, it should always be possible to start some nutrition and health education *pari passu* with capsule use.

It is important to bear in mind that interventions of this nature are taking place in a situation that can never be fully characterized and that is constantly undergoing change. It should there-
fore be evident that it will rarely be possible to attribute any benefit observed directly to the intervention. Good examples of this are provided by the improvements that have been documented in Bangladesh, India and Indonesia (see Figure 12.8).

Those engaged in the control of VADD should constantly keep the wider implications of health interventions in the backs of their minds. Health should be a sustainable state (King 1990). Some communities, by population increase, have outgrown, or are in the process of outgrowing, the carrying capacity of their ecosystem. In other words, there are too many (especially young) mouths to feed. The ethical implications of applying effective health interventions like vitamin A supplementation under such circumstances should not be shirked (King, Elliott 1993).

**TREATMENT**

On several occasions, WHO has made recommendations for the treatment of xerophthalmia. The latest schedule is shown in Table 15.1.

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

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

Because of the known teratogenic effects of large doses of vitamin A, care has to be exercised in the treatment of xerophthalmia in pregnant women. Active corneal lesions should receive the full treatment, but XN and X1B are treated with 10,000 IU (3.0 mg retinol, see Table 15.1) daily for two weeks.

Associated medical conditions, such as PEM, measles, and diarrhea, should receive appropriate treatment. Frequently, secondary infections of the eye are also present and should receive local and/or systemic treatment.

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**Table 15.1:** Treatment schedule for xerophthalmia for all age groups except women of reproductive age\(^A\) (WHO, UNICEF, IVACG Task Force, 1997).

<table>
<thead>
<tr>
<th>Timing</th>
<th>Vitamin A dosage(^B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately on diagnosis:</td>
<td></td>
</tr>
<tr>
<td>&lt;6 months of age</td>
<td>50,000 IU</td>
</tr>
<tr>
<td>6–12 months of age</td>
<td>100,000 IU</td>
</tr>
<tr>
<td>&gt;12 months of age(^A)</td>
<td>200,000 IU</td>
</tr>
<tr>
<td>Next day</td>
<td>Same age-specific dose(^C)</td>
</tr>
<tr>
<td>At least 2 weeks later</td>
<td>Same age-specific dose(^D)</td>
</tr>
</tbody>
</table>

\(^A\) Caution: Women of reproductive age with night blindness or Bitot’s spots should receive daily doses ≤10,000 IU or weekly doses of ≤25,000 IU. However, all women of reproductive age, whether pregnant or not, who exhibit severe signs of active xerophthalmia (i.e. acute corneal lesions) should be treated as above.

\(^B\) For oral administration, preferably in an oil-based preparation.

\(^C\) The mother or another responsible person can administer the next-day dose at home.

\(^D\) To be administered at a subsequent health-service contact with the individual.
PROPHYLACTIC SUPPLEMENTATION

This is often termed vitamin A supplementation. In almost all countries where programs of this nature are in operation, which numbered 103 in 2009 according to UNICEF, the supplement takes the form of a capsule. In India the supplement has been administered as a locally made syrup dispensed by teaspoon.

Children under the age of 6 years and pregnant and lactating women constitute the main vulnerable groups in communities where VAD has been identified as a public health problem. They should participate in a supplementation program wherever this is deemed to be appropriate (Table 15.2).

A number of studies carried out recently in several developing countries have shed additional light on some aspects of vitamin A supplementation programs. In Nepal (Christian, Schulze, Stoltzfus et al. 1998) supplementation with weekly dosage of vitamin A or β-carotene at about the RDA level failed to eliminate maternal night blindness in about 60% of cases. It is possible that some other factors besides vitamin A may also play a part, such as zinc deficiency (see Chapter 11). In a similar study in Bangladesh (Rice, Stoltzfus, de Francisco et al. 1999) vitamin A or β-carotene failed to prevent subclinical vitamin A deficiency in lactating mothers and their infants.

Universal distribution of vitamin A capsules in Bangladesh was reviewed (Bloem, Hye, Wijnroks et al. 1995) and was shown to be an effective strategy for reducing the incidence of night blindness, but weaknesses were revealed in the rural areas of the program.

There are two main areas of concern with regard to the safety of use of vitamin A prophylaxis (Sommer, West 1996:394–9). The first of these relates to acute adverse effects in children. Nausea, vomiting and headache have been reported in several percent of children taking part in large-dose programs (100,000 or 200,000 IU vitamin A). Severe vomiting (1.2%) was limited to the children given 60 mg vitamin A; symptoms lasted in almost all cases no longer than 12–24 hours. In young infants there may also be observed bulging of the anterior fontanelle of the skull, which is still open at that age. Like the other symptoms the effect is transient and there is no evidence of after-effects (de Francisco, Chakraboty, Chowdhury et al. 1993). Several controlled trials have been carried out and the consensus is that the extremely slight risk is fully justified in view of the potential benefit to life and health (Florentino, Tanchoco, Ramos et al. 1990). The effects of neonatal vitamin A supplementation were followed over a period of many months and no evidence was found for an adverse effect on development or growth (van


<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants &lt;6 months of age&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>• Non-breastfed infants</td>
<td>50,000 IU orally</td>
</tr>
<tr>
<td>• Breastfed infants whose mothers have</td>
<td>50,000 IU orally</td>
</tr>
<tr>
<td>not received supplemental vitamin A</td>
<td></td>
</tr>
<tr>
<td>Infants 6–12 months of age</td>
<td>100,000 IU orally, every 4–6 months&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Children &gt;12 months of age</td>
<td>200,000 IU orally, every 4–6 months&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mothers</td>
<td>200,000 IU orally, within 8 weeks of delivery</td>
</tr>
</tbody>
</table>

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

<sup>B</sup> Evidence suggests that vitamin A reserves in deficient individuals can fall below optimal levels 3–6 months following a high dose; however, dosing at 4–6 months intervals should be sufficient to prevent serious consequences of vitamin A deficiency.
A recent review of the vitamin A supplementation of young infants (Humphrey, Rice 2000) concludes that “a regimen in which mothers are given 200,000 IU vitamin A at delivery and their infants receive four doses of 50,000 IU (i.e., at birth and with their immunization contacts) would allow nearly all infants to enter the second half of infancy in adequate vitamin A status.”

The second issue concerns vitamin A prophylaxis during and shortly after pregnancy. Vitamin A and related compounds in large doses are known to be teratogenic in early pregnancy (Nau, Chahoud, Dencker et al. 1994) (see Chapter 14). High-dose vitamin A given at or shortly after delivery has been shown to raise breast milk levels considerably for a number of months. Frequently this is a rare opportunity to apply prophylaxis. Present recommendations are that the high-dose prophylaxis (200,000 IU) would be given only to breastfeeding women at delivery or during the infertile postpartum period, currently thought to last 4–6 weeks. IVACG (1998) has issued a statement on “Safe doses of vitamin A during pregnancy and lactation.” WHO currently recommends that the relatively small increased need for vitamin A during pregnancy should be met by diet, or a supplement not exceeding 10,000 IU daily throughout gestation (WHO, UNICEF, IVACG Task Force 1997). This is a second edition of its guide to the use of vitamin A supplements and prevention of vitamin A deficiency and xerophthalmia.

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

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

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

Before presenting some lessons learned from individual VAS programs it is necessary to draw attention to the source of one of the special problems that is currently in dispute and which is dealt with below. This concerns the lack of uniformity of response to VAS of children in different age groups under the age of 72 months. Between the ages of six and 72 months many studies have shown an increase in survival with VAS of 24% (Mayo-Wilson E., Imdad A., Herzer K. et al. 2011). Between 1–5 months there has been little or no response, for unknown reasons (West K.P. Jr, Katz J., Shrestha S.R et al. 1995, WHO Guideline 2011: Vitamin A supplementation in infants 1–5 months of age).

In newborns dosed within 2–3 days of birth mortality has been reduced in some settings by >15% (Humphrey, Agoestina, Wu et al. 1996, Klemm, Labrique, Christian et al. 2008, Tielsch, Klemm, West et al. 2008), but not in others (Benn, Diness, Roth et al. 2008, Malaba, Iliff, Nathoo et al. 2005).

The Nepal National Vitamin A Program (NNVAP) has been independently evaluated and provides important lessons for all in this field (Fiedler 2000). The NNVAP commenced in 1993 in eight of the country’s 75 districts and planned to implement nationwide coverage by 2003. Table 15.3 summarizes some key aspects of the NNVAP.
It consists primarily of twice-yearly distribution of high-dose vitamin A capsules to all children 6–59 months of age. Female community health volunteers play a key role. The annual cost is US$ 1.7 million. The cost per averted death is US$ 327. The incidence and severity of diarrheal disease and measles have been reduced, saving the government yearly US$ 1.5 million.

A recent update on NNV AP presented at the XXII IVACG Meeting stated that coverage rates of 85% and above had been maintained since inception in 1993 and all 75 districts were covered from October 2002. Bitot’s spot (X1B) prevalence was reduced to 0.33% (WHO cut off <0.5%). NNVAP was also seen as a major factor in the reported decrease in child mortality. This cost-effective program, at just US$ 0.74/child/year, has also contributed significantly to a deworming program at almost no extra cost.

Even with such a successful and apparently cost-effective supplementation program as this, the question must be asked about its continuance. Other countries, especially India, are in a similar position and decisions need to be taken.

In South India (Rahmathullah, Tielsch, Thulasiraj et al. 2003) the impact of supplementing newborn infants with vitamin A on early mortality was assessed. 11,619 newborn infants received 24,000 IU vitamin A by mouth or placebo on days 1 and 2 after delivery. Mortality at age 6 months was recorded. Those in the vitamin A group had a significant 22% reduction in total mortality compared with those in the placebo group. The impact was on mortality between 2 weeks and 3 months after treatment, with no additional impact after 3 months.

Another commendation of extension of the application of periodic vitamin A supplementation comes recently from a demonstrated decrease in mortality rate in HIV-infected children in Uganda (Semba, Ndugwa, Perry et al. 2005).

Recently in the Indian pediatric literature (Kapil, Prakash 2004), the relative merits of delivery of vitamin A by capsule or by syrup have been discussed. In each case, cost is low and efficiency of delivery is high. However, with millions of doses being dispensed annually these considerations merit further attention.

Long-term coverage has long been known to be disappointingly low in many circumstances. A recent study in central Java, Indonesia (Martini, Rice, de Pee et al. 2005) found that vitamin A capsule coverage among postpartum women overall was as low as 19.3%. Mothers active as health cadres, those whose infants were delivered by a midwife or a doctor, or those who delivered their baby in village maternity clinics had higher rates than others. None achieved more than 50%.

Experience with vitamin A prophylactic supplementation in India has been more extensive both over time (the national program started in 1970) and in terms of numbers covered. Detailed accounts of this work have recently been published (Reddy 2002; Kapil, Goindi 2004). In the first of these papers, and in some other sources, discussion has taken place concerning the reports in newspapers in 2001 that vitamin A caused deaths of children taking part in a mass supplementation campaign in the state of Assam. Like other similar reports over the previous years (see earlier) the general consensus was reached that vitamin A supplementation could not be held responsible for the deaths that occurred (Sommer, West 2002).

Accounts have been published of a number of national vitamin A supplementation programs, which report difficulties as well as achievements. As examples, attention may be drawn to the recent India experience (Kapil, Goindi 2004), the vitamin A capsule distribution among infants.
aged 6–11 months in rural Bangladesh (Akhter, Stallkamp, de Pee et al. 2004), and experience with enrichment of breast milk with vitamin A from several Asian countries (Harvey 2002). A recent study of refugee women in Sudan (Stranders, Northrop-Clewes, Thurnham 2004) showed a prevalence of low milk retinol concentrations and elevated mammary epithelial permeability. The postpartum megadose of vitamin A alone did not appear to be sufficient for a substantial and prolonged improvement of vitamin A status.

A recent study in Nepal (Grubesic 2004) looked at the relationship between the prevalence of health indicators of malnutrition, diarrhea, and acute respiratory infection and the consumption of vitamin A rich food and the supplementation status of three groups of children (regularly supplemented, supplemented once, and never supplemented). Regardless of the amount of vitamin A rich foods consumed, those regularly supplemented were better protected against all three groups of diseases than others.

An investigation of breastfeeding in Kenya by mothers with low vitamin A status showed that infants frequently remained deficient when weaned onto diets low in vitamin A sources (Ettyang, Oloo, Lichtenbelt et al. 2004).

In the Philippines, the National Vitamin A Supplementation Program has been operative among preschool children for many years. A recent report (Pedro, Madriaga, Barba et al. 2004) showed that two aspects of this experience were of major concern. Firstly, the impact of vitamin A supplementation on serum retinol was mainly confined to those with severe vitamin A deficiency. Secondly, the effect did not persist for 6 months, which is the interval between doses. It was recommended that in areas of low prevalence of VAD, distribution of capsules should be targeted to stunted children. In areas of high prevalence, capsules should be distributed to children aged 1–5 years three times a year.

The Nutrition Section, UNICEF HQ (2005) issued a monograph entitled “Vitamin A Supplementation: Progress for child survival” that summarizes most of the aspects of the subject.

THE SPECIAL ISSUES OF
1) NEWBORN VAS AND
2) EFFECTS OF VACCINES

1. Newborn VAS

Following upon a number of very large double-blind controlled field trials that have been undertaken in several low-income countries in South Asia and Africa on the effects of a single dose (of 50,000 IU vitamin A) to newborns, no general agreement concerning widespread implementation of VAS has been reached at the present time. The situation was discussed by one of the propenent groups (Tielsch, Klemm, West et al. 2008).

It was suggested that for VAS to reduce mortality significantly, two things need to be present; endemic VAD and high infant mortality rates (most such deaths are attributable to infectious diseases which are usually responsive to vitamin A). On the whole the countries chosen for study in Asia tend to meet these conditions while those in Africa do not.

Apart from infants whose mothers were given vitamin A during pregnancy and infants aged 1–5 months at the time of supplementation, the suggestion holds good: i.e. VAS significantly reduces mortality of newborn infants and those 6–59 months of age. The reasons for the age-related differences in outcome are not known, although it has been observed that the effect results from case fatality rather than from incidence of infection (Tielsch, Rahmathullah, Thulasiraj et al. 2007).

Now, after the event as it were, suggestions are being made and evidence being sought to try to explain the conflicting results. It has been clear for a long time that we have not been comparing like with like here. In general vitamin A status is different, as shown by Thurnham (2010) recently. Also it may be significant that the data previously given in Figure 12.5 show the Africa region to be better off for vitamin A supplies than is South Asia, and infant mortality rates tend to be lower. In this context and from earlier knowledge, data in Tables 12.1 and 12.2 are particularly difficult to interpret. Africa generally has rates up to
twice as high as Southern Asia. On the other hand it might be that application of the FAO food intake data is misleading and Africa deteriorates while Southern Asia improves.

The main contribution of Thurnham (2010) is his analysis of the varying practices regarding the initiation of breastfeeding in the newborn VAS studies. The well-known use or discarding of colostrum, of high vitamin A activity, seems to have been hitherto largely ignored and the relevant data in these studies are very scanty. Vitamin A status of the newborn is obviously enhanced and it should not be difficult to encourage the feeding of colostrum where appropriate. Thurnham rightly draws attention to two papers that show that failure to provide human milk to the neonate in the first hour after delivery will increase the risk of neonatal mortality (Edmond, Zandoh, Quigley et al. 2006, Mullany, Katz, Li et al. 2007). In two related Opinions (Klemm 2010, Benn 2010) it is good to see some common ground being shared.

In the same issue of the *Sight and Life* Magazine (McLaren 2010), in a personal communication a former colleague of mine raises an issue which those involved would do well to take heed of. He asks whether a detailed comparison has been made of the precise chemical formulation of the vitamin A product used, including details of the possible presence of any isomers. Such information appears not to be in any related publications. Differences between studies in this regard might easily influence results. The raising of such questions and the possible existence of others yet undiscovered imply lack of thoroughness in design in this field. A question was asked about monitoring of quality of vitamin A capsules for supplementation programs (Newton, Owusu-Agyei, Kirkwood 2007) and answered (Mannar, Court 2008) but the issues raised were entirely different.

The question of the possibility of differences arising in the effects of VAS supplementation in the presence of the use of vaccines, including sex differences, has been repeatedly raised by the Danish group of researchers in Guinea-Bissau, West Africa. The topic is dealt with below.

Meanwhile, as so often happens, things tend to move on and decisions are taken without all the possible questions to any particular situation being answered. In 2008 the group of research workers at the Johns Hopkins Bloomberg School of Public Health, Baltimore, USA which had been involved in the research in Asia, received support for what is termed the A2Z Program. It is anticipated that about 200,000 lives in South Asia would be saved per year by high coverage of supplementation of newborns with vitamin A (50,000 IU).

2. Effects of vaccines

The group of Aaby, Benn et al. has investigated programs of immunization of young children in West Africa for many years. They took the opportunity of the introduction of VAS to the WHO program, EPI, referred to below to study the possible effects of the combination. They became aware of the apparent differences between different countries with regard to the effect of VAS on newborns and published an hypothesis (Benn, Balé, Sommerfelt et al. 2003). In outline it proposed ‘that the effect of VAS may depend on the amplification of non-specific effects of vaccines on childhood mortality’. They suggested that different effects in different age groups may be explained by different factors operating. They point out that in their own and in two other studies boys are more benefited than girls, who may actually be harmed (Benn, Fisker, Jorgensen et al. 2008). Subsequent reports of this group have tended to strengthen doubts previously raised about some aspects of recommendations WHO continues to make about VAS in the presence of routine vaccinations of young children. In Open Commentary recently (Prentice, Savy, Darboe et al. 2009) the complexities of the issues were revealed. More recent analysis from the same group (Savy, Edmond, Fine et al. 2009) shows how the prevailing multinutrient approach is invading this field also. Yet again from Guinea-Bissau comes evaluation of BCG vaccination which includes adverse effects of vitamin A in
girls (Roth, Benn, Ravn et al. 2010, Shann 2010).

A line has to be drawn somewhere, or two lines here, without a prospect of satisfactory resolution.

PREVENTION AND MANAGEMENT OF INFECTIOUS DISEASES

After its success in eradicating smallpox WHO established its Expanded Program on Immunization (EPI), employing BCG, OPV, diphtheria, tetanus, pertussis, and measles vaccines. By 1990 nearly 80% coverage had been achieved and other vaccines and measures, such as vitamin A, have been added. Fifty percent of global coverage of vitamin A, one dose per year, was achieved in 1999. This rose to 68% in 2004 and 82% in 2008. The greatest gains have been in the least developed countries. The most successful aspect of EPI, or of any other immunization programs in developing countries, usually accompanied by VAS in recent years, has been that against measles, and this can be traced throughout the book through use of the Index.

Attention was drawn earlier to the inter-relationships of infections and vitamin A status (see Chapters 9 and 13) that need to be taken into account in the control of VADD. Immunizations that have been developed for certain infectious diseases, especially measles, may provide an opportunity for a joint approach. The Program WHO EPI advises that “any EPI contact after the age of six months is appropriate for supplementing the infant or young child [with vitamin A]. The visit for measles vaccine at around 9–11 months is especially suitable” (WHO 1994).

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

Local circumstances have varied greatly and socioeconomic and familial characteristics have influenced a program’s success (e.g., Gorstein, Bhaskaram, Khanum et al. 2003; Panganbari-buyan, Scherbaum, Erhardt et al. 2004). Ching, Birmingham, Goodman et al. (2000) made an estimate of the cost of integrating vitamin A supplementation into immunization campaigns. During 1998, in 41 countries, more than 94 million doses of vitamin A were administered. It is estimated that this helped to avert about 169,000 deaths. During 1999, delivery of more than 97 million doses in 50 countries helped to avert an estimated 242,000 deaths. The estimated incremental cost per death averted was US$ 72 (range 36–142) in 1998 and US$ 64 (range 32–126) in 1999. The estimated average total cost of providing supplementation per death averted was US$ 310 (range 157–609) in 1998 and US$ 276 (range 139–540) in 1999. Costs per death varied by campaign, depending on the number and proportion of the child population reached, number of doses received per child, and child mortality rates.

National Immunization Days (NID) were introduced in the 1990s and at that time were particularly focused on the elimination of poliomyelitis as a threat to young child health. It was soon appreciated that this might be an additional opportunity to improve vitamin A status where considered necessary, although optimal results appear to require more frequent dosing than
once a year. While NIDs for polio continued they offered a sound delivery structure and an unparalleled reach for VAS. In 1997 more than 450 million children, almost two-thirds of those under 5 years of age, were immunized during polio NIDs. For VAS human and financial resources are very reasonable (see Chapter 1). In 1998 75% of the 118 countries where VAD is a known or suspected public health problem conducted NIDs and over 60 million children received VAS.

Niger became one of the first countries in Africa to effectively integrate vitamin A supplementation into NIDs for polio eradication. Maintaining high vitamin A supplementation coverage has always proved to be difficult. In this instance, coverage exceeding 80% was achieved. This led in 1999 to the first ever National Micronutrient Days (NMD) in Africa (Aguayo, Baker, Crepin et al. 2005). As NIDs are being phased out, although not as early as expected in some countries, this new approach might prove to be an important way forward.

Vitamin A supplementation has never been incorporated into a program of oral rehydration for the prevention and treatment of diarrheal diseases (see Chapter 9). Beneficial effects of vitamin A were demonstrated years before the same were reported for zinc. It is therefore puzzling to see that UNICEF recommends the latter, while no mention is made of vitamin A (Baqui 2006). This is also contrary to other statements by the UN System (see Chapter 1 and also below) on these micronutrients.

FOOD FORTIFICATION

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

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

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

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

Like supplementation, food fortification may be universal or targeted. In the former, fortification is applied throughout the population. In the latter, it applies to specific groups (e.g., to supplementary feeding programs for pregnant women, welfare recipients, school children, or those receiving complementary foods). Fortification of food aid in emergencies is an important issue (see later). Several research studies (Sommer, West 1996:410–30) have demonstrated conclusively that fortification can significantly improve vitamin A status of a whole population.

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

Sugar in Latin America and monosodium glutamate (MSG, a popular flavor enhancer) in Southeast Asia were the first vehicles for vitamin A fortification to be extensively tested, widely distributed and evaluated for their public health impact (Sommer, West 1996: 411–25). Neither of these food substances is an ideal vehicle from the nutritional point of view, but even so, sugar fortification is in operation at the present time in several countries in Central America and is in the process of being introduced in some countries in Latin America, in Africa, and elsewhere.

From the early 1970s, a number of Central and South American countries fortified white, refined sugar with vitamin A; much of the development took place in Guatemala. Surveillance over a number of years demonstrated a positive impact of the program (Arroyave 1986). Adverse internal and external circumstances halted the program for nearly eight years. In 1988, six months after a program restart there was significant reduction in the number of children with low serum retinol (26% to 10%) and of those with an abnormal RDR (33% to 14%) (Pineda 1993).

In the 1970s, fortification of MSG was pursued in the Philippines and in Indonesia. The programs proved to be efficacious in children to raise serum retinol levels, reduce the prevalence of xerophthalmia, improve linear growth, and reduce mortality (Solon, Latham, Guirriec et al. 1985; Muhilal, Permaesih, Idjradinata et al. 1988) (see Figure 15.1).

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

Despite the limitations and the drawbacks, the examples given here have demonstrated that food fortification can be a very powerful strategy. In order for food fortification to be successful, effective collaboration of all parties involved is essential, including scientists, industry, public advocacy groups, legislators and politicians. Sommer and West (1996:410–30) end their review of the subject as follows: “... implementing a national fortification program is a major undertaking that requires sound scientific rationale, industrial capacity, training, advocacy, adequate legislative support, economic viability, community acceptance and long-term sustainability, monitoring and quality control.”

In recent years food fortification programs have commenced in many countries. There have
been reports on many of these at IVACG meetings and in the *Sight and Life* Magazine. One feature has been the successful fortification of novel food items, for example vegetable oil in Morocco (Borghos, Rahmani, Elkari et al. 2002) and cassava in Nigeria (Asonye 2002).

As evidence continues to accumulate in support of the low bioavailability of carotenoids (see Chapter 5), the need for the inclusion in most traditional diets of some vitamin A in its preformed state (i.e. from animal sources) seems unavoidable (see Chapter 12). In this context mention was earlier made (see Chapter 14) of recent discussion on the possibility of excessive vitamin A fortification contributing to bone damage.

Recently, the case has been made for inclusion of selenium and iodine in multi-micronutrient biofortification programs which already include vitamin A, iron and zinc (Lyons, Stangoulis, Graham 2004).

Home fortification refers to a relatively new approach where vitamins and minerals usually used for food fortification are added to the commonly eaten, prepared food just before consumption (Hyder, Zlotkin 2008; de Pee, Kraemer, van den Briel et al. 2008). Home fortification has been applied at increasingly larger scales since 2002. Through home fortification the micronutrients can be targeted and specifically dosed (one RNI or less per individual), and as they are added to the plate or bowl of food just before eating, they are not subject to processing and preparation losses. The concept of home fortification has been proven to be efficacious for reducing deficiencies among young children under controlled circumstances, and has been piloted in large emergency and refugee settings.

The concept of home fortification was developed in the 1990s with the earliest forms of single-dose sachets containing micronutrients in a powdered form that could be easily sprinkled on any individual portion of food prepared in the household. Designed in such a way as to minimize changes to the taste, color, or texture of the food upon addition of the micronutrient powder, these micronutrient powders contained a mixture of essential vitamins and minerals (such as vitamin A, iron, zinc and iodine) in low doses that contribute to its consumers’ daily needs.

**DIETARY MODIFICATION**

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

1. Nutrition education or communication, often using a social marketing approach, to improve practices related to the consumption of available vitamin A rich food sources.
2. Horticultural interventions (or home food provisioning, e.g. home gardening), that aim to increase availability of vitamin A rich foods.
3. Economic/food policies affecting availability, price and effective demand of vitamin A rich foods.
4. Technological advances concerning food preservation, plant breeding, etc.

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

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

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

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

Dietary interventions are usually targeted towards vulnerable groups – infants, preschool-age children and their pregnant and lactating mothers. School-age children might also be included if shown to be particularly at risk. The food categories to be promoted will vary with the vulnerable group (Figure 15.2).

Surveys have shown that young children frequently eat less than 15 g of green leafy vegetables in a day. It was shown in Bangladesh (Rahman, Mahalanabis, Islam et al. 1993) that 40 g of dark green leaves would be readily consumed daily if prepared attractively. This is sufficient to provide the daily requirement of vitamin A in one meal.

Most yellow fruits and some green leaves are usually available only at certain seasons. Village-based food processing and preservation can extend the availability and acceptability (Sommer, West 1996:355–87).

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

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

Dietary modification can be brought about by a variety of means, and if several can be employed so much the better. In Vietnam (English, Badcock, Giay et al. 1997), a combination of home garden production and nutrition education resulted in highly significant improvement in the incidence of diarrheal disease and respiratory infections. Social marketing of vitamin A rich foods in Thailand, with special emphasis on use of the readily available ivy gourd, could be readily applied in the region to bring about behavioral change (Smitasiri, Attig, Valyasevi et al. 1993).

Recent experience has confirmed that red palm oil is a valuable source of both provitamin A and non-provitamin A carotenoids (Mahapatra, Manorama 1997; Solomons 1998). Other palms have similar potential, for example in Bangladesh the palmyra palm (Borassus flabellifer), which is native to tropical Africa as well as being found in south and southeast Asia, is being promoted (Shamim, Mawla, Islam et al. 2003). Another important contribution comes from Bangladesh.

Figure 15.2: Composite profile of age-specific protection against xerophthalmia and low serum retinol levels conferred by dietary intakes of selected types of foods. Solid bar denotes ages for which epidemiologic evidence is strong. Dashed bar denotes ages at which some evidence of protection exists for a food (Sommer, West, 1996: 130–137).
Roos, Islam, Thilsted (2003) have advocated the incorporation into carp culture of some wild, small indigenous fish species to augment intakes of vitamin A, calcium and iron in rural households. Rodriguez-Amaya (2002) has for long investigated and promoted what she calls the “bounty” of carotenoid sources in Brazil.

Dark green leafy vegetables have received bad press in recent years concerning the low bioavailability and low bioconversion of carotenoids (see Chapter 5). The deuterated-retinol-dilution technique was used to demonstrate that daily consumption of cooked, pureed green leafy vegetables or sweet potatoes has a positive effect on vitamin A stores in Bangaldeshi men (Haskell, Jamil, Hassan et al. 2004).

Again from Bangladesh comes the account of a success story of a homestead gardening program, which resulted in household food security and empowerment of women (Bushamuka, de Pee, Talukder et al. 2005).

Attention has been drawn to the use of “green rice,” traditional in Vietnam (Vuong, Burgess 2004). The local variety of rice is harvested early, when the hulls are still green, and processed into a cereal commonly eaten for breakfast. Analysis of fresh green rice showed concentrations of about 1.9 μg/g of β-carotene and of 2.7 μg/g of lutein. Unlike the hulls of paddy rice, which are removed during processing, those of green rice remain attached. Other vitamins and minerals are therefore available.

It is becoming more widely appreciated that better results from interventions may be expected when more than one adverse factor is counteracted at any one time. Thus, increased fat intake and anthelmintic drug treatment have enhanced the effectiveness of food-based programs (Jalal, Nesheim, Agus et al. 1998).

These and many other reports from around the developing world provide the hope that knowledge-based, careful and persistent efforts to utilize the local resources may go a long way to bringing about the final eradication of vitamin A deficiency in many areas.

**GENERAL ISSUES**

Multiple strategies are now being increasingly employed and this sometimes makes interpretation of results very difficult if not impossible. Many such interventions are being undertaken in countries that are undergoing rapid social and economic change where control, or even identification, of some important variables is not possible.

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

Considerable concern should be expressed about the mixed messages that are emanating from WHO and UNICEF relating to some of the measures being advocated for child survival and health at this time. For example, “vitamin supplementation coverage with at least one dose in last 6 months” is advocated for prevention (Countdown to 2015 Conference 2006), without specifying the vitamin and not including iron or zinc. Another group (Edejer, Aikins, Black et al. 2005) echoes most statements on methods to achieve the fourth Millennium Development Goal (MDG), specific to child health, by including supplementation with vitamin A and zinc. More recently progress has been made towards agreement on the means of meeting MDG requirements, especially as expressed in the Copenhagen Consensus (2008).
Reducing undernutrition is one of the MDGs and is also a key factor underpinning several others. Recent estimates published in The Lancet Series (see Chapter 1) showed maternal and child undernutrition being responsible as the underlying cause for 3.5 m deaths, 35% of the disease burden in children <5 years old, and 11% of the total global DALYs. The considerable contribution to this from VADD was referred to.

A third group (Baqui 2006) advocates addition of zinc to fluids for control of diarrhea in young children, but ignores vitamin A. Most recently, a fourth proposes fortifying milk with zinc and iron, but not vitamin A (spelling disaster in the form of severe marasmus, as was the case through free government milk distribution in some countries in the past) (Bhutta 2007). The consequent confusion is serious, as is the ignoring of all the evidence of the efficacy of vitamin A from the recent past (see Chapter 9).

PLANT BREEDING AND GENETIC MODIFICATION

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

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

Rice (Oryza sativa) is usually milled to remove the oil-rich aleurone layer that turns rancid upon storage especially in tropical areas. This layer contains some β-carotene and some other nutrients that do not occur in the remaining edible portion of the grain, the endosperm. In this research recombinant DNA technology, with a combination of transgenes, was used to enable biosynthesis of provitamin A in the endosperm to occur. Immature rice endosperm can synthesize the early intermediate geranylgeranyl diphosphate (GGPP – see also Chapter 2). This can be used to produce the uncolored carotene phytoene by expressing the enzyme phytoene synthase in rice endosperm. For the synthetic pathway to be completed towards β-carotene three additional plant enzymes are required – phytoene desaturase and ζ-carotene desaturase, each of which catalyzes the introduction of two double bonds, and lycopene β-cyclase, encoded by the lcy gene. Four transgenes were required; all were obtained from daffodil (Narcissus pseudonarcissus).

Earlier studies have shown that the amount of β-carotene available from “golden” rice is quite low, ranging from 0.4 μg/g to 1.6 μg/g (Datta, Parkhi, Rai et al. 2005). The consumption of 200 g of such rice/day should meet daily requirements, but only providing absorption and conversion rates are high. More recently (Paine, Shipton, Chaggar et al. 2005), the daffodil gene encoding phytoene synthase has been replaced by one from maize. In this new Golden Rice 2, total carotenoids were increased up to 23-fold and there was preferential accumulation of β-carotene.

In most cases the transformed endosperms of the rice were yellow in color, indicating the formation of carotenoid. There was some variation in different seed lines concerning the carotenoids produced. Often this was only β-carotene, but occasionally lutein and zeaxanthin were also detected. From the results so far it seems that the yield of provitamin A will be at least 2 μg/g of rice as consumed. The authors suggest that a daily intake of 300 g of rice will provide 100 μg RAE. This would be equivalent to about 25% of a child’s RDA. This assumes that the bioavailability
of β-carotene would be 1/6 that of retinol, but this remains to be fully established. Recent research indicates a conversion factor of 1:4 (Tang, Qin, Dolnikowski et al. 2009). There are still many technological, sociological and other considerations to be addressed before there is any possibility of GM rice becoming the staple food of hundreds of millions of the world’s poor. A diet of ordinary rice adequately supplemented with vegetables and fruits as well as some meat and fish is capable of providing a balanced supply of known nutrients and other health-promoting substances. The overriding goal of attaining full nutrition for all by practical and realistic means should not be allowed to be obscured by any “quick fix” approaches.

Potrykus, the scientist who originally developed Golden Rice, has written about some of the difficulties being experienced in practice (2003). Legal complexities had to be overcome to make Golden Rice freely available to developing countries. The International Humanitarian Golden Rice Network has been formed, with many institutes in rice-growing countries. Challenges include transfer of safe technology, overcoming consumer prejudice and GMO opposition, and deregulation.

Genetically engineered ‘Golden Rice’ was recently shown to be an effective source of vitamin A, containing 35 μg β-carotene per gram of rice in a trial in middle-school children in China (Tang, Qin, Dolnikowski et al. 2009).

The general trend, demonstrated throughout this book, of the emphasis in future moving steadily towards multi-micronutrient, rather than simple nutrient, deficiency control is shown in a recent study involving corn (Naqvi, Zhu, Farre et al. 2009). The three vitamins investigated were β-carotene, ascorbate, and folate. The amount of vitamins contained in the transgenic kernels as compared with normal was 169-fold, 6-fold and double respectively.

**DISASTER RELIEF**

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

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

The experienced team of Aaby and colleagues (Nielsen, Benn, Balé et al. 2005), following the WHO recommendation to provide vitamin A supplementation in emergency situations, evaluated such a program in the war emergency in Guinea-Bissau, 1998–9. They were able to compare wartime with pre-war mortality in over 5,000 children aged 6 months to 5 years in the capital. They found a slight, nonsignificant mortality reduction during the war in those receiving supplementation, but this compared with a significant 12% reduction during three years pre-war. Vitamin A supplementation was associated with a significant reduction in cultural and socioeconomic inequalities.

Where disaster relief is indicated, multiple deficiencies of micronutrients are especially likely to occur and to be sometimes of a severe degree. Concerned UN Agencies recommend supplementation with micronutrient powders.
**Table 15.4:** The composition of multiple micronutrient supplements for pregnant women, lactating women, and children from 6 to 59 months of age, designed to provide the daily recommended intake of each nutrient (one RNI) (WHO, WFP, UNICEF Joint statement 2007).

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Pregnant and lactating women</th>
<th>Children (6–59 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A μg</td>
<td>800.0</td>
<td>400.0</td>
</tr>
<tr>
<td>Vitamin D μg</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin E mg</td>
<td>15.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin C mg</td>
<td>55.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Thiamine (vitamin B₁) mg</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Riboflavin (vitamin B₂) mg</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Niacin (vitamin B₃) mg</td>
<td>18.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Vitamin B₆ mg</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin B₁₂ μg</td>
<td>2.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Folic acid μg</td>
<td>600.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Iron mg</td>
<td>27.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Zinc mg</td>
<td>10.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Copper mg</td>
<td>1.15</td>
<td>0.56</td>
</tr>
<tr>
<td>Selenium μg</td>
<td>30.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Iodine μg</td>
<td>250.0</td>
<td>90.0</td>
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containing 15 micronutrients, shown in **Table 15.4**, which are also being used in multi-micronutrient supplementation research trials (see Chapter 10).
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