XEROPHTHALMIA AND POST-MEASLES EYE LESIONS IN CHILDREN IN TANZANIA

A STUDY OF NUTRITIONAL, BIOCHEMICAL AND OPHTHALMOLOGICAL ASPECTS

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Proefschrift
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The financial support from the Netherlands Foundation for the Advancement of Tropical Research (WOTRO) and the Tanzania Food and Nutrition Centre (TFNC) throughout the study and from the "Fonds Landbouw Export Bureau 1916/1918" (LEB-fonds) for a grant to support this publication is gratefully acknowledged.

STELLINGEN

1. De bruikbaarheid van analytische methodieken welke niet gepubliceerd zijn in wetenschappelijke tijdschriften dienen met argwaan te worden bekeken; dit houdt echter niet in dat alle gepubliceerde methodieken wel bruikbaar zijn.
   (o.a. dit proefschrift)

2. In recente publikaties over de scheiding van verschillende caroteen-frakties uit menselijk serum ontbreekt veelal een verwijzing naar het in de periode 1935-40 door Lanzing en van Veen in het toenmalige Nederlands-Indië verrichte werk. Dit is onterecht.
   - **J.C. Lanzing, Mededelingen Dienst Volksgezondheid Ned-Indië 1938;17:213-23.**
   - **A.G. van Veen en J.C. Lanzing, Geneesk Tijdschr v. Ned-Indië 1940;80:514-37.**

3. Bij de evaluatie van serumwaarden voor albumine, retinol-bindingseiwit, prealbumine en retinol, in kinderen met mazelen dient rekening te worden gehouden met de duur van de mazeleninfektie.
   (dit proefschrift)

4. De prevalentie van corneale littekens ten gevolge van xerophthalmie kan binnen een periode van drie jaar niet met en faktor tien worden verlaagd door aan 10% van de kinderen onder zeven jaar welke op aselekte wijze zijn geselekteerd, capsules met een hoge dosis vitamine A te verstrekken.
   - **Assessment of the prevalence of xerophthalmia in Haiti, American Foundation for Overseas Blind, New York, 1976.**
   - **Evaluation of a programme to prevent xerophthalmia in Haiti, Helen Keller International, New York, 1979.**

5. Het ligt niet in de eerste plaats aan de landen waar xerophthalmie een probleem is, dat de door FAO en WHO ter bestrijding van vitamine A deficiëntie opgezette 10 jaren programma's gedoemd zijn te mislukken.

6. De biologische effektiviteit van op de juiste wijze gebruikte oral-rehydration fluids is goed gedokumenteerd, maar de effektiviteit op gemeenschaps niveau hangt sterk af van de manier waarop deze vloeistof in de praktijk wordt toegepast.
   - L.C. Chen, Lancet 1986,ii,1260-4.

7. Vanaf het moment, dat er op de verpakking van "Boerenlandmelk" de op zichzelf juiste opdruk "extra volle melk" of "vette melk" zou verschijnen, valt een dalende vraag naar dit produkt te verwachten.

8. Alhoewel wetenschappelijk gezien interessant en wellicht leidend tot financiële besparingen valt het niet te verwachten dat er naar het effekt van kransvatchirurgie een dubbelblind onderzoek bij de mens zal worden uitgevoerd.

9. Als auteurs om den brode gedwongen worden zoveel mogelijk publikaties op hun naam te krijgen, zouden zij kunnen overwegen om steeds twee of meer deelpopulaties in hun onderzoek te betrekken ten einde over elke populatie apart, maar wel in het zelfde nummer van een wetenschappelijk tijdschrift, kort te publiceren.
   - J.J. Counsilman, et al. Breastfeeding among poor Singaporeans, J Trop Ped 1986;32:310-2.
   - J.J. Counsilman, et al. Breastfeeding among well-to-do Singaporeans, J Trop Ped 1986;32:313-7.

10. Het verhoogde gezondheidsrisiko van "dikke boeren" in Groningen en Drente wordt wellicht meer veroorzaakt door een falend internationaal landbouwbeleid dan door een ongunstig lichaamsgewicht uitgedrukt in gewicht/lengte^2.

Proefschrift F. Pepping
Xerophthalmia and post-measles eye lesions in children in Tanzania.
Wageningen, 25 november 1987.

Voor mijn ouders

CONTENTS

PREFACE

The concept of detaching someone from the Department of Human Nutrition of the Wageningen Agricultural University at the Tanzania Food and Nutrition Centre (TFNC) arose in the spring of 1981 at a meeting between Professor J.G.A.J. Hautvast, Dr T.N. Maletnlema (Managing Director TFNC) and Dr C.E. West. I gratefully acknowledge the confidence they had in me then to carry out my assignment.

Professor S. Franken joined our group when partly as a result of my stay at TFNC it was decided to submit a research proposal for funding to the Netherlands Foundation for the Advancement of Tropical Research (WOTRO) with the aim to carry out a number of studies on vitamin A deficiency in Tanzania.

To Jo Hautvast, Simon Franken and Clive West: I have enjoyed working with you the last four years and want to express my sincere thanks to you for all the assistance obtained not only during my stay in Tanzania but also during the final year of analyses and writing up of the results.

I am very grateful to Dr Maletnlema for his permission to work at TFNC for three years; his advice on many scientific and technical matters have been of great help.

Without the participation and assistance of many it would not have been possible to carry out the research described in this thesis. With gratitude I want to acknowledge the help of the following persons and organizations.

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- A number of students from Holland participated in the research activities in Tanzania: Mr Geert van Poppel, Ms Erica Hackenitz, Ms Anneke van der Giezen and Ms Karin de Jonge; other students worked in Wageningen on various aspects of the project: Mr Werner Schultink, Ms Ineke Scholte, Mr Rudolf Kaaks, Mr Rob Roggebrand, Mr Andrew Brown, Ms Marion Gijbels and Ms Karina Vencken.

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- The Royal Netherlands Embassy in Dar es Salaam and the Ministry of Foreign Affairs in The Hague assisted in solving housing, transport and communication problems.
- The Foundation for the Battle Against Blindness in Developing Countries (Stichting Blindheidsbestrijding Ontwikkelingslanden) made it possible to carry out the follow up study in Tabora Region and the Harald Quintus Bosz Foundation gave financial support to allow me to attend the International Vitamin A Consultative Group meeting in Hyderabad (India).
- The medical officers, ophthalmologists and paediatricians, then working at the hospitals participating in the hospital-based research on post-measles blindness, Drs D. Masoza, G.L.L. Kassililika, I.A.R. Msigua, L.T. Khan, N. Kinabo, W. Mpanju and A.E. Msengi assisted us greatly. I am especially grateful to Dr K.K.A. Msambichaka for her stimulating assistance at Temeke Hospital during the initial phase of the project.
- Dr Maureen B. Duggan (University of Sheffield) critically reviewed some chapters of this thesis and her experience has been of great help.
- Dr M.A.J. van Montfort (Department of Mathematics) gave me advise on statistical matters and Mr F.S.H.M. Werrij of the same department assisted with the transfer of data from microcomputer to the university mainframe computer.
- The staff of the Central Service Department of the Biotechnion prepared maps, figures and photographs for the thesis.

Finally, I want to thank the staff of the Department of Human Nutrition and the International Course in Food Science and Nutrition (ICFSN) for their interest in the study and specifically Ms Ans Soffers and Mr Frans Schouten for the retinol analyses, Mr Peter van de Bovenkamp for his advice on the food analysis, Ms Ine Halferkamps and Ms Erica Hackenitz for the computer analysis of the data described in Chapters 2, 3, 7 and 8, Jan Burema for statistical advice and proof-reading, Mrs Marlou Mertens for logistic assistance, Dr Frits van der Haar for his help in the early stage of the project both in Dar es Salaam and Wageningen and for his comments on some of the manuscripts, and Mr Marcel van Leuteren, Ms Bianca Dijksterhuis and Mrs Riet Hoogkamer-Weijman for typing the manuscripts.

Wageningen, August 1987 Fré Pepping

ABSTRACT

From 1983 until 1986 a number of studies were carried out in collaboration with the Tanzania Food and Nutrition Centre on vitamin A deficiency and post-measles nutritional blindness.

Prevalence surveys were carried out in four regions in Tanzania in order to estimate the magnitude of xerophthalmia where it was found to be a problem in certain clusters of villages. The retinol and carotenoid content of certain food products were analysed and the results subsequently used in a study to estimate the nutrient intake of children suffering from xerophthalmia. The intake of vitamin A in these children was low.

In children with measles, serious eye lesions which may easily result in blindness, were observed in 3-4% of the children admitted to a number of hospitals. Serum retinol levels in these children were extremely low while in children with measles and not developing such lesions levels were somewhat higher although they were still rather low. These findings confirm the role of vitamin A in the aetiology of post-measles eye lesions.

1. INTRODUCTION

"The state of the world's children"

For some years now the Director of the United Nations Children's Fund (UNICEF) has reported on the state of the world's children (1,2). A number of "low-cost" and "low-risk" strategies have been introduced in order to improve the nutritional and health status of children at risk. Growth monitoring, oral rehydration therapy, prolonged breastfeeding and universal immunization against a number of diseases (GOBI) are given high priority. It is said that these measures can be introduced now because they are independent of the economic and political changes necessary in the longer term to eradicate poverty (1).

Not withstanding such efforts, malnutrition in many forms still threatens the life and health of many children in Africa, Asia and Latin America. Development of the agricultural sector will be necessary to solve the world's food problems although increases in food production will have to be accompanied by improved food distribution in order to increase food consumption and overcome malnutrition (3).

In this thesis the results are presented of a number of studies carried out in Tanzania (East Africa) in which the role of malnutrition in general and a shortage of vitamin A in particular has been examined in preschool-age children. Details of a number of factors which appear to exacerbate the effects of vitamin A deficiency are also given and suggestions for further action are made.

Vitamin A

The generic term vitamin A refers to all fat-soluble compounds present in foods with the biological activity of retinol. Vitamin A occurs in two forms: as pre-formed retinol and as provitamin A compounds such as carotenoids which can be converted in the body to retinol. The best defined function of vitamin A is in vision (4), but it is also involved in cellular differentiation, in the synthesis of glycoproteins including those of the cell surface and in the synthesis of mucous secretion from epithelial tissues. Reproduction, growth and the immune system are also affected by a deficiency of vitamin A. The biological activity of vitamin A is very closely related to molecular structure (5). For example, retinoic acid can replace retinol in the synthesis of glycoproteins but not for vision while only 50 of the 500 known natural

carotenoids have any vitamin A activity.

As with the other fat-soluble vitamins, vitamin A is stored in the body. Thus prolonged periods of reduced intake are necessary in order to deplete body stores before marginal vitamin A status and overt symptoms of deficiency occur.

For many years the monograph written by Moore in 1957 (6) has served as the most complete reference work on vitamin A. In recent years a number of books have been published which provide an excellent overview of the knowledge available on the chemistry, metabolism and nutritional aspects of vitamin A, its precursors and related compounds (7,8). Much attention has been paid to the role of vitamin A in nutritional blindness (9) and the possible measures for the prevention of vitamin A deficiency (10).

There is much confusion on the nomenclature of vitamin A compounds. In this thesis, the nomenclature outlined by DeLuca et al. (7) in their review on recent advances in our knowledge of the metabolism of vitamin A is used.

Vitamin A deficiency and xerophthalmia

The terms xerophthalmia, vitamin A deficiency and vitamin A status are used in accordance with the guidelines laid down in the most recent (1982) WHO publication on this subject (11). The term xerophthalmia includes all ocular manifestations of vitamin A deficiency. The term vitamin A deficiency has a much broader definition relating to any state in which vitamin A status is subnormal.

The current xerophthalmia classification (11), was applied in the work presented in this thesis to describe the ocular symptoms found except for xerophthalmia fundus (XF), which is rarely used under field conditions. The localization of the eye lesions resulting from vitamin A deficiency are indicated in Figure 1.

Revision of the xerophthalmia classification in 1982 (see Table 1) was accompanied by modification of the criteria for determining the public health significance of xerophthalmia and vitamin A deficiency (11). The present criteria are based largely on studies carried out in Indonesia (9). Night blindness (XN), conjunctival xerosis (X1A) and Bitot's spots (X1B) are also referred to as "mild xerophthalmia", while XN, X1A, X1B and corneal xerosis (X2) and corneal ulceration/keratomalacia (X3) are referred to as "active xerophthalmia". During field surveys, conjunctival xerosis is sometimes excluded from the xerophthalmia classification as it is liable to misinterpretation (12).

The prevalence of any of four (of the total of six) clinical criteria in the 0.5 to 6 year age group may be used to determine the magnitude of the public health problem of xerophthalmia. The biochemical criterion of plasma vitamin A levels of 0.35 µmol/l or less indicates that there is significant vitamin A deficiency and may be used in the absence of clinical information if the objective is to improve vitamin A status (11).

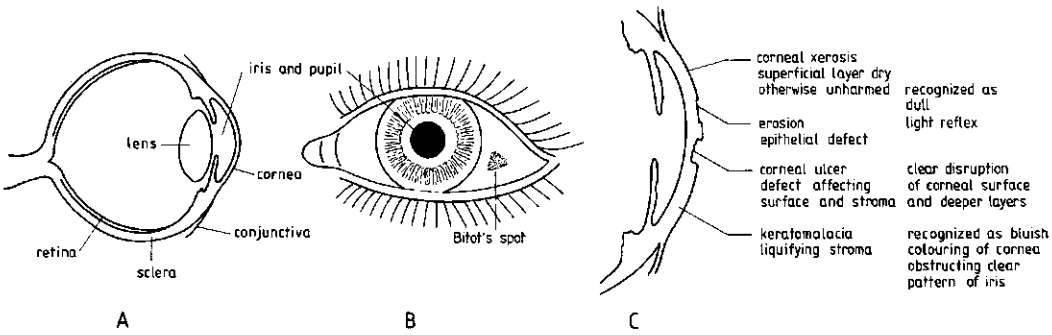

Figure 1: Diagram of the eye (cross-section, A; front view, B) and the localization of the various eye lesions due to vitamin A deficiency with special reference to the localization of corneal defects (C).

Methods for the identification of vitamin A deficiency

Techniques for vital staining were introduced ten years ago as an improved method of identification of conjunctival and corneal xerosis (13). This technique is based on the assumption that degenerated keratinized and dead cells which are present on the ocular surface would be preferentially stained by a suitable dye and thus give an indication of the severity of xerophthalmia. Subsequent reports questioned the specificity and sensitivity of this technique (14,15,16) and the test has been applied less frequently in recent years. More recently, conjunctival impression cytology has been introduced. In this method, a layer of epithelial cells is removed from the conjunctiva by applying filter paper strips to the eye and then stained. An early sign of xerophthalmia is the absence of Goblet cells and an increase in the number of large, keratinized epithelial cells (17). No results are available yet on the applicability of this test in large scale field surveys.

Table 1. World Health Organization scheme for classification of xerophthalmia and for determining the public health significance of xerophthalmia in terms of the percentage of each grade of xerophthalmia in the population 6 months to 6 years old (11)

Classification code	Clinical description	Prevalence levels indicating significant public health problem
XN	Night blindness	> 1% (*)
X1A	Conjunctival xerosis	*
X1B	Bitot's spots	> 0.5% (2%)+
X2	Corneal xerosis	
X3	Corneal ulceration/keratomalacia involving less (X3A) or more than 1/3 (X3B) of the corneal surface	> 0.01% (0.01%)
XS	Corneal scar	> 0.05% (0.1%)
XF	Xerophthalmic fundus	*
Biochemical criterion:	Plasma vitamin A concentration 0.35 μmol/liter (10 μg/100 ml) or less	> 5% (5%)

* No criteria established.
\+ The criteria used prior to 1982 are given in parentheses (18).

Vitamin A status is related to the amount of vitamin A stored in the body principally in the liver but this is difficult to measure. Serum levels of retinol only provide a good indication of vitamin A status when the levels of vitamin A in the liver are very low or reach saturation (19). Thus new techniques for the measurement of vitamin A status have been sought. One such technique is that for measuring the "relative dose response" (RDR). This method is based on the observation that when vitamin A reserves in the body are low, administration of a small dose of vitamin A elevates the retinol concentration in plasma to a maximum after five hours (20). Two disadvantages may limit the use of this technique: two blood samples are required to carry out the test and protein-malnutrition interferes with the interpretation of the results. Other techniques, such as measurement of vitamin A status by isotope dilution, are suitable for laboratory studies but less suitable for studies in the field (21). The development of such techniques is directed towards identification of a simple, realistic method for the early detection of vitamin A deficiency before the development of clinical signs.

The concept of "post-measles blindness"

The term "post-measles blindness" was introduced by Dekkers (22). It is now widely used to describe the largely irreversible eye lesions which occur during or shortly after an attack of measles. In this thesis, the eye lesions observed in children during or soon after measles are referred to as post-measles eye lesions or post-measles blindness. Not all irreversible eye lesions lead to complete loss of vision and strictly speaking, post-measles blindness can only be used for those cases where visual acuity is lost.

Vitamin A deficiency has been indicated by some authors as one of the causes of eye lesions following measles infection (13,23) while others have attributed the lesions to a consequence of measles keratitis (22). Studies carried out in Nigeria indicated a possible role of herpes simplex virus (24,25) while in Kenya, the virus was observed in children with measles only in two per cent of early cases by Sauter (13) and only long after the appearance of the rash by Dekkers (22). The harmful use of traditional eye medicine has been reported from a number of countries such as Zambia (26).

Based on work carried out in northern Nigeria, the possible inter-relationships between measles, secondary infections such as herpes virus, vitamin A intake and protein-energy malnutrition in the aetiology of post-measles blindness were discussed by Inua et al. (27). Measles increases the requirements for vitamin A not only systematically but also locally because of tissue damage while measles, vitamin A deficiency and protein-energy malnutrition all depress various aspects of the immune response which can contribute to increased severity of measles and opportunistic infections such as herpes simplex. These inter-relationships suggest a multifactorial origin of post-measles eye lesions as also proposed by Reddy et al. (28). Recently, Foster and Sommer (29) reconfirmed the role of vitamin A deficiency, measles, secondary infections and traditional medicines in the aetiology of measles-associated corneal ulceration. Their conclusions were based on a study of 130 children admitted to Mvumi Hospital in Dodoma Region (Tanzania) with corneal ulceration of which 48 were associated with measles. The primary cause of post-measles eye lesions was attributed to vitamin A deficiency (50%, n=24), measles keratitis (12.5%, n=6), herpes simplex (20.8%, n=10) and to traditional medicines (16.7%, n=8).

Xerophthalmia and post-measles eye lesions in Tanzania

Earlier work carried out on xerophthalmia and post-measles eye lesions in Tanzania is reviewed below. This subject has been reviewed earlier by Van der Haar (30) and by Kavishe (31).

Xerophthalmia: Two studies in which relatively large numbers of children were screened were carried out prior to 1970. In 1959, McLaren (32) examined a total of 1,510 school children in Mvumi (Dodoma Region, see Figure 2) and in Mwanza (near Lake Victoria). Bitot's spots (X1B) were recorded in 0.5% (5/1,065) of the school children in Mvumi and in 1.4% (6/445) of those in Mwanza. At Mvumi examining a group of preschool children, no Bitot's spots were found in babies (0-2 yr) and toddlers (2-4 yr), but keratomalacia (X3) was found in an extremely high proportion of 1.3% in both groups.

In 1966-1967 a group from the Max Planck Institute (Fed. Rep. of Germany) investigated the situation in Kilimanjaro and Tanga Regions in the north-eastern part of the country (33). As the eye lesions were classified in a different way to that presently used, it is difficult to evaluate the relevance of their finding that 4.6% (55/1,204) of the children examined had keratomalacia plus corneal vascularisation. More importantly they reported a 18% prevalence of serum vitamin A levels below 0.35 μmol/l among children in the 1-5 year age group (n=77) and a similar prevalence among older children (6-10 year, n=156).

In 1962 Latham (34) examined 1,032 children during five surveys, although the number of children in each survey was low, Bitot's spots were frequently found (up to 3.6%) in the Central Region (now Dodoma Region). Other data collected in Tanzania have been excluded from discussion because of small sample size or of selection bias in the study population (35,36).

Integrated nutrition/health surveys have been organized in various parts of the country by the Tanzania Food and Nutrition Centre and in some of these surveys eye screening was included. Fifteen villages in Iringa Region were surveyed in 1979 and 1980 (37). Xerophthalmia was observed in only one out of the 3,278 children under five examined. In six villages where serum levels of retinol-binding protein (RBP) were estimated, the proportion of children with values below 2.4 mg/100 ml (corresponding to 1.1 μmol/l) varied from 2.7 to 20.0%. The survey report concluded: "Our survey thus did not manage to provide any reliable information on the prevalence of vitamin A deficiency in the region the main reason being the absence of a suitable and reliable method of assessment under field conditions" (37). Nutrition surveys were also carried

out in Lindi, Mtwara and Mbeya Regions but no xerophthalmic eye lesions were found (38,39). However, no staff specially trained in the detection and classification of xerophthalmic eye lesions participated in these surveys.

In 1980, 603 preschool-age children were examined in four villages in Dodoma Region (40). No xerophthalmic eye lesions were reported but the prevalence of conjunctivitis and trachoma was reported to be 5.1% and 7.3% respectively. Low and deficient RBP levels were found in 20.8% of the children, while eyes of 25% of the children showed positive staining with lissamine green.

Insufficient data are available from these surveys to conclude whether or not xerophthalmia is a regional or countrywide public health problem in Tanzania. There is evidence of subclinical deficiency and it may be argued that the low number of clinical signs reported were the result of inadequate clinical skill.

A two-year surveillance programme in 15 hospitals all over mainland Tanzania (41) demonstrated that clinical manifestations of vitamin A deficiency were reported from all participating hospitals. However prevalence data cannot be based on hospital studies. It is therefore somewhat unfortunate that the results of the first part of this programme have been misquoted in the WHO ten year programme document (42). The localization of the hospitals where this surveillance programme has been in operation are given in the map of the United Republic of Tanzania (see Figure 2).

Post-measles eye lesions: The problem of post-measles blindness has been addressed by various authors (23,29,41,43,44). Data collected in East Africa in the last 15 years are summarized in Table 2. The widely divergent results reported may be due, in part, to differences in the classification of eye lesions. Post-measles ocular complications are mainly confined to the cornea but complications such as retrobulbar neuritis and retinitis may be observed on occasions (22). However, these complications will not be discussed further.

It has been estimated that every year in Tanzania, 600,000 children suffer from measles of which 30,000 children die (45,46). The proportion of hospitalised children with measles who develop corneal ulceration has been estimated to be in Africa 1 to 4% (29,47,48). Although large numbers of children with measles are admitted to hospital, they are likely to represent a small proportion of all cases. It is therefore difficult to estimate the total toll of blindness due to measles.

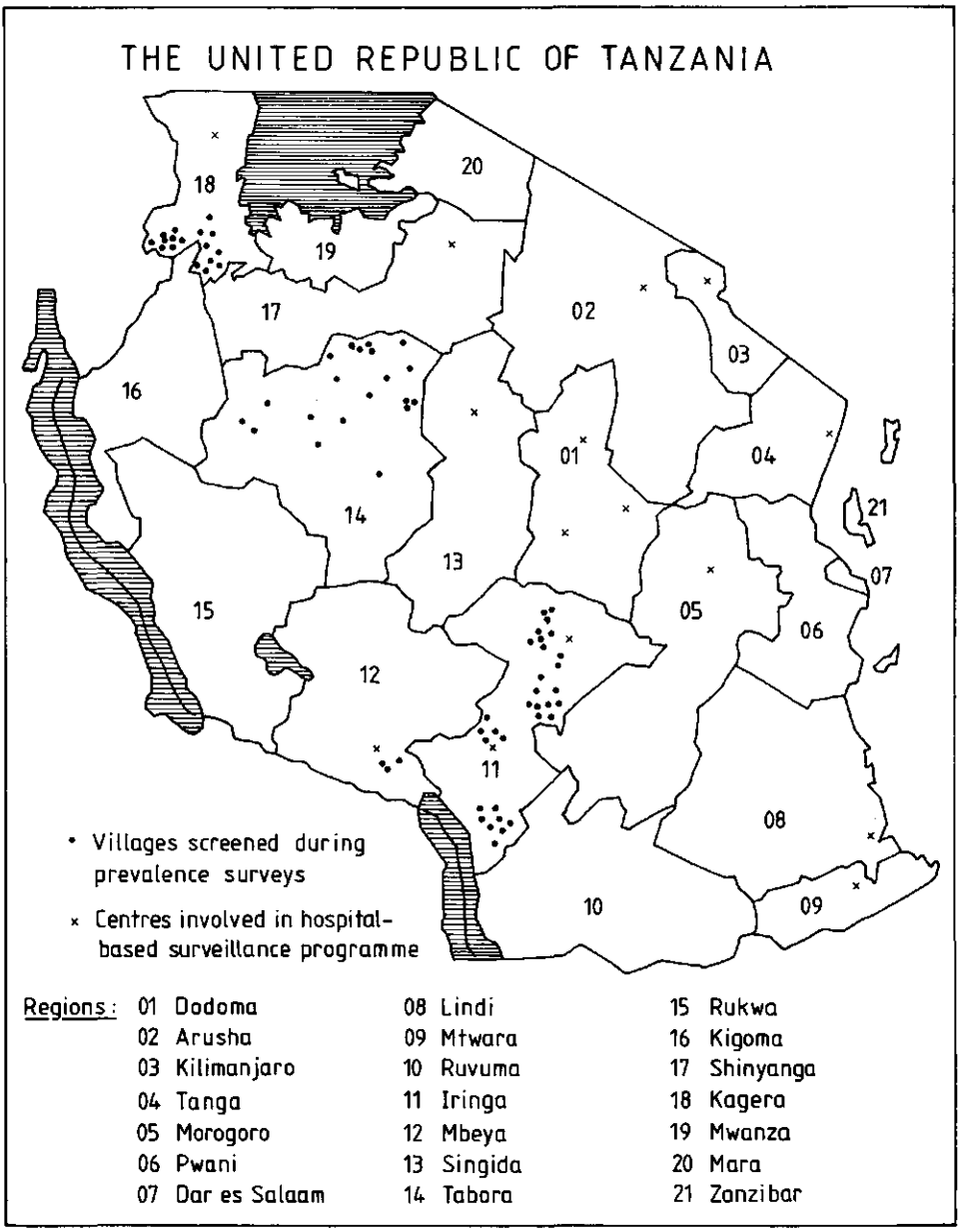

Figure 2: Map of the United Republic of Tanzania indicating the villages surveyed for the prevalence of xerophthalmia and the location of hospitals participating in the hospital-based surveillance programme.

Table 2: Summary of studies carried out on children with measles with special reference to the prevalence of post-measles eye lesions in East Africa

Reference/country	Number of cases examined	Origin of the data	Description of the eye lesions*	Nutritional status and mortality
Franken (23) (Tanzania/Kenya, 1972)	27	out-patients clinic	corneal xerosis and ulceration	-
Gupta and Singh (43) (Tanzania, 1972)	250	hospital	2.8% corneal opacities + ulceration	7% severely malnourished 31% moderately malnourished, 1% deaths
Kimati and Lyaruu (44) (Tanzania, 1973)	624	hospital	0.8% "keratitis and blind"	
Manyanga (51) (Tanzania, 1977)	420	hospital	13.6% "xerophthalmia"	
Burgess et al. (52) (Tanzania, 1981-1983)	913	hospital	few eye changes, not further described	11% severely malnourished, 51% moderately malnourished, 7.9% deaths
Serventi et al. (53) (Tanzania, 1982-1983)	194	hospital	not described	19% deaths
Foster et al. (41) (Tanzania, 1982-1984)	3591	multi center study	XN 0,08%, X1B 0,11% X3 4.6% of which 2.5% unilateral	
Sauter (13) (Kenya, 1974)	140	mainly out-patients	no signs	good general nutritional status,
	356	hospital	7.3% corneal xerosis + kerato-conjunctivitis 7.3% corneal ulceration/keratomalacia	of a sample of 43 out of 234 children, serum proteins, retinol and ß-carotene levels were determined
Dekkers (22) (Kenya, 1976-1978)	248 (main study)	hospital	2.8% corneal erosions 1.2% exposure ulcers	5% severely malnourised 50% moderately malnourished, albumin and retinol binding protein levels determined in 91 children
	356 (main + pilot study)	hospital	3.4% corneal erosions 1.4% exposure ulcers	
Lindtjørn (54) (Ethiopia, 1981-1982)	176	hospital	1.7% corneal ulceration/ keratomalacia	10.6% deaths on community basis

* See Figure 1

In a prospective community-based study in India the consequences of measles were studied in children living in a slum area in Hyderabad (28,49,50). In addition to anthropometric, biochemical and ophthalmological data, the immune response of children with measles was measured and conjunctival swabs were collected for microbiological investigation. The major findings were: that the duration of measles and complications seen were similar for well-nourished and malnourished children: that the cell-mediated immune response was similarly reduced in both well and malnourished children with measles; and that corneal lesions found in these Indian children were relatively mild. The prevalence of corneal eye lesions (xerosis and ulceration) in the community-based study in Hyderabad was 3.1% (10/315). This rate appears to be much lower than that found in hospital-based studies in Africa. In a large hospital-based study also in Hyderabad, it was reported that 0.49% (7/1,426) of the children hospitalised with measles developed corneal xerosis/corneal ulceration (55). It is difficult to avoid the conclusion that the lesions observed in Africa are more severe than those observed in the Hyderabad studies (56).

Vitamin A, morbidity and mortality

The research work carried out in Indonesia already quoted (9) and subsequent work has increased the interest in the inter-relationships between vitamin A, morbidity and mortality. The mortality rate among children with mild xerophthalmia (XN and X1B) was reported to be on average four times the rate reported among children without xerophthalmia (57). Using the same set of data, originating from a prospective, longitudinal study carried out among 3,400 children in West Java, it was shown that children with mild xerophthalmia developed respiratory disease twice and diarrhoea three times as frequently as non-xerophthalmic controls (58). Later on the reverse of this relationship was observed as children with respiratory disease and/or diarrhoea were found to be at an increased risk of developing xerophthalmia (59). A study carried out in 450 villages in Aceh (North Sumatra) claimed that supplementation of vitamin A with high-dose vitamin A capsules (200,000 I.U) resulted in a reduction of mortality in children aged 12-71 months living in the villages where supplementation was introduced (229 villages, n=12,281) compared with the unsupplemented villages (221 villages, n=11,378). The results of this study created not only considerable comment in the scientific press (see for example, ref 60) but also much interest in the lay press. As a result of this, further studies with funds from the U.S. Government are underway or being planned in Bangladesh and the Phillipines to investigate the

possible role of vitamin A supplementation on childhood mortality. In these studies a number of aspects criticized in the Indonesian study on supplementation as for example: supplementation coverage, sex differences, effect of supplementation of the "non-supplemented" group with a small physiological dose, will be further examined.

Work on xerophthalmia and post-measles eye lesions in Tanzania

The work described in this thesis was carried out as part of the cooperation between the Tanzania Food and Nutrition Centre (TFNC) in Dar es Salaam and the Department of Human Nutrition, Wageningen Agricultural University, The Netherlands. The research activities, field surveys and hospital-based studies in Tanzania, were part of TFNC's ongoing programme on vitamin A deficiency within the medical nutrition research programme. The guidelines for this research were laid down at meetings at national level coordinated by TFNC. In order to phase the research work carried out, the major outcome of these meetings is summarized as follows:

February 1981: A meeting was held in Dar es Salaam to review existing information on xerophthalmia. Two committees were established to organize follow-up activities, one on biochemical methods and one to implement the recommendations (61).

November 1981: A further meeting was organized in Dar es Salaam to bring together people from all disciplines involved in possible future research and prevention activities related to vitamin A deficiency and xerophthalmia. The major recommendations of the meeting were: to start studies on the aetiology of post-measles blindness; to improve further the laboratory facilities for measuring vitamin A status; and to establish a xerophthalmia surveillance programme by eye auxiliaries (62,63).

July 1982: The xerophthalmia surveillance programme to be carried out by Assistant Medical Officers in Ophthalmology was begun with a one-week training seminar in Iringa.

July 1983: A follow-up training course of two days was organized in Dodoma for the participants in the surveillance programme.

February 1984: Ongoing surveillance and post-measles blindness research programmes were reviewed and recommendations for further activities were made during a seminar on prevention of

blindness held in Moshi in Northern Tanzania. The seminar was attended by delegates from 12 Central and East African countries as well as by observers from outside the region (47).

September 1985: Progress made since 1981 was reviewed at a meeting in Dar es Salaam. Particular attention was paid to the surveillance programme started in 1982 and to the evaluation of the studies on post-measles blindness and the prevalence surveys. One of the most important results of this meeting was the inclusion in 1987 of high-dose vitamin A capsules in kits of the Essential Drug Programme distributed to rural health centres and dispensaries (64). After this meeting a national programme for the control of xerophthalmia was drafted (65).

Numerous organizations have given financial support to facilitate the organization of the meetings listed above. Apart from the fact that much valuable information has been collected during the last five years, a major achievement is, that within a relatively short period a large number of key persons working in various fields have become well aware of the importance of adequate vitamin A nutriture and are now involved in ongoing activities.

Outline of the thesis

The work reported in this thesis can be divided into three subject areas.

Prevalence studies: Surveys were carried out to estimate the prevalence of xerophthalmia in a number of regions representing the various geographical and agricultural conditions in Tanzania. Over 21,000 children were examined in four regions of mainland Tanzania. The location of the villages involved in these surveys is given in Figure 2 and in Appendix I the list of individual villages with the total number of children examined is presented. In addition to the collection of data on xerophthalmia, the overall health status of the preschool-age population was also assessed. The findings in Mbeya, Iringa and Kagera Regions are presented in Chapter 2 and those from two studies from Tabora Region are presented in Chapter 3.

Food composition and food consumption studies: During the course of the programme, a limited number of foods which form part of the diet in Tanzania

were analysed. Special attention was given to those foods regarded as good sources of vitamin A either because they contained high levels of retinol or carotenoids or because of their prominent role in the diet. Some of the results have already been published (66). The results of these analyses are presented in Chapter 4. In addition, a food composition table based on these analyses and also on data from the literature was prepared (Appendix IV). This table was used to assess the nutrient intake in a food consumption study carried out in a number of villages in Tabora Region, the results of which are reported in Chapter 5.

Hospital-based studies: Two hospital-based studies on the aetiology of post-measles blindness were carried out with assistance of staff from five hospitals both in and around Dar es Salaam. More than 650 children with measles were enrolled in these studies which are reported in Chapters 7 and 8. Details of eye lesions recorded during the two studies are presented in Appendix II and III.

Accurate assessment of biochemical parameters used to describe the vitamin A status was one of the major aims of these studies. Details of the quality control procedures applied in the laboratory work are therefore presented in Chapter 6.

The findings described in this thesis and the implications of these findings for the prevention of vitamin A deficiency, xerophthalmia and post-measles blindness in general, and more specifically in Tanzania, are discussed in Chapter 9.

REFERENCES

1 Grant JP. The state of the world's children, 1982-83. London: Oxford University Press, 1982.

2 Grant JP. The state of the world's children 1984. London: Oxford University Press, 1983 (see also the 1985 and 1987 reports which were published under the same title by OUP).

3 Pacey A, Payne P. eds. Agricultural development and nutrition. London: Hutchinson, 1985.

4 Wald G. Molecular basis of visual excitation. Science 1968;162:230-9.

5 Olson JA. Recommended dietary intakes (RDI) of vitamin A in humans. Am J Clin Nutr 1987;45:704-16.

6 Moore T. Vitamin A. Amsterdam: Elsevier, 1957.

7 DeLuca LM, Glover J, Heller J, Olson JA, Underwood BA. Guidelines for the eradication of vitamin A deficiency and xerophthalmia. Recent advances in the metabolism and function of vitamin A and their relationship to applied nutrition. Washington: The Nutrition Foundation, 1977.

8 Sporn MB, Roberts AB, Goodman DS. The Retinoids, vol. 1 and 2. New York: Academic Press, 1984.

9 Sommer A. Nutritional blindness: Xerophthalmia and keratomalacia. New York: Oxford University Press, 1982.

10 Bauernfeind JC. Vitamin A deficiency and its control. New York: Academic Press, 1986.

11 WHO. Control of vitamin A deficiency and xerophthalmia Report of joint WHO/UNICEF/USAID/Helen Keller International/IVACG meeting Techn Rep Series No. 672. Geneva: WHO, 1982.

12 Kusin JA, Sinaga HSRP, Marpaung AM. Xerophthalmia in North Sumatra. Trop Geogr Med 1977;29:41-6.

13 Sauter JJM. Xerophthalmia and measles in Kenya. Groningen: Drukkerij van Denderen, 1976.

14 Emran N, Sommer A. Lissamine Green staining in the clinical diagnosis of xerophthalmia. Arch Ophthalmol 1979;97:2333-5.

15 Duarte Favaro RM, Vieira de Souza N, Vannucchi H. Evaluation of rose bengal staining. Am J Clin Nutr 1986;43:940-5.

16 Kusin JA, Soewondo W, Parlindungan Sinaga HSR. Rose Bengal and Lissamine Green vital stains: useful diagnostic aids for early stages of xerophthalmia?. Am J Clin Nutr 1979;32:1559-61.

17 Wittpen JR, Tseng SCG, Sommer A. Detection of early xerophthalmia by impression cytology. Arch Ophthalmol 1986;104:237-40.

18 WHO. Vitamin A deficiency and xerophthalmia Report of a Joint WHO/USAID Meeting Techn Rep Series No. 590. Geneva: WHO, 1976.

19 Underwood BA. The determination of vitamin A and some aspects of its distribution, mobilization and transport in health and disease. Wrld Rev Nutr Diet 1974;19:123-72.

20 Loerch JD, Underwood BA, Lewis KC. Response of plasma levels of vitamin A to a dose of vitamin A as an indicator of hepatic vitamin A reserves in rats. J Nutr 1979;109:778-86.

21 Olson JA. New approaches to methods for the assessment of nutritional status of the individual. Am J Clin Nutr 1982;35:1166-8.

22 Dekkers NWHM. The cornea in measles. Den Haag: Junk Publishers, 1981.

23 Franken S. Measles and xerophthalmia in East Africa. Trop Geogr Med 1974;26: 39-44.

24 Whittle HC, Sandford-Smith J, Kogbe OI, Dossetor J, Duggan MB. Severe ulcerative herpes of mouth and eyes following measles. Trans Roy Soc Med Hyg 1979;73:66-9.

25 Sandford-Smith J, Whittle HC. Corneal ulceration following measles in Nigerian children. Br J Ophthalmol 1979;63:720-4.

26 Awdry PN, Cobb B, Adams PCG. Blindness in the Luapula Valley. Centr Afr J Med 1967;13:197-201.

27 Inua M, Duggan MB, West CE, et al. The role of vitamin A, malnutrition and measles in post-measles corneal ulceration in children in Northern Nigeria. Ann Trop Paediatr 1983;3:181-91.

28 Reddy V, Bhaskaram P, Raghurumulu N, et al. Relationship between measles, malnutrition, and blindness: a prospective study in Indian children. Am J Clin Nutr 1986;44:924-30.

29 Foster A, Sommer A. Corneal ulceration, measles, and childhood blindness in Tanzania. Br J Ophthalmol 1987;71:331-43.

30 Van der Haar F. Review of the prevalence of vitamin A deficiency in Tanzania. In: Mrisho F, Pepping F, Lukmanji Z, eds. Proceedings of a national symposium for vitamin A deficiency, Dar es Salaam 16-18 November 1981. TFNC Report No. 735 Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

31 Kavishe FP. The epidemiology of xerophthalmia and vitamin A deficiency in East Africa. Thesis London School of Hygiene and Tropical Medicine, 1982.

32 McLaren DS. Nutrition and eye disease in East Africa Experience in Lake and Central Provinces, Tanganyika. J Trop Med and Hyg 1960;63:101-22.

33 Kreysler J, Schlage C. The nutrition situation in the Pangani Basin. In: Kraut H, Cremer JD, eds. Investigations into health and nutrition in East Africa. Munchen: Weltforum Verlag, 1969, 85-178.

34 Latham MC. Nutritional studies in Tanzania. Wrld Rev Nutr Diet 1967;7:31-71.

35 Burgess HJL, Maletnlema TN, Burgess AP. The nutritional status of young children in Hombolo, Tanzania. East Afr Med J 1968;45:605-12.

36 Kondakis XG, Marealle ALD, Kazungu M. Cross-sectional studies on protein-calorie malnutrition in Tanganyika. J Trop Med 1964;67:224-9.

37 Ljungqvist B. Iringa nutrition survey 1979-1980 TFNC Report No. 692. Dar es Salaam: Tanzania Food and Nutrition Centre, 1981.

38 Kisanga P, Bunga BE. Lindi/Mtwara Regions rapid nutrition survey December 1980 TFNC Rep No. 844. Dar es Salaam: Tanzania Food and Nutrition Centre, 1983.

39 Lukmanji Z, Materu M. Nutrition status survey of under five population in five villages - Kyela District, Mbeya Region (March 1982) TFNC Report No. 922. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

40 Malimi L. A report on the survey on corneal scars and vitamin A deficiency in Dodoma. TFNC Report No. 538. Dar es Salaam: Tanzania Food and Nutrition Centre, 1981.

41 Foster A, Kavishe F, Sommer A, Taylor HR. A simple surveillance system for xerophthalmia and childhood corneal ulceration. Bull Wrld Health Org 1986;64:725-8.

42 WHO. Prevention and control of vitamin A deficiency, xerophthalmia and nutritional blindness: proposal for a ten-year programme of support to countries Nut/84.5. Geneva: WHO, 1985.

43 Gupta BM, Sing M. Mortality and morbidity pattern in measles in Tanga District, Tanzania. Trop Geogr Med 1975;27:383-6.

44 Kimati VP, Lyaruu VP. Measles complications seen at Mwanza Regional Consultant and Teaching Hospital in 1973. East Afr Med J 1976;53:332-40.

45 Tanzania Public Health Association. Measles in Tanzania. Dar es Salaam, 1982.

46 UNICEF. Analysis of the situation of children and women, volume 1 and 2 Government of the United Republic of Tanzania and United Nations Children's Fund (UNICEF). Dar es Salaam, 1985.

47 Foster A, ed. Focus on blindness in Africa, Proceedings of the sub-regional prevention of blindness seminar for East and Central Africa, Moshi, Tanzania, Feb 13-18 1984. Moshi: Africa Region Medical Office of Christian Blind Mission International, 1984.

48 Pepping F, Hackenitz EA, Mroso DM, Franken S, West CE. The role of nutritional status with special reference to vitamin A in the development of post-measles eye lesions II. Eye lesions, and other clinical complications in relation to nutritional status (submitted for publication).

49 Bhaskaram P, Madhusudhan J, Radhrakrishna KV, Reddy V. Immune response in malnourished children with measles. J Trop Ped 1986;32:123-6.

50 Bhaskaram P, Mathur R, Rao V, et al. Pathogenesis of corneal lesions in measles. Hum Nutr:Clin Nutr 1986;40c:197-204.

51 Manyanga JSN. Ocular involvement in relation to general complications in severe measles. Elective period research project, Muhimbili Medical Centre, Dar es Salaam (unpublished report), 1977.

52 Burgess W, Mduma B, Josephson GV. Measles in Mbeya, Tanzania 1981-1983. J Trop Ped 1986;32:148-53.

53 Serventi M, Byalugaba A. Report on measles morbidity and mortality in Bukoba Government Hospital in a period of six months (1/9/82-28/2/83), and considerations on its prevention by vaccination. Bukoba (unpublished report), 1983.

54 Lindtjørn B. Severe measles in the Gardulla area of southwest Ethiopia. J Trop Ped 1986;32:234-9.

55 Bhaskaram P, Reddy V, Raj S, Bhatnagar RC. Effect of measles on the nutritional status of preschool children. J Trop Med Hyg 1984;87:21-5.

56 Pepping F, Hackenitz EA, West CE, Duggan MB, Franken S. Relationship between measles, malnutrition and blindness: a prospective study in Indian children. Letter to the editor. Am J Clin Nutr 1987 (in press).

57 Sommer A, Hussaini G, Tarwotjo I, Susanto D. Increased mortality in children with mild vitamin A deficiency. Lancet 1983;1:585-8.

58 Sommer A, Katz J, Tarwotjo I. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 1984;40:1090-5.

59 Sommer A, Tarwotjo I, Katz J. Increased risk of xerophthalmia following diarrhea and respiratory disease. Am J Clin Nutr 1987;45:977-80.

60 Cohen N. Vitamin A supplementation and child mortality. Xerophthalmia Club Bulletin 1986;34.

61 Vitamin A deficiency in Tanzania, Report of a national seminar Dar es Salaam, TFNC Report No. 650. Dar es Salaam: Tanzania Food and Nutrition Centre, 1981.

62 Mrisho F, Pepping F, Lukmanji Z. Proceedings of a national symposium for vitamin A deficiency, November 16-18 1981 Dar es Salaam, TFNC Report No. 735. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

63 Upungufu wa vitamin A Tanzania, Expert Committee Report, TFNC Report No. 718. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

64 Kisanga P, Pepping F, Kavishe FP. Proceedings of a workshop on the control of vitamin A deficiency and xerophthalmia in Tanzania held at the Salvation Army in Dar es Salaam, 9th-11th September 1985, TFNC Report No. 980. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

65 A national programme on the control of vitamin A deficiency in Tanzania. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

66 Schultink JW, West CE, Pepping F. ß-carotene content of Tanzanian foodstuffs determined by high performance liquid chromatography. East Afr Med J 1987;64:368-71.

2. PREVALENCE OF XEROPHTHALMIA IN RELATION TO NUTRITION AND GENERAL HEALTH IN PRESCHOOL-AGE CHILDREN IN THREE REGIONS IN TANZANIA

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ABSTRACT

Data are presented from integrated nutrition/health surveys carried out in Mbeya, Iringa and Kagera Regions in Tanzania in which a total of 12,880 children were examined for the presence of xerophthalmia. Of this total 2,380 children were screened during two follow-up surveys in Iringa Region. Xerophthalmia was found to be a problem of public health significance in two of the three regions surveyed where the prevalence of active corneal xerophthalmic lesions was above the criteria set by WHO. However, because there was clustering of the children with Bitot's spots, corneal xerosis/ulceration or corneal scarring, only certain areas within the districts comprising the regions could be regarded as areas where xerophthalmia may be a problem. The results of the ophthalmological examinations are discussed in relation to the nutritional status of the children, as measured by anthropometric indices, serum levels of retinol-binding protein and prealbumin, haematological parameters, and vaccination status.

INTRODUCTION

The severity and magnitude of the problem of xerophthalmia in Africa remains unclear (1), and insufficient data are available from most countries in this region (2). Up until recently, data on the prevalence of xerophthalmia in Tanzania were scarce and limitation in sample size hindered regional comparisons. In the period between 1964 and 1967, data were collected in the northern part of the country and from small groups of children scattered over other areas (3,4). A relatively high prevalence of Bitot's spots (3.6%, 13/360) was recorded in the Central Region, now Dodoma Region, where McLaren had also reported xerophthalmia in 1959 (5). In studies carried out in 1979 and 1980 in 15 villages in Iringa Region, only one case of active xerophthalmia was identified among 3,278 children under five years of age and no other eye lesions related to vitamin A deficiency were reported (6).

It has been estimated that xerophthalmia leads in Tanzania to two to four thousand new cases of blindness every year so that a total of 10,000 children in Tanzania are likely to suffer from nutritional blindness (7). Based on an expected total preschool-age population (< 6 years), of 4.5 million this would be equivalent to a prevalence of blindness of 0.22% in this age group.

In this paper, we describe the magnitude of xerophthalmia observed during three nutrition surveys and two follow-up studies carried out in the United Republic of Tanzania between November 1983 and September 1985. These surveys were set up as integrated nutrition and health surveys and most were designed not only to obtain data on xerophthalmia but as part of larger ongoing programmes carried out by Tanzania Food and Nutrition Centre (TFNC) and other organizations (i.e. UNICEF/WHO).

A hospital-based surveillance system for recording eye lesions in children with and without measles operated from 1982 until 1984 (8). The results from this are discussed with special reference to the three regions in which the present surveys were carried out.

SUBJECTS AND METHODS

Background information and study areas

The studies from which the data are derived and the areas where these studies were carried out (see also the map on page 18 in Chapter 1) are described briefly and related to data which have been published.

A. Mbeya Rural District in Mbeya Region. In November 1983, a survey on the prevalence of xerophthalmia was carried out as part of a joint survey on iodine deficiency disorders (IDD) and xerophthalmia in three villages in Mbeya Rural District which comprises, with the districts of Chunya, Mbozi, Rungwe, Mbeya Urban and Kyela, Mbeya Region which is located in the Southern Highlands in southwestern Tanzania. All women aged between 15 and 45 years living in the villages under study were injected with iodinated oil and were asked to bring all their preschool-age children with them. In this way it was possible to study 188 children. Mbeya Rural District has a total population of 260,000 (9) and is largely a mountainous area with altitudes up to 2,500 meters. Goitre is frequently seen in the district.

No community-based data on xerophthalmia are available from the region but in a number of surveys, the nutritional status of children has been examined. In a study carried out in 1977 and 1978 in 11 villages in Chunya District, stunting was observed in 31.6% of the children while 3.4% and 1.7% were

observed to be wasted or wasted and stunted respectively (10).
In Kyela District, 35% of the children (i.e. 500 children) living in five villages were screened in 1982 (11). Stunting was observed in 29.3% and wasting in 9.4% of the children studied. Several studies have been carried out in Mbozi District. Corneal ulceration and keratomalacia was found in 1.5% (16/1,100), of the children attending a nutrition rehabilitation unit (12). Mortality among the malnourished children with corneal eye lesions was high (9/16). In 1983 a survey was carried out in 14 villages in the district in which a total of 3,167 children below six years of age were examined (13). The overall nutritional status in the cash-crop area (20% stunting and 2.5% wasting) was better than in the poorer more isolated food-crop area (41% stunting and 1.8% wasting).

B. Iringa Region. From March until June 1984, data on the prevalence of xerophthalmia were collected in 27 villages in four districts. Six and 11 months later (phase two and three), the children in seven villages were re-examined. These studies were carried out as part of the Joint WHO/UNICEF Nutrition Support Programme (JNSP), financed by the Italian Government, which started in December 1983 in Iringa Region. The programme involves 167 villages in total, located in all of the five rural districts of the region. A mass screening and vaccination campaign was part of the initial phase of the programme. Data on the prevalence of xerophthalmia were also obtained in a further village in Iringa Rural District which did not participate in the JNSP. In this village (Ilula) a study was carried out by TFNC on determinants of reproductive performance and child survival (14). A total of 5,975 children were thus screened for the prevalence of xerophthalmia in this region, while 2,380 children were examined during phase two (November 1984) and phase three (April 1985).

Iringa Region is to the east of Mbeya Region and is also part of the Southern Highlands. There is a range of agro-economic and ecological zones including the dry savannah bushlands of the northern part of the region where the annual rainfall is between 200 and 400 mm/year. The total population in 1984 was around 1 million. The survey carried out in 1979 and 1980 by Ljunggvist (6), indicated that 5-6% of the children were severely underweight (weight-for-age < 60%) while about 50% of the children were moderately underweight. In the southern part of the region, the nutritional status was found to be worse than in the northern part. A gap of one year between the screening in both areas might have been responsible for this because the harvest prior to the second survey (1979/80) was very poor in the country as a whole (6). A nutritional surveillance programme has been operating in Iringa

Region for a number of years prior to the start of the JNSP (15).

C. Biharmulo and Ngara Districts in Kagera Region. In July and August 1985 a child survival programme was started by the regional authorities, and supported by UNICEF, in Ngara and Biharamulo Districts which are in the south of Kagera Region. Fifty four villages were covered by the programme and in 20 villages the prevalence of xerophthalmia could be estimated in 4,437 children. The population in these villages was estimated at 85,000 with 16,000 below the age of five of which 11,577 were enrolled at the start of the programme (16).

Kagera Region forms the extreme northwestern corner of Tanzania, bordering on Rwanda, Burundi and Uganda with a population in 1985 of 1.3 million inhabitants and an infant mortality rate estimated in 1978 of 133 (17). Since then, Kagera Region has suffered more than any other part of Tanzania from the ravages of war and from economic recession.

In 1985, Dahlin (18) screened 400 children in Izimbya ward (Bukoba Rural District) and observed that 25% of the children were underweight with an additional 1.5% being severely underweight. Malaria was found to be a tremendous problem evidenced by a splenomegaly rate of at least 56%.

Survey methodology

As during the two surveys carried out in Tabora Region (19) screening of children started with an examination of the eyes. Then children with eye lesions and a 20% sample of those without eye lesions were selected for detailed anthropometric, biochemical and haematological investigations and a short questionnaire on morbidity, vaccination status and family size was administered to the mother or guardian by a member of the survey team which, on most occasions was a public health nurse.

Ophthalmological examination. The eyes of all children were examined immediately after registration in the study by an ophthalmologist, Assistant Medical Officer Ophthalmology or eyenurse. All, except one, of the seven persons carrying out the ophthalmological work had participated in the hospital-based surveillance programme including the initial training which was designed to provide a high degree of uniformity in diagnosis (7). A normal hand torch or a illuminated magnifier (X5, International Centre for Eye Health, London, England) was used to examine the eyes. Fluorescein paper strips were used to stain the eyes if this was considered necessary for diagnosis. The WHO classification was used to classify the eye lesions, with the exception that no attempt was made to estimate the prevalence of night blindness (2). In some communities, night blindness has been used as a sensitive and specific

indicator of xerophthalmia (20), and the existence of an expression in the local language for this condition may facilitate its use in epidemiological studies. Among the tribes living in the areas presently studied, only the Wabena who live mainly in Njombe District have a word for it, "Kitinala". During the surveys only one child with night blindness and no other eye lesions was recorded. All children with xerophthalmia were given high-dose vitamin A capsules (55 mg retinyl palmitate equivalent to 200,000 I.U. vitamin A + 40 I.U. vitamin E). In one village in Iringa Rural District, these capsules were distributed to all children above the age of 12 months attending phase three.

Anthropometry. Length and weight were measured using standard procedures (21) and weight-for-age (WA), weight-for-length (WL) and length-for-age (LA) indices were calculated using approperiate reference standards (22). The nutritional status was estimated using WL and LA as nutritional indicators. The proportion of children showing wasting and stunting was calculated for age groups (23). In the absence of data on length or height (as in the initial survey in Iringa Region), the 80% and 60% value of the median reference weight was used as cut-off point to identify children underweight or severely underweight for their age respectively (22).

Biochemical, parasitological and haematological investigations. During the prevalence surveys fingerprick blood samples were collected using Microvettes CB 1000 (Sarstedt, D 5233 Nümbrecht, FRG). After collection, samples were stored in a coolbox in the dark. Samples were centrifuged in the clinic within four to six hours after sampling and serum was stored at -18°C. Retinol-binding protein (RBP) and prealbumin (also referred to as transthyretin, TTR) levels were determined by immunochemical methods (24,25). An internal quality control system was applied to all the analytical procedures (26).

Thick blood smears were examined for malaria parasites. Haemoglobin was estimated with a portable haemoglobin photometer (Leo Diagnostics, Helsingborg, Sweden) with an internal standard of 12.6 g/100 ml (7.8 mmol/l). During phase one of the investigations in two districts in Iringa, a manual oxyhaemoglobin method was used. Packed cell volume (PCV) was measured using a Compur 101 Minicentrifuge (Compur Electronic GmbH, Münich, FRG).

Table 1: Prevalence and severity of xerophthalmia in Mbeya, Iringa and Kagera Regions of Tanzania (November 1983- August 1985)

Survey location* REGION (Date)/ District	Number of children examined	Number (n) and proportion (%) of children									
		X1A+		X1B		X2		X3		Corneal scarring	
		n	%	n	%	n	%	n	%	n	%
MBEYA (November 1983)											
Mbeya Rural	188	0		0		0		0		0	
IRINGA (March-June 1984)											
Iringa Rural	1763	2	0.11	2	0.11	5	0.28	2	0.11	11	0.62
Mufindi	1450	2	0.14	0		0		0		22	1.52
Njombe	881	1	0.11	2	0.23	2	0.23	0		13	1.48
Ludewa	1881	0		0		0		0		23	1.22
	5975										
IRINGA (November 1984)											
Iringa Rural	967	2	0.21	2	0.21	0		0		27	2.79
Njombe	364	1	0.27	1	0.27	0		0		9	2.47
	1331										
IRINGA (April 1985)											
Iringa Rural	617	0		0		0		0		4	0.64
Njombe	432	0		2		0		0		0	
	1049										
KAGERA (July 1985)											
Biharamulo	2661	12	0.45	3	0.11	0		1	0.04	1	0.04
Ngara	1776	6	0.34	2	0.11	0		0		0	
	4437|									

* For further details, see Appendix I.
\+ Classification of xerophthalmic eye lesions as established by WHO, see Subjects and Methods (2).
| The total number of children seen in these villages was 4,592 but the eyes of 155 children were not examined.

RESULTS

During the programmes carried out in Iringa and Kagera Region coverage of the eligible child population raged from 37 to 100% for the respective villages while the overall coverage rate was 76%.

Eye lesions

Nearly all children (99.7%) examined in Mbeya, Iringa (phase one, March-June 1984) and Kagera Regions were below the age of 60 months. The total prevalence of signs of active xerophthalmia (X1A, X1B, X2, X3) was 0.30% (95% confidence interval, CI 0.20-0.53%) for Iringa Region and 0.54% (CI, 0.37-0.86%) for Kagera Region. No xerophthalmia was found in Mbeya Region (Table 1). The age and sex-specific prevalence of xerophthalmia and the reported causes of the corneal scars recorded are given in Tables 2 and 3.

Table 2: Age and sex-specific prevalence of xerophthalmia as found in Mbeya, Iringa and Kagera Regions of Tanzania (November 1982 - August 1985)

Age (months)	Total+ examined	Number of children: Conjunctival xerosis (X1A) Boys	Girls	Bitot's spots (X1B) Boys	Girls	Corneal xerosis/ulcers (X2/X3) Boys	Girls	Corneal scarring Boys	Girls
0 - 11	2,299	0	0	0	0	3	1	3	0
12 - 23	2,999	4	0	1	0	2	2	3	3
24 - 35	3,051	2	0	2	0	2	1	7	5
36 - 47	2,675	7	2	1	0	0	0	6	5
48 - 71	1,956	5	2	5	4(1)*	0	0	11	4
Age/sex unknown			5		0		0		63
Total	12,980		27		13 (1)		11		110

* One child identified in phase one and phase two in Iringa Region.

\+ Age distribution of the total population based on that of the respective samples.

Although conjunctival xerosis (X1A) is included in the xerophthalmia classification scheme, it is liable to misinterpretation and it proved to be impossible to reach a sufficient inter-observer standardization. Thus, the differences between the districts are mainly due to this inter-observer variation. Conjunctival xerosis was more prevalent in boys and mainly observed in children above the age of 36 months.

At the regional level Bitot's spots (X1B) were identified at almost identical rates of 0.07% and 0.11% in Iringa and Kagera Region and were seen twice as often in boys than in girls. In Iringa Region, the highest prevalence at the district level was found in Njombe District, 0.22% (2/881). In the village in

Table 3: Causes of corneal scarring specified according to age as recorded among 12,980 children examined in Mbeya, Iringa and Kagera Regions (November 1983-August 1985)

Age	Number of children					
(months)	Xerophthalmia	Measles	Trauma	Others	Unknown	Total
0 - 11	1	1	0	1	0	3
12 - 23	3	3	0	0	0	6
24 - 35	6	5	0	1	0	12
36 - 47	7	2	0	2	0	11
48 - 71	9	3	0	3	0	15
Unknown	0	4	3	7	49	63
Total	26	18	3	14	49	110

Njombe in which these two children were seen, the prevalene rate of 0.61% (2/328) exceeded the WHO-limit of 0.05% (2). The total of five children with Bitot's spots recorded in the villages surveyed in Kagera Region were clustered in three of the 20 villages studied. High-dose vitamin A capsules were distributed in one village in Iringa Rural District where a prevalence of X1B of 0.95% (2/211) was found during phase two. The mean age of the children with Bitot's spots was 50 months.

Corneal xerosis (X2) and corneal ulceration/keratomalcia (X3) were found more among boys (n=7) and girls (n=4) and only in children below the age of three years, with the xerosis predominantly present in younger children.

During the first and second survey in Iringa Region, an over-reporting of corneal scarring must have occured. Most of the smaller lesions, ascribed by the mother to causes other than vitamin A deficiency were excluded from further investigation. Corneal scars were seen more often among boys, 43% of the children with corneal scars of which the history could be assessed were classified as xerophthalmic (26/61) while 30% (18/61) were attributed to measles.

Clustering of xerophthalmia was observed in three areas of Iringa Region. Two of these areas were in Iringa Rural District, where two children with X3, and one child with X1B were found in Ilula village (n=670), while in the two neighbouring villages of Mfyome and Itagutwa (Kalenga Division), four children with X2 and five with XS were found in phase one and two children with X1B and four with XS were found during phase two. The third area comprised the two

neighbouring villages of Kijombe and Katenga in Wanging'ombe Division of Njombe District, where during phase one: one child with X1A, two children with X1B, two with X2 and two with XS were found (n=543). During phase two one child with X1B and three with XS were recorded (n=364). Thus, 72% of the active cases of xerophthalmia recorded in phase one, were found in these five villages in a child population of 1,612 which is 27% of the total number of children examined.

Nutritional status

In Mbeya Rural District, 188 preschool-age children were screened. The original intention to screen at least 1,000 children was impossible to achieve because of fuel shortages during the last months of 1983. The age and sex distribution presented in Table 4 show that the age group of 48-59 months was under-represented. Complete anthropometric data are available on 179 children and 15.4% showed stunting, 3.9% wasting and 1.7% wasting plus stunting. Using weight-for-age as an indicator, a total of 30.0% of the children were underweight and 1.1% severely underweight.

In the first phase of the study in Iringa, anthropometric data were collected from 2,003 children. This number included 1,333 children from a 20% population sample as described previously and all of the 670 children examined in Ilula village. The age and sex distribution of the children are presented in Table 4.

Table 4: Age and sex distribution of preschool-age populations examined in Mbeya and Iringa Regions(November 1983-June 1984)

Age	Mbeya				Iringa			
(months)	Boys	Girls	Total		Boys	Girls	Total	
	n*	n	n	%	n	n	n	%
< 12	28	23	51	27.1	185	176	361	18.0
12-23	16	24	40	21.2	225	247	472	23.6
24-35	17	17	34	18.1	227	228	455	22.7
36-47	18	12	30	16.0	206	207	413	20.5
48-59	2	10	12	6.4	147	143	290	14.5
60-71	3	8	11	5.9	3	4	7	0.4
⩾ 72	1	3	4	2.1	0	0	0	
Age/unknown	3	3	6	3.2	2	3	5	0.3
Total	88	100	188	100,0	995	1008	2003	100,0

* n = Number of children examined; % indicates the proportion.

The proportion of children who were underweight was 28% for the children below the age of 12 months and this proportion increased to a level of about 60% in the third to fifth years (see Figure 1). No differences between boys and girls were observed (data not shown). For those villages within the JNSP area, differences between the districts with regard to nutritional status were small (see Table 6) even when correction was made for differences in the age profiles existing between the districts. The only exception was that severe malnutrition was more prevalent in Iringa Rural District than in the other districts in the region (X^2=12.9, p<0.01).

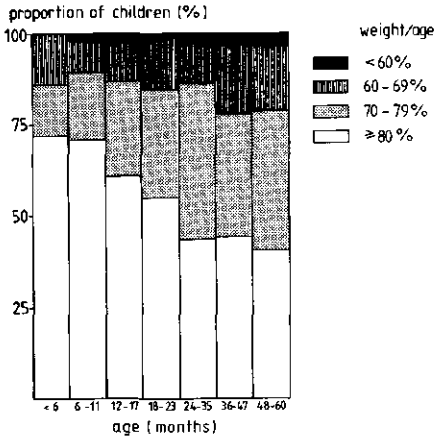

Figure 1: Nutritional status expressed in terms of weight-for-age of children according to age, studied in Iringa Region

During phase two and phase three the eyes of 1,331 and 1,049 children were examined, and of these children anthropometric data were collected from 233 and 179 children respectively. Children from seven villages were examined in all the three phases of the work carried out in Iringa. A comparison of the nutritional status as recorded in the villages in the population sample shows that the proportion of severely underweight children increased progressively from 0.8% (2/240) during phase one to 1.8% (2/114) during phase two to 3.2% (6/185) during phase three. At the same time, the proportion of children showing moderate underweight also increased progressively from 39.2% in phase one to 41,2% and 46.0% for phases two and three respectively. The proportion of children showing stunting was 25% during phase two and 32.4% during phase three, wasting was observed in 5.2% and 3.2% and wasting combined with stunting in 3.0% and 2.7% during phase two and phase three, respectively.

Of the 20 villages screened in Kagera Region anthropometric data are available for all children examined in 11 villages in Biharamulo District and in

Table 5: Summary of data on the prevalence of xerophthalmia, nutritional levels in children examined in eight districts in Tanzania between November

	Number (n) and proportion (%) of children					
	Mbeya Rural		Iringa Rural		Mufindi	
	n	%	n	%	n	%
Total examined*	188		2266		1450	
Eye examination	188	100	1763	77.8	1450	100
Active xerophthalmia+	0		11	0.62	2	0.14
Malnutrition						
Children examined	179		1003		328	
Moderate (60-80% WA)	54	30.0	461	46.0|	153	46.6
Severe (<60% WA)	2	1.1	43	4.1	7	2.1
Total	56	31.1	504	50.1	160	48.8
RBP levels						
Number determined	-		50		61	
< 10 µg/ml	-		1	2.0	2	3.3
10-20 µg/ml	-		13	26.0	16	26.2
Prealbumin levels						
Number determined	-		31		71	
< 100 µg/ml	-		4	12.9	0	
100-200 µg/ml	-		23	74.2	42	59.2
Haemoglobin						
Number determined	-		188		271	
< 10 g/100 ml	-		36	19.3	31	11.3
Packed Cell Volume						
Number determined	-		272		206	
< 35 %	-		184	67.7	168	81.6
Malaria						
Number examined	59		844		262	
Positive	0		149	17.7	82	31.3
Measles status						
Not vaccinated¶	-		51/293	17.4	12/250	4.8
History of measles	-		8/328	2.4	30/303	9.9

* For the number of children examined per village, see Appendix 1.

\+ Active eye lesions defined as conjunctival xerosis (X1A), Bitot's spots (X1B), corneal xerosis (X2) and corneal ulceration/keratomalacia (X3), see Table 1.

| Severe malnutrition (<60% WA) was more prevalent in Iringa Rural District when compared with the other districts in the region (Mufindi, Njombe, Ludewa, Makete), $p<0.01$.

status, malaria, measles vaccination and RBP, prealbumin and haemoglobin 1983 and August 1985

Number (n) and proportion (%) of children									
Njombe		Ludewa		Makete		Biharamulo		Ngara	
n	%	n	%	n	%	n	%	n	%
1157		1881		444		2743		1849	
881	76.1	1881	100	0		2661	97.0	1776	96.1
5	0.57	0		–		16	0.60	8	0.45
254		304		93		2559		1616	
100	39.4	126	41.4	45	48.4	1230	48.1	837	51.8
3	1.2	7	2.3	1	1.1	213	8.3§	79	6.8
103	40.6	133	43.7	46	49.5	1443	56.4	916	58.6
9		–		–		57		20	
0		–		–		9	17.5	1	5.0
3	11.1	–		–		33	57.9	6	30.0
–		–				–		–	
–		–		–		–		–	
–		–		–		–		–	
102		215		93		107			
42	41.0	22	10.2	7	7.5	96	54.2	–	
126		191		–		–		–	
107	84.9	126	66.0	–		–		–	
204		294		92		384		269	
90	44.1	65	22.1	0		93	24.2	98	36.4
38/160	23.8	7/252	2.8	2/62	3.2	–		–	
41/214	19.2	8/261	3.0	0		–		–	

§ Severe malnutrition was more prevalent in Biharamulo District when compared with Ngara District ($p<0.001$).

¶ Not vaccinated includes only those children who were eligible for vaccination (i.e. older than 9 months) and who had not been vaccinated after the programme had started.

seven villages in Ngara District. Severe underweight (< 60% WA) was observed more in Biharamulo than in Nzega District ($p<0.001$, see Table 5): in Biharamulo District a high proportion of 11.7% (76/649) of the children aged 36-60 months showed severe malnutrition (data not shown). The age distribution of the child population in both districts showed no differences. Moderate underweight was seen most in the children aged between 12 and 35 months.

Biochemical, parasitological and haematological parameters

Data on serum retinol-binding protein (RBP), prealbumin, haemoglobin, packed cell volume were collected in Iringa and Kagera Regions from children in the population sample. Only data from analyses for RBP and prealbumin completed within 18 months after collection are included in the statistical analyses (Table 5). There was no influence of age on RBP and prealbumin levels and the correlation coefficient between individual RBP and prealbumin levels was 0.25 for the initial survey (n=72, CI 0.02-0.45) and 0.46 (n=93, CI 0.28-0.60) during phase three in Iringa. The proportion of children with RBP levels below 10 µg/ml was 2.5% (3/120) for the initial survey in Iringa, 5.1% (5/99) during phase three and 13.0% (10/77) in Kagera Region. The results obtained at district level are presented in Table 5. Low prealbumin levels were recorded in 3.9% (4/102) of the children during the initial survey in Iringa and for 7.4% (6/81) during phase three. There was a significant correlation between RBP and nutritional status expressed as the proportion weight-for-age ($p<0.05$, n=99). Serum levels for RBP and prealbumin and haemoglobin levels for each district are also shown in Table 6.

The geographical location of the districts had a marked influence on the prevalence of malarial parasitaemia (see Table 5). For Iringa Region the villages in Pawaga Division (Iringa Rural District) and in Wanging'ombe Division (Njombe District) had a much higher proportion of children with malaria (44%) than was observed in the other areas (18-31%). Malaria was absent in the villages in Makete District of Iringa Region, and Mbeya Rural District of Mbeya Region which were surveyed. During phase two in Iringa Region, 22% (47/210) of the children were shown to be carrying malaria parasites. This survey was carried out in November 1984 at the end of the dry season and the prevalence of children with malaria was lower in all three divisions examined. For Kagera Region the prevalence of malaria was higher in Nzega District (36.4%) when compared with Biharamulo District (24.2%).

The cut-off point to diagnoze anaemia was set at a haemoglobin level of 10 g/100 ml (6.2 mmol/l) and the porportion of children diagnozed to be anaemic

Table 6: Mean concentration of haemoglobin in blood and of RBP and prealbumin in serum in children studied in seven districts in Iringa and Kagera Regions.

REGION/ District	Blood haemoglobin g/100 ml		Concentration in serum (mean±SE) RBP µg/ml		Prealbumin µg/ml	
IRINGA						
Iringa Rural, phase 1	12.1±0.2	(188)	21.3±0.5	(63)	175±6.9	(51)
Iringa Rural, phase 3	9.7±1.9	(101)	20.2±0.7	(56)	167±7.1	(54)
Mufindi	11.8±0.1	(271)	-		-	
Njombe, phase 1	10.1±0.2	(102)	20.3±0.8	(46)	164±7.7	(42)
Njombe, phase 3	10.3±1.4	(32)	20.3±1.1	(43)	157±6.9	(42)
Ludewa	11.9±0.1	(215)	-		-	
Makete	12.2±0.1	(93)	-		-	
KAGERA						
Biharamulo	-		16.3±0.7	(57)	-	
Ngara	-		22.3±1.3	(20)	-	

varied at district level from 7.5% to 54.2% (see Table 5). Low mean haemoglobin levels were found to correlate with malaria parasitaemia: Pawaga Division and Wanging'ombe Division were as for the prevalence of malaria the areas where the proportion of anaemic children was high (41%). The high proportion of anaemic children in Biharamulo District cannot be explained by the high rate of malaria-positive children and might be more than in Iringa Region of nutritional origin.

Vaccination

One of the general aims of the JNSP programme carried out in Iringa Region was to increase the vaccination coverage. With regard to measles vaccination, there was a marked difference between the five districts (see Table 5). The mass vaccination campaign at the start of the programme was carried out in two periods and in Iringa Rural and Njombe Districts, which were covered during the first (March-April 1984), organizational problems are likely to have reduced the rate of coverage.

The proportion of children not vaccinated against BCG, diphtheria/pertussis/tetanus (DPT) and poliomyelitis after completion of the mass campaign was 7.8%, 2.7% and 2.7% respectively at regional level.

Xerophthalmia and nutritional status

For children with eye lesions, mean serum levels for RBP and for the anthropometric indices are given in Table 7. Despite the rather small number of children for whom data are available, RBP levels for xerophthalmic children were lower than those observed in the population sample.

Table 7: Serum concentration of RBP and anthropometric indices for children with eye lesions

	Mean ± SE (n)			
Eye lesion	RBP µg/ml	WA (%)	WL (%)	LA (%)
Conj. xerosis (X1A)	17.3±2.4 (9)	72.2±5.8 (15)	88.9±4.0 (12)	93.3±2.3 (12)
Bitot's spots (X1B)	15.8±3.2 (3)	73.1±3.5 (13)	89.2±2.9 (9)	85.7±2.2 (9)
Corneal xerosis (X2)/ ulceration (X3)	13.4±3.2 (3)	77.9±5.6 (10)	94.3±3.1 (4)	93.5±1.4 (4)
Corneal scars, total	15.4±1.3(15)	79.2±2.4 (30)		
Xerophthalmic (XS)	14.8±1.6(10)	78.7±3.2 (17)	-	-
Measles	16.3±7.6 (2)	82.6±3.9 (7)	-	-
Other causes	16.3±3.8 (3)	75.7±5.8 (6)	-	-

Stunting was observed in 42% of the children with conjuctival xerosis and in 67% of the children with Bitot's spots. Despite the low RBP levels recorded in the children with corneal xerosis/ulceration the nutritional status was better than of those children with conjunctival xerosis or Bitot's spots. A single child with X2 and a weight-for-age of 35% of the reference standard reduced the group average by 4%. Mean serum RBP levels and also the mean weight-for-age were lower in children with xerophthalmic scars when compared with those children with corneal scarring due to measles. A severe deficit of weight-for-age was recorded in 13.3% (4/30) of the children with corneal scarring, which is two to ten times the rate recorded in the population sample in the various districts.

DISCUSSION

The surveys reported in this paper were not specifically designed as surveys to determine the prevalence of xerophthalmia and we acknowledge the implications of this on the evaluation of the data. We feel however, that despite a number of shortcomings concerning coverage and diagnosis in the early stages of the work,

it is justified to conclude that a higher prevalence of xerophthalmia was seen in Iringa Region than has been previously reported (6), while no data have been available uptil now on the prevalence of xerophthalmia in Kagera Region. A high degree of clustering of active lesions was observed so that in several isolated villages or clusters of villages, the prevalence criteria of the WHO for determining whether xerophthalmia is a problem of public health significance were exceeded. These foci of xerophthalmia were located in Iringa Rural District and in Njombe District.

Of the total of 13 children with Bitot's spots, there were more males (n=9) than females (n=4) as has been reported from Tabora Region (19) and elsewhere (27). As also already observed in Tabora, Bitot's spots were predominantly seen in children above the age of 48 months.

Of a total of 12,980 children examined, corneal ulceration was observed in three children (0.23%) which is above the WHO limit of 0.01% (2), set for corneal xerosis and corneal ulceration.

During the first survey carried out in Iringa Region, survey procedures did not ensure a proper description of the various types of corneal scarring. As in the Indonesia Nutritional Blindness Project (28) xerophthalmic scarring was diagnozed by exclusion of those children with a history of traumatic, infectious or congenital lesions. In our work this method did not prove to be satisfactory and during later studies the eye examination form presented in a manual on conducting xerophthalmia surveys was preferred (29). The overall prevalence of 0.20% (26/12,980) for xerophthalmic scars is four times the WHO limit (2). The proportion of corneal scars which could be attributed to measles (30%) was somewhat lower than that observed in Tabora (19).

From the regions involved in the studies described here, four hospitals participated in the hospital-based surveillance programme referred to above (8). The results of this programm, as far as non-measles cases are concerned, showed that Bitot's spots were recorded at Mbeya Regional Hospital at a rate of 0.12% (5/3,951). For Ilembula Hospital (Njombe District of Iringa Region) and Iringa Regional Hospital these proportions were 6.1% (5/82) and 0.52% (2/385) respectively while for corneal scars the proportions were 9.4% and 5.2%. Among the 551 non-measles children enrolled in the surveillance programme at Ndolage Hospital (Muleba District, Kagera Region), four (0.73%) showed corneal scarring. It cannot be overemphasized that the data obtained through the surveillance programme are not prevalence data. However, the data from Ilembula Hospital and Iringa Regional Hospital, which serve those areas indentified as having a high prevalence of xerophthalmia, show a much higher proportion of (xerophthalmic)

eye lesions than in the other thirteen participating hospitals.

Although anthropometry is a useful tool for assessing the nutritional status of children, there has been much discussion on the use of international reference values for weight and height/length. The situation in Tanzania has been reviewed by Van Roosmalen-Wiebenga (13). We fully agree with the recent recommendations that nutritional status should be described in terms of standard deviation (SD) scores instead of a proportion of the reference weight-for-age or weight-for-length (30). As the surveys described here were part of large scale health programmes emphasizing the proper use of growth cards to monitor the childs' weight gain, results were expressed in term corresponding to those used on the growth card (i.e. weight-for-age) or when possible in terms of wasting and stunting.

The proportion of children with moderate and severe underweight observed at district level ranged from 31% (Mbeya Rural) to 58.6% (Ngara). Severe underweight was observed more in Kagera Region than in the other areas. The results from Njombe District are difficult to explain as, on one hand the prevalence of malnutrition was comparatively low (40.6%), while on the other hand the prevalences of anaemia (41%), low PCV (85%) and malaria (44%) were relatively high as was the proportion of children with active xerophthalmia (0.57%). The nutritional status reported for the population under study in Mbeya District appears to be more favorable than the results of other studies in the area (11,19), although the number of children studied was limited. The proportion of children with moderate and severe underweight in Mbeya is also low (31.1%) when compared with the situation in neighbouring Iringa Region. The present study does not support the results of the surveys carried out in 1979 and 1980 in which malnutrition was found to be more prevalent in the south of Iringa Region (6). In the present study a positive relationship between RBP levels and weight-for-age was recorded, while in earlier studies in Tabora Region such a relationship was not found (19).

After completion of the JNSP mass vaccination campaign, a high level of vaccination was achieved and a major challenge to the programme would be to keep the vaccination coverage at this level. Subsequent reports on the progress of the programme conluded that the vaccination coverage had increased to 80%, with many of the villages having rates of 90% or above. At the beginning of 1986, the measles mortality had dropped to 6% from 20-25% before the programme started (31). However a detailed study on the impact of measles vaccination has shown that the proportion of children protected was 60%, possibly because of poor functioning of the cold chain (31). It would be interesting to record the

incidence of post-measles corneal scars in this area where measles vaccination coverage is high.

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REFERENCES

1 Tielsch JM, West KP, Katz J, et al. Prevalence and severity of xerophthalmia in Southern Malawi. Am J Epid 1986;124:561-8.

2 WHO. Control of vitamin A deficiency and xerophthalmia Report of joint WHO/UNICEF/USAID/Helen Keller International/IVACG meeting. Techn Report Series No. 672. Geneva: WHO, 1982.

3 Kreysler J, Schlage C. The nutrition situation in the Pangani Basin. In: Kraut H, Cremer JD, eds. Investigations into health and nutrition in East Africa. Munchen: Weltforum Verlag, 1969:85-178.

4 Latham MC. Nutritional studies in Tanzania. Wrld Rev Nutr Diet 1967;7:31-71.

5 McLaren DS. Nutrition and eye disease in East Africa Experience in Lake and Central Provinces, Tanganyika. J Trop Med Hyg 1960;63:101-22.

6 Ljungqvist B. Iringa Nutrition Survey 1979-1980 TFNC Report No. 692. Dar es Salaam: Tanzania Food and Nutrition Centre, 1981.

7 Foster A,ed. Focus on blindness in Africa, Proceedings of the sub-regional prevention of blindness seminar for East and Central Africa, Moshi Tanzania, Feb 13-18 1984. Moshi: Africa Region Medical Office of Christian Blinden Mission International, 1984.

8 Foster A, Kavishe F, Sommer A, Taylor HR. A simple surveillance system for xerophthalmia and childhood corneal ulceration. Bull Wrld Hlth Org 1986;64:725-8.

9 Population census 1978, vol II. Bureau of Standards Ministry of Planning and Economic Affairs, Dar es Salaam, 1981.

10 Ooms A. Report of the nutrition and health survey among preschool children in Chunya District, Tanzania. Department of Human Nutrition, Wageningen Agricultural University, 1979.

11 Lukmanji Z, Materu M. Nutrition status of under five population in five villages - Kyela District, Mbeya Region (March 1982) TFNC Report No. 922. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

12 Van Roosmalen-Wiebenga MW, Kusin JA, De With C. Nutrition rehabilitation in hospital-a waste of time and money? Evaluation of nutrition rehabilitation in a rural district hospital in Southwest Tanzania. I. Short-term results. J Trop Ped 1986;32:240-3.

13 Van Roosmalen-Wiebenga MW, Kibona WN, Kusin JA, De With C, Buning M. Action-oriented assessment of nutritional status of young children in Mbozi-Sw Tanzania. East Afr Med J 1985;62:640-9.

14 Kavishe FP, Ballart A, Ngonyani M, Ljungqvist BG, Maletnlema TN, Gebre Medhin M. Determinants of reproductive performance and child survival in an African rural community TFNC Report No. 927. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

15 Mwikongi SS, Ndengerio A, Bategeki WB. Iringa Region Nutritional Surveillance Project, Second Report (covering the period October 1982-March 1983) TFNC Report No. 820. Dar es Salaam: Tanzania Food and Nutrition Centre, 1983.

16 UNICEF. A programme for women and children in Kagera Region. Dar es Salaam, 1985.

17 UNICEF. Analysis of the situation of children and women, volume 1 and 2 Government of the United Republic of Tanzania and United Nations Children's Fund (UNICEF). Dar es Salaam, 1985.

18 Dahlin K. Health in Izimbya ward Tanzania A study on health, health service and expressed needs in the Kagera Region, 1985.

19 Pepping F, Hogeweg M, Mroso DM, West CE. A nutritional survey, with special reference to the prevalence of xerophthalmia in Tabora Region (West Tanzania) (submitted for publication).

20 Sommer A, Hussaini G, Muhilal, Tarwotjo I, Susanto D, Sulianti Saroso J. History of nightblindness: a simple tool for xerophthalmia screening. Am J Clin Nutr 1980;33:887-91.

21 Jellife DB. The assessment of the nutritional status of the community. Geneva: WHO, 1968.

22 WHO. Measuring change in nutritional status. Geneva: WHO, 1983.

23 Waterlow JC. The presentation of height and weight data for comparing the nutritional status of groups of children under the age of 10 years. Bull Wrld Hlth Org 1977;35:489-98.

24 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235-54.

25 Arroyave G, Chichester CO, Flores H, et al. Biochemical methodology for the assessment of vitamin A status. Washington: IVACG/The Nutrition Foundation, 1982.

26 Pepping F, Soffers AEMF, West CE. Quality control of clinical chemical analysis in research on vitamin A deficiency and xerophthalmia (submitted for publication)

27 Sommer A. Nutritional blindness: Xerophthalmia and keratomalacia. New York: Oxford University Press, 1982.

28 Indonesia Nutritional Blindness Prevention Project. Characterization of Vitamin A deficiency and Xerophthalmia and the Design of Effective Intervention Programme Final Report Ministry of Health Republic of Indonesia and Helen Keller Int., 1981.

29 Tielsch JM. A generalized xerophthalmia survey package. Baltimore: International Center for Epedemiologic and Preventive Ophthalmology, 1984.

30 WHO Working Group. Use and interpretation of anthropometric indicators of nutritional status. Bull Wrld Hlth Org 1986;64:929-41.

31 WHO and UNICEF. Joint WHO/UNICEF support for the improvement of nutrition in the United Republic of Tanzania Annual Report 1986. Dar es Salaam, 1986.

3. A NUTRITIONAL SURVEY WITH SPECIAL REFERENCE TO THE PREVALENCE OF XEROPHTHALMIA IN TABORA REGION (WEST TANZANIA)

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ABSTRACT

A xerophthalmia prevalence survey carried out in 1985 in Tabora Region (West Tanzania) in which 5,266 children were examined revealed that at regional level xerophthalmia could not be regarded as a problem of public health importance based on the criteria established by WHO. However in several villages in two districts the prevalence of corneal ulceration/ keratomalacia (X3) or corneal scarring (XS) exceeded the WHO limits. A marked clustering of xerophthalmia was observed in two of the fifteen villages surveyed, both villages located in the north of the region. In a subsample of 700 preschool-age children severe malnutrition was observed in 2.6%.

In 1986, a follow-up study was carried out in the areas most affected by xerophthalmia the previous year in which 3,177 children were examined for the prevalence of xerophthalmia. Identical to the first study 27% of the total number of children examined exceeded the age of six years. Bitot's spots were more common among the children between six and nine years (1.47%) than below the age of six years (0.26%) and a regression of these lesions after treatment with vitamin A was recorded in half of the cases. Serum retinol levels increased after treatment with vitamin A more than RBP levels did. Corneal scars were predominantly seen in children below the age of six years and seemed to be the most useful indicator of xerophthalmia at the population level.

INTRODUCTION

"Regional or countrywide probability surveys are the only unbiased means of determining the frequency (prevalence) and severity of vitamin A deficiency and xerophthalmia in a population. These surveys should include both clinical and biochemical determinations, whenever possible".
This quotation is from a report of the World Health Organization (1) and has stimulated the much-needed collection of data on the prevalence of

xerophthalmia in many regions of the world including Africa.

A recent survey in Malawi demonstrated that the prevalence of active xerophthalmia was 3.9% (210/5,436) in preschool-aged children (2). The information available from Kenya was summarized by Jansen and Horelli in 1981 (3) who concluded that xerophthalmia was not a major health problem in Kenya.

A research programme was set up in 1981 with the aim of describing the magnitude and severity of the xerophthalmia problem in Tanzania (4,5). In this paper, we report on health status and the prevalence of xerophthalmia in Tabora Region. This region was not included in a hospital-based xerophthalmia surveillance system which operated for two years from 1982 to 1984 and from which the results were published recently (6). One year after completion of the initial survey, a follow-up study was carried out in those wards in the region with the highest prevalence of xerophthalmia in order to re-examine the situation. The application of the widely used WHO classification for xerophthalmic eye lesions will be discussed (1).

METHODS

Study area

Tabora is one of the twenty regions of Tanzania mainland which together with the Island of Zanzibar comprise the United Republic of Tanzania (see Fig. 1). Tabora region is part of the central highlands. It is situated in the western part of the country and has an area of 73,500 km^2.

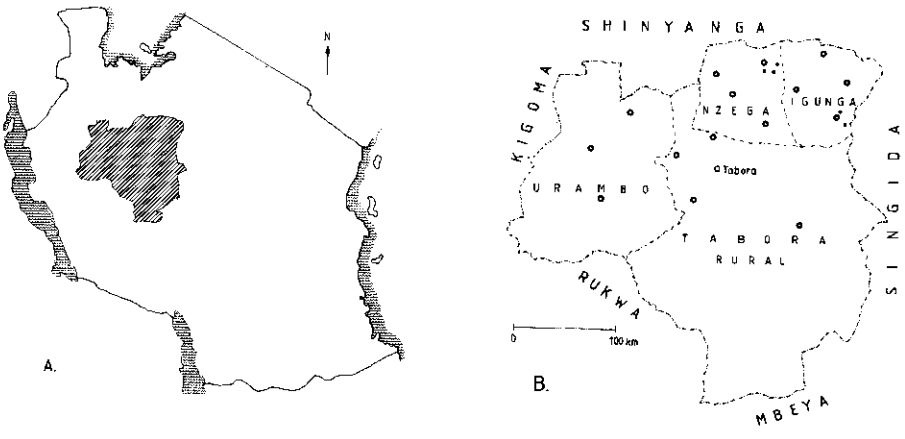

Figure 1: Map of the United Republic of Tanzania (A) indicating the location of Tabora Region and a map of Tabora Region (B) indicating the district boundaries and villages screened in 1985 (o) and the villages added in 1986 (●).

Miombo woodland covers 60% of the area of the region. Rainfall is rather variable with the main rains falling between November and April. Tabora is divided into the administrative districts of Tabora Rural, Urambo, Nzega, Igunga and Tabora Urban. The population of the region was estimated in 1985 to be 1.1 million. Thus, the regional population density is 15 persons/km^2 which is below the national average of 20 persons/km^2. Nzega and Igunga, two districts in the north of the region are more densely populated (30 persons/km^2). The dominant tribe in the region is the Nyamwezi. Maize is the principal staple food; other important food crops are sorghum, groundnuts, cassava, sweet potato and rice. Tobacco, cotton and sunflower seed are grown as cash crops. Infestation with tsetse fly has excluded livestock production from many parts of the region with the exception of Nzega and Igunga. The region has seven hospitals, eleven health centres and in excess of one hundred dispensaries. The infant mortality rate was estimated in the 1978 census to be 130-145 per 1,000 live births (7).

Survey procedure

The regional health authorities agreed to select 15 villages on the basis of population density and geographical spread. Tabora Urban District and the vast and scarcely populated woodlands in the southern part of the region were excluded by the selection process. It was necessary for all of the villages selected for the study to be accessible by car within two or three hours from the respective district headquarters.

Survey procedures and the questionnaires used in the surveys were based on the instructions given in a manual on xerophthalmia surveys (8). A short questionnaire aiming at determining the number of adults and children living in the village and the organization of health facilities, was completed for each village. The initial survey was carried out in March 1985 and the follow-up survey was carried out in February 1986.

All families living in the selected villages were requested to attend a clinic organized at a local dispensary or primary school together with their preschool-age children. After intake administration, the eyes of all children were examined carefully. The children with xerophthalmic eye lesions and every fifth child of the remaining child population were selected for more detailed examination, including anthropometry, fingerprick bloodsampling, and collection of information on breast-feeding, measles history, recent morbidity and vaccination status. A thick blood smear was immediately examined for malaria parasites. Serum was prepared and stored according to the guidelines

established by the International Vitamin A Consultative Group (9).

In this survey, as in all integrated nutrition/health surveys carried out by the Tanzania Food and Nutrition Centre, objectives were not limited to the investigation of a single health problem. All primary schoolchildren were therefore examined for iodine deficiency disorders (10), and vaccination and growth monitoring were also carried out in collaboration with the local health authorities.

Ophthalmological examination

At least one fully qualified ophthalmologist was always available for the eye examinations. Eyes were examined with a standard torch or a magnifying eyeloup (5X, International Centre for Eye Health, London, England). Inspection of the inner surface of the upper eyelid was done whenever trachoma was suspected. Fluorescein filterpaper strips (Haag Streit AG, CH-3097 Liebfeld, Switzerland) were used to stain the ocular surface when any abnormalities were suspected. Mothers or guardians of those children with evidence of corneal scarring were questioned in detail about the possible cause of the lesion. Children with active xerophthalmia were treated with high-dose vitamin A capsules (200,000 I.U. vitamin A + 40 I.U. vitamin E) as recommended by WHO (1).

Anthropometric measurements

Body weight was measured using standard procedures (11). Supine length was recorded with the child lying on a measuring board. The anthropometric indices, weight-for-length, weight-for-age and length-for-age, were calculated using the National Centre for Health Statistics (NCHS) reference standards (12).

Biochemical measurements

Retinol levels were estimated by high performance liquid chromatography (HPLC) as described by Driskell et al. (13,14). Total retinol-binding protein (RBP) was measured by radial immunodiffusion (15) with antiserum from Dakopatts a/s (DK 2600 Glostrup, Denmark) and commercial standards from Behringwerke AG (Marburg, FRG). Quality control was ensured by including a pooled serum sample in each series of analyses for both retinol and RBP. Details of this quality control programme have been described elsewhere (16). Results are expressed in convential units and to convert these into μmol/l the following conversion factors should be used: 1 μg RBP/ml = 0.047 μmol/l

and 1 μg retinol/100 ml = 0.035 μmol/l.

Follow-up study

A follow-up survey was carried out in the two administrative units (wards) where xerophthalmia was found to be a problem of public health significance in certain villages. In each case the whole ward (3-5 villages) rather than the index village was examined. The procedures followed were identical with those of the initial survey except that pupils of the two lowest primary school classes were also included in the investigations. One of the ophthalmologists (DMM) participated in both surveys.

In addition haemoglobin levels were measured in a population sample, which was selected in the same way as during the initial survey, by means of a portable haemoglobin meter with an internal standard (Leo Diagnostics, Helsingborg, Sweden). Venous blood samples were collected from a number of children with Bitot's spots and corneal scars before and/or after the administration of vitamin A and from a number of selected control children enrolled in a separate food consumption study (17) for the analyses of retinol and RBP in serum.

Data analysis

Data were entered on diskettes in Dar es Salaam. For final analysis of the data in Wageningen, data processing was carried out with SPSS-X software (18) run on a VAX-8600 computer. Chi-square and Student's t-test were used for statistical tests of significance and differences between districts were adjusted for age using a multiple regression model.

RESULTS

Study population

Details of the study populations are given in Table 1. Despite the stated difference in the survey procedure the proportion of children above the age of 6 years was almost identical in both surveys. As indicated above, primary school children in the first and second class were included in the 1986 survey but not in the 1985 survey.

The coverage was between 70 and 80% of the target population. Somewhat lower coverage in two villages was due to the fact that the geographical area covered by the village was too large to expect all people to attend.

Table 1: Age and sex distribution of children studied in two surveys on xerophthalmia carried out in Tabora Region in 1985 and 1986

	March 1985				February 1986			
	Males	Females	Total		Males	Females	Total	
	n*	n	n	%	n	n	n	%
Population screened			5,266	100			3,177	100
< 72 months of age			3,842	73.0			2,294	72.2
⩾ 72 months of age			1,424	27.0			883	27.8
Population sample+	372	338	710		188	173	361	
Age (months)								
<12	105	91	196	27.5	42	35	77	21.6
12-23	112	101	213	30.0	43	40	83	23.3
24-35	69	79	148	20.8	39	34	73	20.5
36-47	57	39	96	13.5	30	17	47	13.5
48-59	26	27	53	7.5	12	10	22	6.2
60-71	3	1	4	0.6	13	19	32	9.0
⩾ 72	0	0	0		7	15	22	6.2
Unknown	0	0	0		2	3	5	

* Number (n) and proportion (%) of children.
\+ Every fifth child below the age of six years was selected for detailed investigation.

One of these villages was a refugee settlement established in 1972 for inhabitants from the neighbouring country Burundi. The age distribution of the population sample was biased with an excess of children below 24 months of age.

Nutritional status

Using weight-for-length and length-for-age as nutritional indicators, 24.1% of all children showed wasting and/or stunting. The prevalence of stunting (low length-for-age) increased in children above the age of 24 months, while wasting (low weight-for-length) was less prevalent between the ages of 18 and 35 months (see Figure 2). The prevalence of stunting was higher in Tabora Region and Urambo District (22 and 28%) than in Nzega and Igunga (15 and 8%). The difference observed between the latter two districts is partly due to a difference in mean age. After adjustment in a multiple regression model the difference in mean length-for-age was no longer significant (p=0.08). Severe PEM

as classified by weight-for-age was observed in 2.6% of the study population (Table 2). Underweight (below 80% of weight-for-age) was more prevalent in boys than in girls (X^2=5.1, p<0.025).

Table 2: Distribution of children classified by weight-for-age and sex in a population sample studied in Tabora Region in 1985

Weight-for-age % reference	Boys		Girls		Total	
	n*	%	n	%	n	%
⩾ 80%	204	55.4	212	63.9	416	59.4
70-79%	109	29.6	87	26.2	196	28.0
60-69%	40	10.9	30	9.0	70	10.0
< 60%	15	4.1	3	0.9	18	2.6
Total	368		332		700+	

* Number (n) and proportion (%) of children.
\+ Of 10 children (4 boys and 6 girls) no weight recorded.

The proportion of children below 18 months of age still being breastfed was high (95.6%, n=174) except in Nzega District where children were weaned relatively early from the breast (88.4%, n=114). This difference was significant (p<0.1).

Morbidity

A history of measles was recorded in 10.4% of the children. Of these children nearly 50% suffered from the disease during the second year of life. Of the remaining children with a history of measles, about 20% contracted the disease before 12 months and about 20% on 24 to 35 months of age. Of the 20% sample studied, the proportion vaccinated against measles was 61%, with a considerable variation among the districts (47-71%). Of those children who were vaccinated against measles, 56.5% received the vaccination during the first year of life (< 12 months), with an additional 33.2% in the second year.

Of the 610 blood slides examined for malaria parasites, 242 (39.7%) were positive. The proportion of children with malaria varied at village level from 25 to 64%. Malaria was most prevalent in the 6-11 months and 12-17 months age groups. Of those children with malaria 43.3% were underweight (below 80% of the reference weight-for-age) compared with 39.1% of those without malaria.

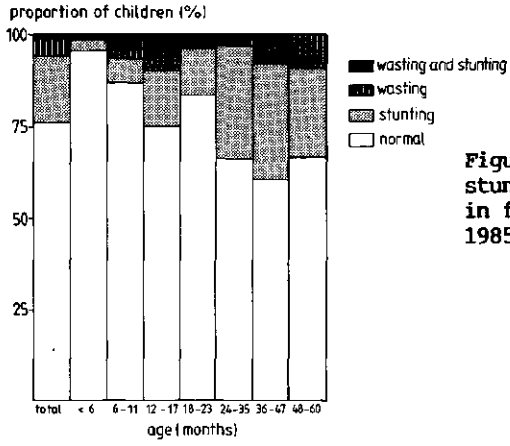

Figure 2: Distribution of wasting and/or stunting by age in 700 children studied in four districts in Tabora Region in 1985

This difference was not significant, nor was it significant when the effect was studied separately in children of 0-1, 2-3 and 4-5 years of age.

Forty-two per cent of the children had been free of recorded illness for six months. Fever was reported in 36.7% of the children although this did not correlate with the presence of malaria parasites. Other frequently reported illnesses were respiratory tract infections (9.3%) and diarrhoea (4.9%).

Eye lesions

Active xerophthalmia (X1A, X1B, X2/X3) was found in 0.27% of the study population (95% confidence interval (CI) 0.17-0.52%). Bitot's spots (X1B, n=11) were always bilateral and located on the temporal side of the limbus (see Appendix II, photographs no. 1-4). The mothers reported that four of these 11 children had a history of night blindness while five children definitely did not. The regional prevalence of X1B was 0.21% (Table 3). Marked clustering of cases was observed: 8 of the 11 children with X1B lived in two of the total of fifteen villages. The prevalence in Mwaluzwilo village (Lusu ward, Nzega District) was 0.6% (5/787), and in Itumba village (Itumba ward, Igunga District) 1.6% (3/188). All X1B cases were boys. The prevalence of Bitot's spots in children below the age of six years was 0.16% (6/3,842; CI 0.08-0.36%).

One 13 months old baby-boy with photophobia showed limited areas of bilateral corneal haziness without signs of inflammation. The clinical picture was suggestive of keratomalacia. Of the 18 children with corneal scarring, two children had unilateral phthisis bulbi (see Appendix II, photograph no. 7). Five children had bilateral corneal scars. One of these children was

Table 3: Eye lesions recorded in children studied in two surveys on xerophthalmia carried out in Tabora Region in 1985 and 1986

Classification of eye lesions	March 1985*						February 1986+					
	Males		Females		Total		Males		Females		Total	
	n	rate	n	rate	n	rate|	n	rate	n	rate	n	rate
Conj. xerosis (X1A)			1	0.40	1	0.19						
Bitot's spots (X1B)	11	3.98			11	2.09	13	7.86	6	3.94	19¶	5.98
Corneal ulcers/ keratomalacia (X3)	1	0.36			1	0.19						
Corneal scarring	12		6		18		8		7		15	
- measles	4	1.45	2	0.80	6	1.14	4	2.42	5	3.29	9¶	2.83
- xerophthalmia (XS)	2	0.72	1	0.40	3	0.57	1	0.60			1	0.31
- others/unknown	6	2.17	3	0.80	9	1.71	3	1.81	2	1.31	5	1.57
Total	24	8.68	7	2.80	31	5.89	21	12.68	13	8.54	34	10.79

* Total number of 5,266 children examined: 2,765 males and 2,501 females.
\+ Total number of 3,177 children examined: 1,655 males and 1,522 females.
| Rates are expressed as number of cases per 1,000 children.
¶ Four children with Bitot's spots and one child with a corneal scar due to measles had also been observed during the previous survey.

identified as blind in this series with an estimated visual acuity of 1/60 to 3/60 (see Appendix II, photograph no. 5). In the remaining four other children, the bilateral scarring was reckoned to produce slight impairment of visual acuity. In seven cases there was a history of purulent conjunctivitis, and in a further two children trauma or foreign body were implicated. The three children with xerophthalmic scarring (XS) were all from the two villages with the high rates for Bitot's spots. Acute purulent conjunctivitis was present on examination in one to three percent of the children. Trachoma was seen in 1% of the children in Tabora Rural District while it was rare in the other districts. Seven children showed miscellaneous acute eye conditions requiring treatment. Two children had congenital deformities of the eye, of whom one child was blind.

Observations one year after the initial survey

In the follow-up survey, coverage figures for the population under the age of six years were 93% for Lusu ward and 83% for Itumba ward. The nutritional status of the population sample did not differ from that observed during the

initial survey. Stunting and/or wasting was observed in 24.2% (85/351) of the children under six years of age. Further analysis revealed that after correction for age differences there remained a significant difference in the mean length-for-age between both wards (Lusu 95.4% vs. Itumba 99.4%, $p<0.001$).

In Lusu ward, 47.6% (81/170) of the children had a haemoglobin level below 10 g/dl and for Itumba ward this was 62.4% (63/101). This difference in prevalence of anaemic children was due to an extremely high prevalence (80%) of anaemia in one village in this ward, whose inhabitants belong to a pastoral tribe (the Taturu) which had not yet widely accepted health services.

Vaccination coverage for BCG, diphtheria/pertussis/tetanus (DPT) and poliomyelitis was also recorded during the follow-up study. The results demonstrated enormous variation between well and poorly served areas. In Lusu, 88.1% of the children under six years of age had received a BCG vaccination, while 93.4% were vaccinated against DPT, 90.4% against poliomyelitis and 82.1% against measles. For Itumba these proportions were 42.0%, 38.4%, 38.4% and 35.0% respectively.

During the second survey, 19 (0.60%) children with Bitot's spots were identified (see Table 3). Bitot's spots were unilateral in five children and in one child the bilateral lesions were located on the temporal and nasal side of the limbus. Two brothers with Bitot's spots persisting since the earlier survey were accompanied by their 10 year old sister who also had Bitot's spots.

The number of children examined in Lusu and Itumba wards in 1986 (2,380 and 797) was three to four times the number examined in the respective single villages in 1985 (787 and 188 respectively). Nevertheless in Lusu ward the prevalence of Bitot's spots found in 1986 (0.67%, 16/2,380) was identical with that observed in 1985 (0.64%). The prevalence for children under the age of six years was 0.26% (6/2,294; CI 0.12-0.36%). Of the total of 11 children with Bitot's spots in March 1985, six were re-examined in February 1986 and the lesions had vanished in two children. In June 1986 the 19 children who were seen with Bitot's spots in February (including the four unhealed cases of 1985) were re-examined. At this time, the lesion was reduced in size in seven children and had completely disappeared in three children. Thus a regression of the lesions was observed in 48% (12/25) of the re-examined children after treatment with vitamin A.

Of the 15 children with corneal scars four had bilateral lesions, of whom one was totally blind and one had an estimated visual acuity of 1/60 to 3/60.

Unilateral phthisis bulbi was seen in two children (one of whom was also included in the earlier survey) in both cases measles was blamed as the cause.

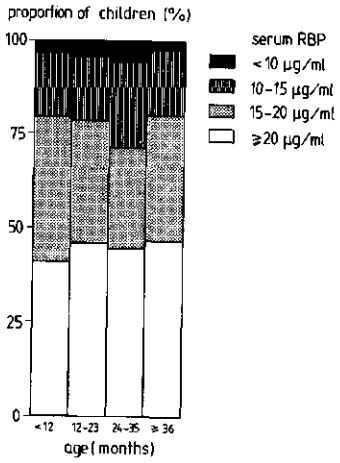

Figure 3: Distribution of RBP levels according to age for children in Tabora Region (n=566)

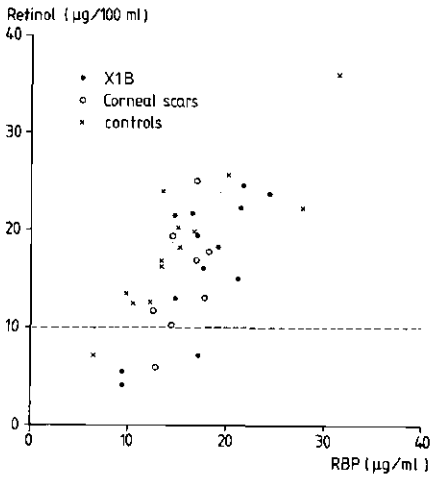

Figure 4: Relationship between the concentration in serum of retinol and RBP in children with Bitot's spots (•), corneal scars (o) and control children (x) studied in two surveys in Tabora Region.

Serum levels of RBP and retinol in relation to anthropometric indices

Over 700 fingerprick samples from the population sample and those with xerophthalmia were analysed for total RBP (582 first study, 134 follow-up study). The distribution of RBP levels in the population sample was not influenced by age (see Figure 3). Of those children with malaria 5.1% had low RBP levels (< 10 µg/ml or 0.48 µmol/l) compared with only 3.1% of those without malaria (n.s., $p<0.25$). Low levels of RBP were found in 4.1% (23/568) of the children examined in 1985, the proportion was higher in boys than in girls although this difference was not significant (4.6%, 14/303 vs 3.2%, 9/265). During the follow-up study RBP levels below 10 µg/ml were found in 7.1% of the children examined in Lusu but in none examined in Itumba, however relatively few samples (n=30) were collected in Itumba.

Investigation of the relationship between serum RBP and the anthropometric indices (weight-for-age, weight-for-length and length-for-age) showed that there was no significant correlation between these parameters irrespective of age or malaria status.

Table 4: The concentration of retinol-binding protein (RBP) and retinol in serum of children with Bitot's spots or corneal scars and before and after receiving a high oral dose of vitamin A, and in a population sample

	Serum concentration*							
Eye lesions/	RBP (μg/ml)				Retinol (μg/100 ml)			
Nutritional status	n	Before	n	After	n	Before	n	After
Bitot's spots								
- all	22|	19.1±1.1	16	20.3±2.6	15|	17.6±2.3	16	23.3±2.3§
- normal	14	18.6±1.6	12	19.5±1.5	10	17.2±3.3	14	23.6±2.4
- stunted	6	19.0±1.5	4	22.8±2.6	4	17.5±3.4	2	21.6±6.9
Corneal scars								
- all	21	18.4±1.3	6	19.1±1.3	8	15.1±2.2	2	18.2±1.6
- normal	12	19.7±2.0	4	17.7±1.1	7	14.8±2.5	2	18.2±1.6
- stunted	9	16.8±1.2	2	22.0±2.0	1	17.1		
20% population sample								
- all	551	19.7±0.3			13¶	18.9±2.1		
- normal	410	19.7±0.3			11	19.5±2.3		
- stunted	103	19.8±0.7						
- wasted	34	20.6±1.1			2	15.4±2.9		
- wasted + stunted	4	18.3±1.7						

* Results are expressed as mean ± SE, and n = number of children.
\+ The data are based on the surveys carried out in 1985 and 1986. Blood samples were taken before and after the oral administration of vitamin A
| In these two groups the nutritional status of two children (for RBP) and one child (for retinol) was not recorded.
§ $p<0.05$ for the comparison between retinol levels before and after dosing.
¶ These children belong to the control group in a food consumption study (see Chapter 5).

The overall nutritional status of the children with eye lesions was only for the children with corneal scars significantly different from that of the population sample ($p<0.01$). Stunting was seen in 29% (7/24) of the children with Bitot's spots recorded during both surveys. Mean levels of RBP and retinol in children with Bitot's spots and corneal scars sampled before administration of a high dose of vitamin A and RBP levels in the population sample are illustrated in Table 4. Levels of retinol indicating deficiency (< 10 μg/100 ml or 0.35 μmol/l) were found in three of the 22 children with Bitot's spots and in two of the 13 controls (results of both surveys combined). High doses of vitamin A had little effect on the levels of RBP although the effect seems to be greater in malnourished children. Retinol

levels showed a clear increase as a result of the vitamin A suppletion. The relationship between RBP and retinol observed in cases and controls is shown in Figure 4 (r=0.8).

DISCUSSION

All the ethnic groups living in Tabora Region (the Nyamwezi, Sukuma, Taturu, Nyeramba and Burundese) were represented in this survey. No home visits were made and information is available only on those who attended the clinic. A study design in which the problem of non-attendance is addressed (2), has obvious advantages but is far more costly. The age distribution suggests that children aged four and five years were underrepresented, although to a lesser extent during the follow-up survey. As many older children were seen it could be speculated that mothers without a child below the age of four years were less likely to attend. The follow-up survey in the two respective wards was not designed as a longitudinal study and no attempt was made to correlate data from both surveys on individual level except for those children with eye lesions.

Severe malnutrition was observed in 2.6% of the children and this is relatively low. A summary of studies carried out in Tanzania compiled in 1986 by UNICEF (7) concluded that "on average the frequency of severe PEM is 4-9% and moderate PEM 40-60%". The influence of age seen is also in agreement with findings in other studies (7). During the first two years of life, stunting was found at rates of 4.8% and 13.9% while in the older age categories this was about 30%. However this was an expected finding (19).

Differences between districts were small and most marked for the length-for-age indicator showing more chronic malnutrition in the two districts in the southern part of the region, Tabora Rural and Urambo.

The influence of malaria infection on nutritional status was not significant and less marked than that observed by Wenlock in preschool-aged children in Zambia (20). Malaria lowered RBP levels and increased the number of children with RBP levels below 10 μg/ml but both effects were not significant. The overall prevalence of 39.7% of the children with malaria is above the prevalence rates found in other surveys in Tanzania (7) except in one survey also carried out in Tabora Region in which the health and nutritional status of 463 preschool-aged children from 10 villages in Nzega and Mpanda Districts (now part of the neighbouring region but then part of Tabora) was examined in 1967 (21). In this survey, protein-energy

malnutrition, based on weight, height and clinical signs of malnutrition, was diagnosed in 8% of the children and malaria in 45% of the children, suggesting that the situation in 1985 was better, at least from a nutritional point of view, than in 1967.

The overall impression is that health facilities are better organized in Nzega District than in other districts, as indicated by the higher vaccination coverage. The vaccination coverage against measles of 61% at the regional level in 1985 is an encouraging figure and might explain the rather low proportion (10.4%) of children with a history of measles, although the age distribution of the 20% sample might have biased this figure. It has been estimated that measles vaccination coverage ranges from 40% to 67.0% throughout the country depending on the availability of primary health care services (7). A recent coverage survey by the Expanded Programme of Immunization (EPI) showed that, in rural areas, coverage was 67% for children in the second year of life and 52% for those one year older (22). In the follow-up study described in this paper the coverage in Lusu ward was comparatively high (82.1%) while the coverage in Itumba ward in Igunga District was comparatively low (35.0%).

Mothers ceased to breastfeed their children earlier in Nzega District than in the other three districts. This difference might be related to the relatively high cow milk production in the district. Anaemia was widely prevalent and although the overall impression was that among the semi-nomadic tribe living in Igunga District the nutritional status was comparable to that of other children they did have a higher prevalence of anaemia. It is tempting to suggest that the dietary pattern characterized by a high milk consumption does not provide sufficient iron but the poor iron status could also be attributable to a higher burden of intestinal and other parasites.

Active xerophthalmia was found predominantly in Nzega and Igunga Districts in the northern part of the region. In Tabora Rural and Urambo Districts, only one child with conjunctival xerosis and six with corneal scarring, non of these xerophthalmic, were found out of a total of 1,910 children. During the 1985 survey much efforts were made to investigate whether a diagnosis of xerophthalmia could be based on night blindness. This failed because mothers were unaware of the phenomenon. During our field studies in other regions of Tanzania it also proved to be impossible to use night blindness for this purpose. Other studies from the African region have produced the same conclusion (23), although among 152 cases of xerophthalmia identified in Malawi 130 (85.5%) had night blindness (2). We feel that the use of the 1%

criterion for night blindness as part of the WHO prevalence criteria for xerophthalmia might be of limited use in many parts of Africa.

The WHO prevalence criteria for Bitot's spots and for xerophthalmia in general are intended to apply to children up to 72 months of age (1). In 1985, six of the 11 children with X1B were within this age range whereas in 1986 10 out of the 15 new cases were of older age. For the under-six population the prevalence of Bitot's spots found in 1985 and 1986, 0.16% and 0.26% respectively, are below the WHO criterion of 0.5% (1). Such relatively low prevalence rates are common in Africa. Tielsch et al. (2) reported a rate of 0.33% (18/5,436) in an area long known for its high prevalence of blindness and noted like others (24) a higher prevalence among males which finding was confirmed once more in this study. Just recently it was reported from South Ethiopia that Bitot's spots were observed in 3.9% (103/2,647) of the preschool-age children (23). The relatively high proportion of the Bitot's spots in children aged between 6 and 10 years as observed in this study was also noticed in Nepal (25) and a prevalence of up to 15% in schoolchildren up to the age of 15 years has been reported from India (26).

Retinol and RBP levels observed in the children with Bitot's spots were not deficient and retinol levels clearly increased as a result of suppletion with vitamin A and the increase persisted up to at least three months after suppletion. The observation that less than half (12/25) of the Bitot's spots responded to the administration of vitamin A in combination with the age profile of these children suggests that the majority of the Bitot's spots found, were inactive cases (24).

The data on corneal scarring suggest that measles is responsible for almost half (14/32) of these lesions. Of the 14 "measles scars" recorded during both surveys, six were bilateral. We must therefore conclude that measles is an important factor in the aetiology of childhood blindness, perhaps responsible for half of the number of cases (27). Only in four children the corneal scarring seemed to be attributable to uncomplicated xerophthalmia. This leads to prevalence rates for the under-six population of 0.08% (3/3,842; 1985) and 0.04% (1/2,294; 1986) which are of the same magnitude as the WHO-criterion of 0.05% (1) but should be regarded as conservative estimates. In children who developed corneal scarring after purulent eye infection with or without the use of traditional eye medicine, or after severe coughing with or without diarrhoea, a possible role of xerophthalmia cannot be ruled out. A history as described above was observed in seven children in 1985 and in two children in 1986 and except one, all these children were below the age of six years.

Inclusion of these children in the XS-group would increase the prevalence rates twice and thus bring these for both surveys above the WHO prevalence criterion.

It cannot be inferred from the finding of a 0.03% prevalence of keratomalacia in the population under six years of 3,842, that xerophthalmia is a public health problem in Tabora, hence the WHO criterion for X2/X3 is 0,01%. Regarding the size of the study population it could be expected to find a child with keratomalacia and that this child was just found in Igunga District should not lead to the conclusion that xerophthalmia is more prevalent in this district or that xerophthalmia is a public health problem in the whole region (24). However accumulation of all children with Bitot's spots and of the children with keratomalacia and xerophthalmic scarring made Igunga District and the northern part of Nzega District highly suspected of having a xerophthalmia problem.

In comparison with other studies (28,29) RBP levels as found in the 20% population sample were low, 4.1% of the children having very low levels (< 10 µg/ml). Among a group of 55 healthy control children selected in Dar es Salaam we found a mean total RBP level of 21.6 µg/ml in conjunction with a mean serum retinol level of 22.1 µg/100 ml (30). Correlation between RBP levels and anthropometric indices in this study was poor which may be due to the relatively small number of children found with acute/severe malnutrition. The nutritional status of children with corneal scars irrespective of the origin of the scars, was less than that of the children with Bitot's spots and of those without eye lesions. Although the majority of the Bitot's spots were inactive cases in older children, the serum retinol levels responded to the suppletion of vitamin A. Serum total RBP however could not be used as a sensitive indicator of the nutritional status.

In conclusion we feel that our attempt to describe the xerophthalmia situation in Tabora Region was carried out according to the guidelines established for this which were quoted earlier (1). In the south of the region the prevalence of xerophthalmia is insignificant, whereas in the north the prevalence reaches or exceeds the respective WHO criteria for the evaluation of the public health significance of xerophthalmia due mainly to clustering of cases in certain villages. Paradoxically malnutrition appears to be more prevalent in the southern part of the region. Bitot's spots were predominantly seen in children above the age of six years making these lesions less suitable for the evaluation of the magnitude of xerophthalmia in the region. Corneal scars including those attributable to xerophthalmia were more prevalent in

younger children, making these lesions a suitable indicator of the xerophthalmia status. The importance of measles as the main contributing factor in corneal scarring was reconfirmed.

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REFERENCES

1 WHO. Control of vitamin A deficiency and xerophthalmia Report of joint WHO/UNICEF/USAID/Helen Keller International/IVACG meeting Techn Rep Series No. 672. Geneva: WHO, 1982.

2 Tielsch JM, West KP, Katz J, et al. Prevalence and severity of xerophthalmia in southern Malawi. Am J Epid 1986;124:561-8.

3 Jansen AAJ, Horelli HT. Vitamin A deficiency in Kenya past and present. East Afr Med J 1982;59:107-12.

4 Mrisho F, Pepping F, Lukmanji Z. Proceedings of a national symposium for vitamin A deficiency, November 16-18 1981 Dar es Salaam, TFNC Report No. 735. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

5 Upungufu wa vitamin A Tanzania, Expert Committee Report, TFNC Report No. 718. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

6 Foster A, Kavishe F, Sommer A, Taylor HR. A simple surveillance system for xerophthalmia and childhood corneal ulceration. Bull Wrld Hlth Org 1986;64:725-8.

7 UNICEF. Analysis of the situation of children and women, volume 1 and 2 Government of the United Republic of Tanzania and United Nations Children's Fund (UNICEF). Dar es Salaam, 1985.

8 Tielsch JM. A generalized xerophthalmia survey package. Baltimore: International Center for Epidemiological and Preventive Ophthalmology, 1984.

9 Arroyave G, Chichester CO, Flores H, et al. Biochemical methodology for the assessment of vitamin A status. Washington: IVACG/The Nutrition Foundation,1982.

10 Kavishe, FP. Iodine deficiency disorders in Tanzania In: Van der Haar F, Kavishe FP. eds. Iodine deficiency disorders in the region Eastern, Central and Southern Africa, Symposium Gaborone (Botswana). Wageningen: NINI/ICFSN, 1987.

11 Jellife DB. The assessment of the nutritional status of the community. Geneva: WHO,1968.

12 WHO. Measuring change in nutritional status. Geneva: WHO, 1983.

13 Driskell WJ, Neese JW, Bryant CC, Bashor MM. Measurement of vitamin A and vitamin E in human serum by high-performance liquid chromatography. J Chrom 1982;231:439-44.

14 Driskell WJ, Bashor MM, Neese JW. Loss of vitamin A in long-term stored, frozen sera. Clin Chem Acta 1985;147:25-30.

15 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235-54.

16 Pepping F, Soffers AEMF, West CE. Quality control of clinical chemical analysis in research on vitamin A deficiency and xerophthalmia (submitted for publication)

17 Van der Giezen AM, De Jonge KI. A food consumption study in children with xerophthalmia in a rural area in Tabora Region, Tanzania TFNC Report No. 1070. Dar es Salaam: Tanzania Food and Nutrition Centre, 1987

18 SPSS Inc. Release 1 of SPSS-X programme. Chicago, Illinois USA, 1984.

19 WHO Working Group. Use and interpretation of anthropometric indicators of nutritional status. Bull Wrld Hlth Org 1986;64:929-41.

20 Wenlock RW. Endemic malaria, malnutrition and child deaths. Food Policy 1981;6:105-12.

21 Maletnlema TN, Marealle ALD. The health and nutritional status of children in Tabora Region. Env Child Hlth 1973;19:14-8.

22 Wulffsberg H, Blass E. EPI coverage survey 1986 for rural areas in Tanzania, internal report Ministry of Health (unpublished). Dar es Salaam. 1986.

23 De Sole G, Belay Y, Zegeye B. Vitamin A deficiency in southern Ethiopia. Am J Clin Nutr 1987;45:780-4.

24 Sommer A. Nutritional blindness: Xerophthalmia and keratomalacia. New York: Oxford University Press,1982.

25 Upadhyay MP, Gurung BJ, Pillai KK, Nepal BP. Xerophthalmia among Nepalese children. Am J Epid 1985;121:71-7.

26 IVACG. Minutes of the Xth IVACG meeting held in Hyderabad October 1985. New York: The Nutrition Foundation, 1986.

27 Foster A, Sommer A. Childhood blindness from corneal ulceration in Africa: causes, prevention and treatment. Bull Wrld Hlth Org 1986;64:619-23.

28 Ingenbleek Y, Van den Schriek H, De Nayer P, De Visscher M. Albumin, transferrin and the thyroxine-binding prealbumin/retinol-binding protein (TBPA-RBP) complex in assessment of malnutrition. Clin Chem Acta 1975;63:61-7.

29 Reddy V, Mohanran M, Raghuramulu N. Serum retinol-binding protein and vitamin A levels in malnourished children. Acta Paediatr Scand 1979;68:65-9.

30 Pepping F, Hackenitz EA, West CE. The role of nutritional status with special reference to vitamin A in the development of post-measles eye lesions I. Nutritional status (submitted for publication).

4. RETINOL AND CAROTENE CONTENT OF FOODS CONSUMED IN TANZANIA DETERMINED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

F. Pepping, C.M.J. Vencken and C.E. West

ABSTRACT

The vitamin A activity of a number of fresh and dried foods important in the diet of people in East Africa was determined by high performance liquid chromatography (HPLC). The analytical results obtained were combined with data from the literature for inclusion in a comprehensive food table which has been used in a study on xerophthalmia in the United Republic of Tanzania. The importance of adopting standard procedures for reporting retinol and carotenoid values is discussed.

INTRODUCTION

There is a need for reliable information not only on the content of retinol but also of carotenoids in foods eaten both in countries where xerophthalmia is a health problem and in countries with a high cancer incidence. Epidemiological studies have indicated that carotenoids may play a role in the prevention of cancer particularly of epithelial tissues, which is independent of that expressed after their conversion to retinol (1,2). Increased attention is also being paid to the problem of vitamin A deficiency and xerophthalmia. Recently, the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) launched a ten-year plan of action to combat the problem of vitamin A deficiency, xerophthalmia and nutritional blindness (3,4). The consequences of vitamin A deficiency are not limited to the eye (xerophthalmia) but are also found in other epithelial tissues such as those of the respiratory and gastrointestinal tracts and there is evidence that vitamin A deficiency may lead to increased morbidity from respiratory infections and diarrhoea (5) and to increased mortality (6). In many developing countries, the principal sources of vitamin A activity are plant carotenoids especially ß-carotene and to a lesser extent α-carotene and other provitamin A carotenoids. It is therefore important to have information on the carotenoid content of foods which could possibly play a role in combatting

vitamin A deficiency and consequently xerophthalmia. In western countries, the principal source of vitamin A activity is retinol present in animal products particularly dairy products, eggs and meat.

Unfortunately, many of the data on carotenoid content of foods presently available are of limited value because the methods used did not take into account that the various carotenoids have different biological activities relative to vitamin A. Most of the earlier data were obtained by measuring the extinction at 450 nm of a fat extract with or without prior chromatographic separation on an alumina column and not by the more specific technique of high performance liquid chromatography (HPLC). It has been shown that the degree of overestimation by traditional methods such as the AOAC-method (7) depends on the proportion of the total carotenoids present as ß-carotene. Overestimation increases progressively when higher proportions of other compounds are present in the samples being analysed (8). Most data on the provitamin A content of food included in food composition tables are not based on HPLC analysis. For example for foods from Africa, heavy reliance is placed upon analyses carried out some twenty years ago (9,10).

As part of a study carried out in conjunction with the Tanzania Food and Nutrition Centre on the prevalence and aetiology of xerophthalmia and post-measles blindness, data have been collected on the retinol and carotene content of foods consumed in Tanzania. In an initial publication, the amount of retinol and ß-carotene found in maize, legumes and some species of fish were presented (11). Only in yellow maize and green peas were considerable amounts of ß-carotene found. In this paper, the results of further analyses carried out by us and of those carried out by HPLC and reported in the literature are presented. These data have been included in a food composition table prepared for use in our studies in Tanzania (12).

MATERIALS AND METHODS

Collection of samples

The food samples were collected at various places in Tanzania. Most of the cereals, vegetables and fruits were gathered in Nzega District (Tabora Region, West Tanzania; see also map on page 18) from household stores from families participating in a food consumption study (13). Depending on the product, a sample of 200-300 g was collected. The moisture content of fruits and vegetables was assessed immediately after collection and also prior to analysis. This enabled correction for loss of water during transport and

storage to be made. All samples were stored in sealed polyethylene bags from which excessive air was removed with a vacuum-sealer. Fish oil was processed from two species of fresh water fish, Nile Perch (Lates niloticus) and Haplochromis spp, caught in Lake Victoria near Mwanza. Palm oil samples were collected in Kigoma Region near Lake Tanganyika and in Dar es Salaam. It can be assumed that the palm oil obtained in Dar es Salaam also came from Kigoma.

For the samples of fish oil, fresh fish were selected, weighed, washed in running water, and then gutted. The fat was separated from gut and gonads, washed, and placed in polyethylene bags, tagged and weighed. Fat samples were stored in a deepfreeze. For preparation of the oil, the fat samples were thawed, cut into small pieces and boiled in water for approximately 45 minutes. Water evaporated as boiling continued and the oil started to form a separate layer. When most of the water had evaporated, extraction continued for five minutes to complete the process. The oil was filtered through a metal sieve, cooled and put in polyethylene bottles.

Food samples were collected between April and July 1986 and stored for four months at -20°C before analysis.

Extraction of carotenes and retinol

For all of the analyses presented in this paper, a saponification step was included in the extraction procedure. Although this is necessary for samples containing a high proportion of lipid such as the oil samples, analysis of products such as vegetables do not necessarily require this step. Under non-oxidizing conditions, both hot and cold saponification have been reported to result in little loss of carotenoids (14). However losses depend also on the carotenoid profile present in the sample under analysis. More than one third of the lutein, a carotenoid having no vitamin A activity, is reported to be lost during saponification (15).

Carotene analysis were carried out according to the method described by Speek et al. (16) which was also used for the work described earlier (11). After alcoholic saponification, carotenoids were extracted with diisopropylether.

The method used for the analysis of the retinol content of the fish oil was largely based on existing methods (17,18). Alcoholic saponification was carried out for 45 minutes on a waterbath at 90°C followed by extraction with diethylether. Hydroquinone was added as antioxidant, dl-tocol as internal standard and retinyl acetate was used to plot a calibration curve.

All reagents were of analytical or chromatographic grade. Diisopropylether

stabilized with butylated hydroxytoluene (BHT) (0.01% w/v) was obtained from Merck. α-carotene and all-trans retinyl acetate were obtained from Sigma, ß-carotene from Merck and all-trans retinol from Fluka. Dl-tocol and 15,15'-cis-ß-carotene were gifts from Hoffmann-La Roche.

Chromatographic separation and quantitation

The HPLC system comprised the following components: a metering pump (model: Constametric III) and a variable wavelength detector (model: Spectromonitor D) from LDC/Milton Roy, Riviera Beach, FL 33404, USA) and an injection valve (type 7010; Rheodyne Inc., Cotati, CA 94928, USA). Stainless steel columns (250 x 4.6 mm i.d.) were used: for the separation of the fish oils, a 10 μm C18 reversed phase column (Chrompack, Middelburg, The Netherlands) and for the separation of the carotenoids, a 5 μm C18 column (Brownlee Lab Inc., Santa Clara CA 95050, USA). For both types of analyses, a Chrompack reversed phase guard column (75 x 2 mm i.d.) was used. Absorption measurements were made using a spectrophotometer (Carl Zeiss, FRG).

A 50 μl sample of the extract dissolved in the mobile phase (acetonitrile: methanol:hexane:dichloromethane, 65:15:10:10 by volume) was isocratically eluted at a flow rate of 1.5 ml/min. The absorbance at 445 nm was recorded and peak heights of three working standards for α- and ß-carotene were used to plot calibration curves. For the analysis of retinol, the mobile phase consisted of a water:methanol mixture (96:4 v/v) with detection at 294 nm which is the absorption maximum of the internal standard.

RESULTS

Analytical results

Recovery of carotenoids was checked by adding various amounts of ß-carotene prior to saponification and extraction and was in excess of 91%. A comparative study carried out with another laboratory in The Netherlands in which six samples of yellow maize were analysed in both laboratories gave results within 6% of the mean values (19).

The number of samples analysed per product varied from one to ten. The results for the plant foods are presented in Table 1.
Considerable amounts of α-carotene were found only in red palm oil, approximately half of the amount of ß-carotene, and in pumpkin squash where

Table 1: The content of α- and ß-carotene in 17 plant foods consumed in Tanzania determined by high-performance liquid chromatography

Food	n[a]	Water content g/100g	Carotene content[b] µg/100 g edible portion	
			α-carotene	ß-carotene
Amaranthus (Amaranthus spp)				
leaves, fresh	1	84	85	2400
Cassava (Manihot esculenta)				
leaves, fresh	1	71	Ø	2820
Cowpea (Vigna spp)				
leaves, fresh	1	77	Ø	730
leaves, dried	4	10	Ø	3600 (2300-5700)
"Kayeba" leaves, fresh	1	78	Ø	3500
Millet (Eleusine spp)				
grain	2	8	Ø	26 (23-29)
Oil palm(Elaeïs guineensis)				
oil, red	10	trace	9430 (5820-14350)	21338 (9300-33000)
Okra (Hibiscus esculentus)				
fruits	2	91	Ø	173 (160-270)
local variety, leaves	1	80	Ø	830
Papaya (Carica papaya)				
fruit, fresh	1	89	Ø	160
Peas green (Pisum sativum)				
fresh	1	70	26	110
Pumpkin (Cucurbita moschata)				
squash, fresh	1	93	1100	1170
leaves, fresh	2	82	trace	1050 (1040-160)
leaves, dried	2	8	250	9650 (9050-10200)
Sorghum (Sorghum spp)				
grain	2	9	Ø	20
Sweet potato (Ipomoea batatas)				
yellow, tuber, fresh	1	83	Ø	1820
white, tuber, dried	2	9	Ø	75

a Number of samples analysed.
b Results are expressed as mean and range. Results calculated on the basis of the original water content of the sample as shown in the table.
Ø Quantity too small to be of any importance.

both carotenes occurred in almost equivalent amounts. Very small amounts of α-carotene were found in green peas, amaranthus and dried pumpkin leaves. There was a considerable variation in the carotene contents of the different oil samples. From some of the samples it was not known from which variety of oil palm (E. guineensis) the fruits were obtained for preparation of the oil. Most oils are prepared from fruits from the dura and tenera variety. The single sample exclusively processed from the tenera variety gave rather low values, 6,400 µg α- and 9,400 µg ß-carotene per 100 g edible matter and these low values were accompanied by a relatively high proportion of α-carotene (40%). For eight of the other nine samples, the proportion of α-carotene varied from 27 to 34% of the total carotenes. Typical HPLC elution profiles for red palm oil and pumpkin squash are given in Figure 1. The two peaks which were eluted immediately after ß-carotene correspond to 15,15'-cis-ß-carotene and an unidentified compound.

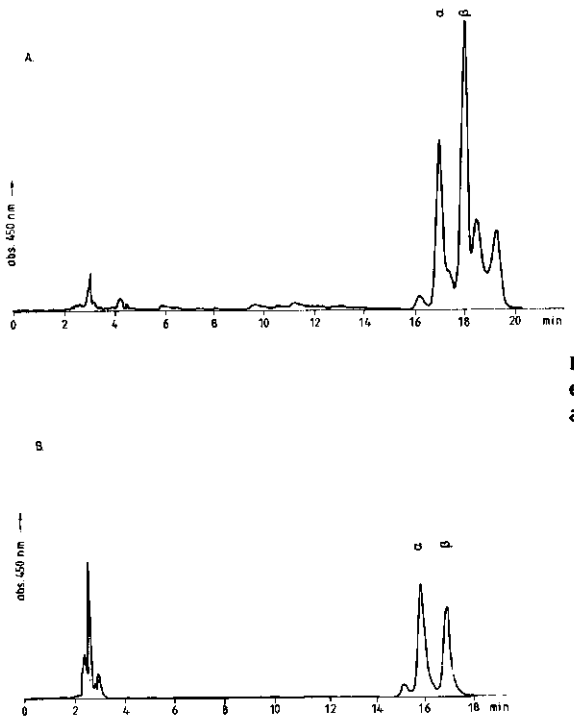

Figure 1: Elution profiles of extracts of red palm oil (a), and pumpkin squash (b) with α- and ß-carotene peaks.

Of the various green leafy vegetables only a limited number of fresh samples were available for analysis. The carotene content of the fresh vegetables was low compared with values reported in food composition tables. Two indigenous types of vegetables eaten in the western part of Tanzania have also been analysed. These included "kayeba" (scientific name unknown) which was a rich source of ß-carotene and a local variety of okra (Hibiscus spp). Dried leaves of cowpea and pumpkin, and dried sweet potato are widely consumed in several parts of Tanzania. The amount of ß-carotene present in staple foods such as sorghum and millet is extremely low.

In Table 2, the amounts of retinol and dehydroretinol (vitamin A2) in six samples of fat from Nile perch and in one sample of fat from Haplochromis are given. The oil prepared of the Haplochromis had a yellow/brown colour, while that of the Nile perch had a white appearance. For the samples from Nile perch the average retinol equivalent was 5,150 µg per 100 g but as can be seen from Table 2, the range was considerable.

Table 2: Retinol and dehydroretinol content of fat from fresh water fish taken from Lake Victoria

Sample	Content per 100 g of fat		
	Retinol (µg)	Dehydroretinol (µg)	Retinol equivalents[a] (µg)
Nile perch (L. niloticus)			
1	7960	5510	10160
2	3860	2950	5040
3	5020	2400	5980
4	3420	3020	4630
5	2870	2440	3840
6	1030	490	1220
Haplochromis spp	5600	2780	7360

a Dehydroretinol (vitamin A2) has a biological activity of 40% of all-trans retinol (vitamin A1) (29).

In Table 3, the α- and ß-carotene content of plant foods determined by HPLC both by us and by other workers are presented together with the values reported in the tables prepared by Platt (10) and FAO (9). These values have been incorporated in a food table created for use in studies on vitamin A deficiency in Tanzania.

Table 3: Summary of available data on the α- and ß-carotene content of selected Tanzanian foods determined by high performance liquid chromatography

Food	Carotenoid content, µg/100 g edible portion							
	"ß-carotene" (non-HPLC)		Individual carotenoids					
			Own analyses		Other analyses		Data used in studies	
	Platt[a]	FAO[b]	α-carotene	ß-carotene	α-carotene	ß-carotene	α-carotene	ß-carotene
Amaranthus (Amaranthus spp)								
leaves, fresh	1800	5715	85[c]	2400[c]	-	2140[e]	85	2300
Bambara groundnut (Voandzei subterranea)								
seeds	-	10	-	0[d]	-	-	0	10
Bean (Phaseolus vulgaris)								
seeds, dried	90	10	0[d]	0[d]	-	-	0	0
Carrots (Daucus carota)								
roots	1800	5480	-	-	1050[e] 3480[f] 3790[g]	6000[e] 10500[f] 7600[g]	1050	6000
Cassava (Manihot esculenta)								
leaves, fresh	600	11755	0[c]	2800[c]	0[h] 0[i]	3100[h] 9730[i]	0	3000
Cowpea (Vigna spp)								
leaves, fresh	-	7970	0[c]	700[c]	-	-	0	700
leaves, dried	-	-	0[c]	3600[c]	-	-	0	3600
seeds, dried	12	70	0[d]	15[d]	-	-	0	15
Grapefruit (Citrus paradisi)								
fruit, peeled	0	25	-	-	1[g]	248[g]	0	250
Horse radish (Moringa pterygosperma Gaertin), leaves	0	-	-	-	-	7800[i]	0	7800
"Kayeba", leaves, fresh	-	-	0	3500[c]	-	-	-	-
Kidney beans (Phaseolus vulgaris), red seeds	0	10	0[d]	4[d]	-	-	0	4
Maize (Zea mays)								
grain, white	0	5	-	0[d]	-	-	0	0
yellow	90	100	-	124[d]	-	-	0	125
flour, white	0	-	-	0[d]	-	-	0	0
yellow	90	25	-	142[d]	-	-	0	140
Mango (Mangifera indica)								
flesh, ripe	360	3200	-	-	tr[i]	2370[i]	0	2400
unripe	-	1020	-	-	-	58	0	60
Millet (Eleucine spp), grain	0	tr	0	25[c]	-	-	0	25
Oil palm (Elaeis guineensis)								
oil, red	12,000	37,000-128,000	9500[c]	21,000[c]	13,170[i]	52,680[i]	10,000	25,000
Okra (Hibisus esculentus)								
fruit	90	185	0[c]	190[c]	28[g]	432[g]		
leaves, fresh	-	385	0[c]	800[c]	-	2470[i]	0	300
Orange (Citrus sinensis)	18	75	-	-	0[e] 20[g]	730[e] 40[g]	0	730
Papaya (Carica papaya)								
fruit, flesh	600	950	0[c]	300[c]	0[e]	440[e]	0	300
Peas, green (Pisum sativum)	60	15	25[c]	110[c]	- 16[g]	180[h] 557[g]	25	150
Pepper (Capsicum annuum)								
leaves, fresh	-	-	-	-	1050[i]	3500[i]	1050	3500
Pumpkin (Cucurbita moschata)								
squash, flesh	210	3565	1100[c]	1200[c]	0[e] 12[g]	5090[e] 178[g]	1100	1200
leaves, fresh	600	3600	0[c]	1050[c]	0[h]	600[h]	0	1000
dried	-	-	250[c]	9600[c]	-	-	0	9600
Rosella (Hibiscus sabdariffa)								
leaves, fresh	-	4135	-	-	-	2470[i]	0	2500
Sorghum (Sorghum spp), grain	0	10	0[c]	20[c]	-	-	0	20
Sweet potato (Ipomoea batatas)								
yellow, tuber, fresh	60 (0-2400)	1255	0[c]	1800[c]	-	8610[g]	0	1800
leaves, fresh	600	5870	-	-	-	2620[j]	0	2620
white, tuber, dried	-	35	0[c]	75[c]	-	-	0	35

Table 3 (continued)

0 below level of estimation; -, not measured; tr, trace.

a In the original table (10), values are expressed in terms of International Units (IU) vitamin A/100 g edible portion. For ß-carotene, these values have been converted to μg by multiplying by 0.6 and only values for total carotene were available, the values have been converted to μg of "ß-carotene" by multiplying by 0.2.

b For the original table (9), where necessary ß-carotene equivalents were obtained from International Units by using the following factors: 1 IU = 0.3 μg retinol = 0.6 μg ß-carotene = 1.2 μg of other total mixed carotenoids with vitamin A activity.

c Data from present paper.

d Data from previous paper from this laboratory (ref 11).

e-i Data from other laboratories: e, unpublished data from Dr K.L. Simpson (University of Rhode Island, Kingston RI, USA); f, unpublished data from Dr G.R. Beecher (Nutrient Analysis Laboratory, USDA, Beltsville MD, USA); g, ref 21; h, ref 16; i, ref 22; j, ref 24.

DISCUSSION

The large differences between the provitamin A content of foods which were reported in the past with the values reported more recently, including those in this publication, need to be examined carefully. Although considerable variation in the carotene content can be attributed to differences in varieties, maturity, soil fertility, handling and storage of samples and other factors, some authors have reported high values consistently. For example, data on the carotene content of vegetables in East Africa, obtained using methods other than HPLC, by Gomez (23) and McLaren (24) are rather high when compared to the values reported in this article. Overestimation of the values for ß-carotene possibly could be attributed to interference from other compounds such as lutein, chlorophylls and xanthophylls although column chromatography was used to separate ß-carotene from the other components prior to measurement. We have been able to analyse the α- and ß-carotene content of a number of foods taking into consideration the loss of water which may occur during shipment and storage. Fresh cassava and pumpkin leaves for example, contained 5,950 and 2,360 μg of ß-carotene per 100 g on analysis and 44% and 60% of water respectively. However immediately after collection the water content for these products was estimated to be 71% and 82% respectively. Recalculation resulted in the values presented in Table 1.

A number of studies have been carried out to examine loss of ß-carotene on drying. Maeda and Salunkhe (25) reported that from 4.2% to 41.7% of the ß-carotene content of four types of tropical leafy vegetables remained after drying. From their data it would appear that the fresh vegetables analysed had an extremely high ß-carotene content ranging from 8.4 up to 25.5 mg/100 g. Devades et al. (26) reported that 40% of the ß-carotene in drumstick leaves (Moringa oleifera) and fenugreek leaves (Trigoneller foenum-graecum) was lost

on drying. The carotene content of the fresh and dried vegetables was further evaluated by feeding them to school children and measuring the increase in serum retinol values. There was no difference in the response based on the analysed ß-carotene content of the vegetables suggesting that the estimated losses in drying were correct.

It is known that extensive drying of vegetables in open sunshine does destroy part of the ß-carotene, although studies on the subject have been carried out under different conditions and are therefore not comparable. However, the contribution which dried green leafy vegetables can make to the total dietary intake of provitamin A should not be underestimated. Our analysis of dried vegetables showed that considerable amounts of ß-carotene were still present after the traditional drying process. Traditionally some 10-20 grams of dried vegetables might be added to a child's meal. If cowpea leaves were eaten, these could provide 360-720 μg of ß-carotene which would mean that a substantial proportion of the vitamin A requirement could be provided from this source.

For red palm oil high values for ß-carotene were reported from Nigeria (27), especially for oil prepared from the tenera variety. Other carotenoids found in ripe fruit of the oil palm were γ-carotene, ß-zeacarotene, lycopene, neurosporene and phytofluene but all in relatively small amounts, i.e. less than 0.5 mg/100 g and not all these compounds do have vitamin A activity (27). The ratio of α- to ß-carotene found in red palm oil by us was very similar to that reported from the Gambia although the total levels found in the Gambia were higher (22).

Fish liver oil is known to be a rich source of retinol. Twenty five years ago McLaren (24), reported data on the concentration of vitamin A in the livers of fish commonly found at the southern end of Lake Victoria. There was much between-species variability in the vitamin A content, the average content of vitamin A in liver oil from eleven species of fish was 92.6 mg of vitamin A per 100 g oil (range 21.8-170.6 mg). Our results also indicate that the vitamin A content of oil prepared from fat may vary considerably within one species. The extraction procedure does not destroy the vitamin or at least leaves an important amount of retinol and dehydroretinol. The high fat content of Nile perch may make it possible to produce industrially fats and oils, suitable for human consumption and which would provide a reasonable amount of vitamin A.

The manner in which data on food composition in general and on vitamin A in particular are presented requires more attention (28). In many

publications, omission of data on the water content makes it difficult to compare results. This is especially so for products with a high water content such as fresh vegetables. In many food tables, it is customary to assume that 6 μg of ß-carotene and 12 μg of mixed dietary carotenoids are equivalent to 1 μg of retinol (9). Other authors use the term "remaining carotenoids" for the carotenoids other than ß-carotene and assign half of the vitamin A activity of ß-carotene to these "remaining carotenoids" (16). The error introduced by this depends on the proportion of total carotenoids which is ß-carotene, and as this might be less then 30% (15,16), considerable overestimation of the vitamin A activity could be introduced. It would therefore be more appropriate to express analytical data on carotenoid content of foods not in terms of retinol equivalents, but in terms of the content of the individual carotenoids which have been measured. This would allow different factors for the conversion of the amount of ß-carotene and other provitamin A carotenoids into retinol equivalents to be applied as has been suggested by Brubacher and Weiser (29). In fact, little is known about the extent of conversion of various carotenoids to retinol under different conditions. This conversion is influenced by many factors, such as the amount of fat, protein and antioxidants in the diet (30). The recommended equivalence of 6 μg ß-carotene and 12 μg other provitamin A carotenoids with retinol is according to Brubacher en Weiser applicable when the ß-carotene intake is between 1,500 and 4,000 μg and for lower intakes the conversion would be more efficient (29). Other conversion factors for ß-carotene, i.e. 4 instead of 6, were proposed as long ago as 1970 (31) but this suggestion has not yet resulted in modification of the guidelines. Further research in this area is required.

The carotenoid profile of vegetables is much more complex than previously recognized and the proportion of carotenoids as ß-carotene may vary considerably. In this respect it is interesting to note that it has been reported recently that ß-carotene comprises only 15% of the total carotenoids in human serum (32,33). Other authors have reported that the proportion of serum carotenoids which could be identified as ß-carotene was 28% (34). What these data mean in terms of the conversion of various carotenoids to retinol remains to be studied. However, it may be possible with the techniques now available to relate the individual intake of carotenoids to the individual carotenoid profile. Some work was done on this subject 50 years ago with less sophisticated techniques (35).

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REFERENCES

1 Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature 1981;290:201-8.

2 Shekelle RB, Liu S, Raynor Jr WS, et al. Dietary vitamin A and risk of cancer in the Western Electric study. Lancet 1981;ii:1185-90.

3 WHO. Prevention and control of vitamin A deficiency, xerophthalmia and nutritional blindness: proposal for a ten-year programme of support to countries Nut/84.5, Geneva, 1985.

4 FAO. Prevention and control of vitamin A deficiency, xerophthalmia and nutritional blindness FAO contribution to a ten-year UN action programme. Rome: FAO, 1985.

5 Sommer A, Katz J, Tarwotjo I. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 1984;40:1090-5.

6 Sommer A, Hussaini G, Tarwotjo I, Susanto D. Increased mortality in children with mild vitamin A deficiency. Lancet 1983;i:585-8.

7 Official Association of Analytical Chemists, Official methods of analysis, 12th ed. Washington: AOAC, 1980.

8 Simpson KL, Chichester CO. Metabolism and nutritional significance of carotenoids. Ann Rev Nutr 1981;1:351-74.

9 FAO. Food composition table for use in Africa U.S. Department of Health Education and Welfare. Rome: FAO, 1968.

10 Platt BS. Table of representative values of foods commonly used in Tropical countries. London: H.M.S.O., 1962.

11 Schultink JW, West CE, Pepping F. ß-carotene content of Tanzanian foodstuffs determined by high performance liquid chromatography. East Afr Med J 1987; 64:368-71.

12 West CE. Food composition table for use in a research programme on vitamin A deficiency in Tanzania, Interim edition August 1985. Wageningen Agricultural University, 1985.

13 Pepping F, Van der Giezen AM, De Jonge KI, West CE. Food consumption of children with and without xerophthalmia, in rural Tanzania (submitted for publication).

14 Arroyave G, Chichester CO, Flores H, et al. Biochemical methodology for the assessment of vitamin A status. Washington: IVACG/The Nutrition Foundation, 1982.

15 Khachik F, Beecher GR, Whittaker NF. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. J Agric Food Chem 1986;34:603-16.

16 Speek AJ, Temalilwa CR, Schrijver J. Determination of ß-carotene content and vitamin A activity of vegetables by high performance liquid chromatography. Food Chemistry 1986;19:65-74.

17 De Leenheer AP, De Bevere VORC, De Ruyter MGM, Claeys AE. Simultaneous determination of retinol and α-tocopherol in human serum by high-performance liquid chromatography. J Chrom 1979;162:408-13.

18 Brubacher G, Muller-Mulot W, Southgate DAT. Methods for the determination of vitamins in food. New York: Elsevier Applied Science Publ,1985.

19 Schultink JW. Food composition table for use in a research programme on vitamin A deficiency in Tanzania: report of work to improve the quality of data on vitamin A and provitamin A through analysis of selected foods and a search of the literature. Wageningen, Department of Human Nutrition, Rep. 84-49, 1984.

20 Moore T. Vitamin A. Amsterdam: Elsevier, 1957.

21 Bureau JL, Bushway RJ. HPLC determination of carotenoids in fruits and vegetables in the United States. J Food Sci 1986;51:128-30.

22 Villard LF. Vitamin A status in human pregnancy and lactation. PhD Dissertation University of Cambridge, Cambridge, 1985.

23 Gomez MI. Carotene content of some green leafy vegetables of Kenya and effects of dehydration and storage on carotene retention. J Plant Foods 1981;3:231-44.

24 McLaren DS. Sources of ß-carotene and vitamin A in Lake Province, Tanganyika. Acta Tropica 1961;18:78-80.

25 Maeda EE, Salunkhe DK. Retention of ascorbic acid and total carotene in solar dried vegetables. J Food Sci 1981;46:1288-90.

26 Devadas RP, Premakumari S, Subramaniam G. Biological availability of beta-carotene from fresh and dried green leafy vegetables on preschool children. Ind J Nutr Dietet 1978;15:335-40.

27 Ikemefuna J, Adamson I. Chlorophyll and carotenoid changes in ripening palm fruit, Elaeis guineensis. Phytochem 1984;23:1413-5.

28 Beecher GR, Khachik F. Evaluation of vitamin A and carotenoid data in food composition tables. J Nat Cancer Inst 1984;73:1397-1404.

29 Brubacher GR, Weiser H. The vitamin A-activity of ß-carotene. Int J Vit Nutr Res 1985;55:5-15.

30 Moore T. The biochemistry of vitamin A in the general system. In: Morton RA (ed). International encyclopaedia of food and nutrition, vol 9. Oxford: Pergamon Press, 1970:223-45.

31 Rao Nagaswaro C, Rao Narasinga BS. Absorption of dietary carotenes in human subjects. Am J Clin Nutr 1970;23:105-9.

32 Thompson JN, Duval S, Verdier P. Investigation of carotenoids in human blood using high performance liquid chromatography. J Micronut Anal 1985;1:81-91.

33 Thurnham DI, Smith E, Flora PS. Plasma carotenes in the British population. Abstract Fifth European Nutrition Congress, Warschaw 20-23 May 1987. Federation of European Nutrition Societies, 1987:82.

34 Stacewisz-Sapuntzakis M, Bowen PE, Kikendall JW, Burgess M. Simultaneous determination of serum retinol and various carotenoids; their distribution in middle-aged men and women. J Micronut Anal 1987;3:27-45.

35 Lanzing JC. Over de analyse van bloedserumcarotinoïden. Med Dienst Volksgezondheid Ned-Indië 1938;17:213-23.

5. FOOD CONSUMTION OF CHILDREN WITH AND WITHOUT XEROPHTHALMIA IN RURAL TANZANIA

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ABSTRACT

Food intake of 26 children (4-9 years old) of whom nine had xerophthalmic eye lesions (Bitot's spots), was recorded over a period of four days by the precise weighing method during the months of March to June at the end of a short period without rain and during the main rainy season. The principal staple foods were maize and sweet potatoes, while sorghum and cassava were also used as staple foods. The intake of energy was rather low partly due to the bulkyness of the diet. Protein intake was above the recommended intake but mainly derived from vegetable sources with a low biological value. The intake of retinol, ß-carotene, folic acid and iron was low in all children with the children with xerophthalmia having the lowest intakes. Dried green leafy vegetables contributed about 20% of the total ß-carotene intake during the period the survey was carried out.

INTRODUCTION

A prolonged insufficient intake of vitamin A, either as preformed vitamin A (retinol) or as provitamin A (various carotenoids) may ultimately result in a state of vitamin A deficiency leading to xerophthalmia and nutritional blindness (1).

The difficulties met in determining the food intake of individual young children are widely recognized. Detailed reports on the vitamin A intake of children suffering from xerophthalmia are very scarce. Quantitative estimates have been made by Blankhart in Indonesia (2) among healthy children and malnourished children with or without night blindness. The vitamin A intake of the healthy children was one third of the recommended intake and that of the xerophthalmic children less than one fifth. In Indonesia, it has also been found that children with xerophthalmia consumed foods rich in vitamin A and provitamin A less frequently than children without xerophthalmia (3). Recently, qualitative data on the diet of

xerophthalmic children and their controls living in Malawi were published (4). Although remarkable differences were observed in history of breast feeding cases and controls were reported to have similar frequencies of the consumption of common sources of vitamin A. Detailed studies on the food intake of preschool-age children belonging to the Akamba tribe living in a rural area in Kenya have been made (5). The vitamin A intake of children in the first three years of life was about 25% above the recommended intake. No evidence of xerophthalmia was found in these children.

In conjunction with a survey to estimate the prevalence of xerophthalmia, in the western part of Tanzania (6), the food intake of children in a rural area with xerophthalmic eye lesions was compared with control children without xerophthalmia.

BACKGROUND AND STUDY POPULATION

As part of the research programme on vitamin A deficiency of the Tanzania Food and Nutrition Centre (TFNC), nutrition surveys aimed at describing the prevalence of xerophthalmia have been conducted in a number of regions in Tanzania (6,7). In March 1985, 5,266 children were screened for the prevalence of xerophthalmia in Tabora Region. Chronic malnutrition, as indicated by a low height or length-for-age was observed in 15% of the preschool children in Nzega District. A follow-up study was carried out in February 1986 in which some 3,200 children were examined in the areas found in the 1985 survey to be most affected. In four villages in Lusu ward (Nzega District), 16 out of 2,380 (0.67%) of the children examined were found to have Bitot's spots. The group of children included those too young to attend school and those in classes one and two. Four of the 16 children with Bitot's spots were below the age of six years. Thus it was decided to conduct a food consumption study in this ward among children with xerophthalmia and control children matched for sex, age and nutritional status.

Lusu is one of the 37 wards in Nzega District and is typical of low-rainfall areas commonly found in the East African savannah (see map of Tabora Region in Chapter 3). The late rains are expected to start in March but are rather unreliable. Thus there is a risk of drought from December until the next early rains. The ward comprises four main villages; Mwaluzwilo, Bujulu, Ifumba and Mwasala inhabited by some 9,000 people according to the 1985 census. The predominant tribe in the study area are

the Sukuma who are the largest tribe in Tanzania and closely related to the Nyamwezi who are the predominant tribe over most of Tabora Region.

There are five primary schools in the ward and in Mwaluzwilo, there is a dispensary which has a Maternal and Child Health (MCH) unit. The number of deliveries at the MCH unit went up from 100 in 1977 to 240 in 1983, indicating the increased acceptance of delivery outside the home. Mean birthweights could be calculated over a period of nine years for those children born at the MCH unit. Mean birthweights observed in the study area increased between 1977 (n=102) and 1981 (n=155) from 2,720 g to 3,010 g after which the number of deliveries remained fairly constant at about 200 per year while the mean birthweight showed a slight continuous decrease to 2,880 g in 1985 (n=185). These observations are similar to those obtained elsewhere in Tanzania (8,9), although the recent decline in birthweight deserves further investigation.

SUBJECTS AND METHODS

In the surveys, 16 children with Bitot's spots were studied. Of these, only nine could be enrolled in the food consumption study and for each of these children, two controls were selected. The study started at the beginning of March 1986 and lasted for two and a half months. At the end of April two villages, i.e. Mwasala and parts of Ifumba, could no longer be reached by car or bicycle because of the heavy rains. As these villages were more than three hours by foot from the main village, it was not possible to include four suitable children with Bitot's spots in the study. Controls (n=17) were chosen after selection of the cases from the nearest household in the same village. Two cases were brothers, as were two controls, therefore the 26 children studied came from 24 families which had on average 10 members, while the number of family members below the age of 20 years was on average six per family. Sixteen out of the 24 families could be regarded as extended families. Four of the families belong to the Nyamwezi tribe, while 20 belong to the Sukuma tribe.

The method chosen to measure food consumption was the precise weighing method combined with the aliquot sampling technique and the recall method (10). For two periods, each of two consecutive days within a two-week period, records were made of food preparation in each family and the food consumption of the particular child under study. From this the equivalent raw ingredients were calculated. For recording the intake of the evening

meal, the method was modified because it was not always possible for one of the interviewers to be present during this time (around 9 p.m.). Food prepared in the evening for a child included in the study was quantified before being offered to the child and any food left over was measured the next morning. Foods were weighed on a Soehnle weighing scale to the nearest 10 g. Measurement of the food intake was done by two trained nutritionists (AMvdG and KIdJ) assisted by two interpreters who were born and had spent most of their lives in the study area. Relevant information about the families, their agricultural production and living conditions was obtained through a questionnaire and from interviews with local leaders and health personnel. All interviews were done using the local language (Kisukuma).

A number of food samples were collected for the determination of the α- and ß-carotene content by high performance liquid chromatography (HPLC). The values obtained were used in the calculation of the nutrient intake. The following is a list of food items for which these analytical results were used: yellow maize; fresh sweet potato (yellow variety); dried sweet potato (white variety); sorghum; millet; pumpkin squash; fresh green leafy vegetables including amaranthus, cowpea leaves, pumpkin leaves, cassava leaves, and two indigenous green leafy vegetables and dried cowpea leaves. The results of these analyses have been published in detail (11,12). Although the carotene levels found were on average lower than those found in existing food tables the results were in good agreement with other analytical data from Tanzania which had also been determined by HPLC (13).

A comprehensive food composition table incorporating 154 food items was established especially for this project (see Appendix IV). The analytical values as far as these were not available from our own analyses were derived from existing food tables for which preference was given the FAO table for Africa (14). Calculations were made with a computer programme developed for use with an Apple computer (15). For the total study population, the results from six days were not complete and have therefore not been included. Thus results from 98 recording days were available for the final analysis.

RESULTS

General characteristics of the study population are presented in Table 1. The mean difference in age between cases and controls was 3 months.

The principal staple foods in the study area were maize, which was often cultivated in combination with groundnuts and sweet potatoes. Sorghum and

Table 1: Age, sex and nutritional status of nine children with Bitot's spots and 17 controls enrolled in the food consumption study in Lusa Ward (Nzega District Tabora Region), March-May 1986

	Sex		Mean age and range (months)	Nutritional status*		
	Boys	Girls		WH (%)	WA(%)	HA (%)
Cases	7	2	80 (64-108)	95.6	87.6	95.2
Controls	13	4	77 (52-110)	94.1	83.6	93.8

* Nutritional status expressed as the mean of the individual proportion of weight-for-height (WH), weight-for-age (WA) and height-for-age (HA).

some cassava were also used as staple foods, mainly in periods of scarcity while in one village, there was a remarkable preference for the cultivation of rice. On average, the families studied had 2.5 acre of maize, 1.6 acre of sorghum and 1.5 acre of rice under cultivation. The rice surplus was used as a cash crop and three families used their maize surplus for the same purpose. Cotton was grown as a cash crop by three families. For all crops there was only one harvest a year. All families, except one, kept livestock and the number of animals kept per family varied from one chicken to over 200 cows, goats and sheep. Eggs were not consumed but left for breeding, and chicken meat was highly appreciated.

The overall food consumption pattern was that the main meal was prepared early in the afternoon and usually consisted of stiff porridge (ugali) with a relish (mboga) which was often based on groundnuts. The evening meal comprised boiled sweet potatoes, maize cobs, rice or cassava, and was not accompanied by a relish. In the morning, the food left over from the previous evening was eaten.

Mean intake of energy and nutrients measured over four days is presented in Table 2. No differences were found between the results obtained during the first day and the remaining three days when the food intake was measured (data not shown). The intake of energy and most nutrients was practically identical in both groups, taking into consideration the difference of 3 months between the mean age of cases and controls. Carbohydrates provided just over 80% of the energy intake. Three nutrients did not follow the general pattern of a slightly higher intake by the cases. The intake of vitamin A, expressed as the intake of retinol and ß-carotene, and also of folic acid and iron were higher in the control group. This indicates a lack

Table 2: Energy and nutrient intake of children with Bitot's spots (cases, n=9) and their controls (n=17) in Tabora Region*

	Energy (kJ)	Protein (g)	Fat (g)	Carbo-hydrates (g)	Retinol+ (µg)	ß-carotene (µg)	Other provitamin A carotenoids (µg)	Thiamin (mg)	Ribo-flavin (mg)	Folic acid (µg)	Iron (mg)
Cases	6708	50	23	321	26	902	69	1.4	0.9	180	16
	(4407-11321)	(32-88)	(5-55)	(234-533)	(0-132)	(103-3142)	(0-753)	(0.5-2.7)	(0.4-1.4)	(16-383)	(5-25)
Controls	6402	46	22	310	72	994	218	1.3	0.8	253	20
	(3578-9569)	(24-89)	(8-64)	(175-391)	(0-334)	(238-4988)	(0-1823)	(0.5-2.2)	(0.4-1.7)	(29-460)	(8-46)
RDI|											
4-6 years	7600	20			300			0.7	1.1	100	5-10
7-9 years	9200	25			400			0.9	1.3	100	5-10

* Results are expressed as mean values with the range given in parentheses.
\+ Mean intake of total vitamin A activity expressed in retinol equivalents [RE (µg) = retinol (µg) + ß-carotene (µg)/6 + other provitamin A carotenoids (µg)/12] was 182 µg for the cases and 256 µg for the controls. Difference in total vitamin A intake between cases and controls was not significant.
| RDI's from ref 16. Values for vitamin A are expressed in terms of retinol equivalents.

of specific foods containing these nutrients in the diet of the xeroph-thalmic children. The intake of thiamin and folic acid was satisfactory. For thiamin, 78% of the cases and 82% of the controls had an intake which was above the Recommended Daily Intake (RDI), while for folic acid this was the case for 70% and 76%, respectively. The intake of riboflavin was inadequate as only 22% of the cases and 18% of the controls showed an intake above the RDI. For iron the recorded intake was less than 10 mg/day in two cases (22%) and two controls (12%) while the mean haemoglobin levels (± SD) for the two groups were 11.5±1.1 g/dl and 10.8±1.1 g/dl respectively.

Table 3: Frequency of consumption of dairy products and leafy vegetables of children with and without Bitot's spots in Tabora Region

	n	Total survey days	Number of days on which product consumed*					
			Whole milk	Skim milk	Butter	Dried cowpea leaves	Fresh leaves	No leaves, milk or butter
Cases	9	35	8	16	2	10	19	5
			(23%)	(40%)	(6%)	(29%)	(54%)	(14%)
Controls	17	63	23	18	13	24	33	3
			(37%)	(29%)	(21%)	(38%)	(52%)	(5%)

* Number of days on which the products were eaten are given with the proportion (%) of the total number of survey days in parentheses.

The frequency of consumption of some particular foods was investigated in more detail (see Table 3). The control group showed a higher frequency of consumption of whole milk, butter and dried vegetables while skim milk was more frequently consumed by the cases. Despite this more frequent consumption of whole milk and butter the amount of fat consumed did not differ between both groups. Retinol contributed only 14% of the total vitamin A intake of the cases compared to 28% of that of the controls with dairy products (mainly milk) being the most important sources of retinol. This indicates once more the importance of vegetable sources in the provision of vitamin A. The contribution by various products to the ß-carotene intake is presented in Table 4. Leafy vegetables were the main contributors to ß-carotene intake, although for the control group, the contribution was only 57%. Apart from the vegetables listed in Table 4,

Table 4: Contribution of various food products to the ß-carotene intake in children with xerophthalmia (cases, n=9) and controls (n=17)

	Relative contribution, % (range)			
Food product	Cases		Controls	
Leafy vegetables	71	(8-98)	57	(10-90)
Pumpkin	41	(0-98)	18	(0-77)
Amaranthus	6	(0-31)	11	(0-88)
Okra (local variety)	4	(0-19)	6	(0-41)
Dried cowpea leaves	20	(0-75)	22	(0-64)
Staple foods	22	(0-89)	29	(3-88)
Sweet potatoes, yellow variety, fresh	9	(0-85)	9	(0-88)
Sweet potatoes, pale variety, dried	6	(0-19)	15	(0-44)
Maize	7	(0-26)	4	(0-16)
Sorghum	0	(0-2)	1	(0-4)
Cassava	-		1	(0-8)
Vegetables and fruits	3	(0-15)	5	(0-74)
Tomato	2	(0-9)	1	(0-10)
Pumpkin squash	-		4	(0-74)
Okra	1	(0-15)	-	
Dairy products	3	(0-20)	9	(0-41)
Meat	1		0	
Total	100		100	

fresh sweet potato leaves and fresh cucumber leaves were eaten in small amounts. Hence dried leaves were used although fresh leaves were available. Pumpkin squash was only consumed by one child and contributed 74% to his ß-carotene intake over the four-day period. No explanation can be provided to explain the limited consumption of the vegetable. The relatively high contribution of both varieties of sweet potatoes to the intake of ß-carotene of both cases and controls (15% and 23% respectively) is rather surprising. The ß-carotene content of dried sweet potatoes was 75 µg/100 g. Fruits, such as mango and papaya, were not consumed to any extent by the villagers and the huge mango trees, which dominate the landscape for example in Tabora Rural and Urambo District in the southern part of the region are not seen in Lusu.

The method used for drying vegetables varied from household to household. In general, the leaves were first dried in bright sunlight, then cooked, followed by pounding and a second period of drying. The time taken for the various components of the process varied. The first drying period could take from two hours to one and a half day while the second period could take one to two days. Cooking time varied from two to twelve hours.

Data on the extent and quality of maternal care are presented in Table 5. Although it is difficult to draw definite conclusions from the small number of children investigated it would appear that the cases were less well cared for than the controls.

Table 5: Characteristics of children with xerophthalmia (cases) and their controls

	Number of children (proportion, %)	
	Cases (n=9)	Controls (n=17)
Not living with mother	3 (33%)	2 (12%)
Living in extended family	4 (44%)	3 (18%)
Possessing MCH card	3 (33%)	10 (59%)
Proper use of MCH card	1 (11%)	5 (29%)

DISCUSSION

The method used to estimate food intake in this study was a combination of three methods; the precise weighing method, the aliquot sampling technique (food analysis) and the recall method. Each method used to estimate the food intake has its advantages and disadvantages and we acknowledge the problems which may arise when a study team of two persons observes food preparation and food consumption. However, the aim of obtaining quantitative data on food consumption of children with and without xerophthalmia (17) dictated the approach used. The presence of the interviewers did not influence food preparation practices.

Despite the relatively small size of the study population, it would appear that the diet of the children with Bitot's spots lacked those foods which provide retinol, and also folic acid and iron. As cases consumed leaves, milk and butter less frequently than did controls (5% vs 14% of child survey-days respectively), it is suggested that the vitamin A intake could be increased by a higher consumption of these foods.

The absorption of retinol and ß-carotene is known to be reduced when the diet contains less than 5 g fat per day (18). A fat intake of 22 to 23 g/day however should be sufficient to promote the absorption of carotenoids and retinol. The WHO recommended daily intake (RDI) for vitamin A is 300 μg RE for children from 4-6 years and 400 μg RE for those between 7-9 years (16). Only one case and four controls (24%) had an intake above these levels, while four cases (45%) and nine controls (53%) had an intake of less than half of the RDI.

With respect to the Tanzanian standards for energy (19), 14 of the 26 children (5 cases, 56%; 9 controls, 54%) had intakes of energy above the recommended intake, while for the corresponding WHO/FAO/UNU standards (20) only one case (11%) and two controls (12%) reached the recommended intakes. The RDI for vitamin A in Tanzania introduced in 1974 are 660 and 930 μg RE per day for children aged 4-6 and 7-12 years respectively (19). These values are unrealistically high and the RDI for vitamin A should be brought in line with those recommended by WHO.

A very important finding from the food analysis carried out in conjunction with this food consumption study was that dried cowpea and cowpea leaves contained reasonable levels of ß-carotene indicating that the drying process did not render the leaves useless as sources of provitamin A (12). In fact dried leaves provided one fifth of the total ß-carotene intake among the children studied. Information on the consumption of dried leaves has been

reported from other parts of Tanzania (21,22) and from other studies in East Africa such as the use of pigeon pea leaves by the Baganda in Uganda (23).

The present study was carried out during a relatively favorable period of the year, at the end of the short dry period between the two rain periods and during the beginning of the latter of these rainy periods. Main contributors to the vitamin A intake, such as pumpkin leaves and amaranthus leaves, are only available in smaller quantities during other periods of the year (the dry season) when the milk output of the cattle would also be reduced (24). Thus, the contribution of dried green vegetables may even be higher in the dry season as indicated by the findings of a dietary survey carried out on 32 families in Kilombero District (Morogoro Region) in February and August 1983 (25). In that study, the intake of vitamin A for the 4-9 year old children decreased from 1,079 μg/day (n=33) in February to 389 μg/day (n=34) in August.
Green leafy vegetables, mainly cassava leaves and amaranthus, were regularly consumed in February but were replaced by beans and legumes in August. The variation between the relative contribution of various products between the seasons was also clearly demonstrated in a study using a 24-hour recall method carried out in 1982 in Ngara District during February/March and September/ October (26). Generally speaking, pumpkin squash contributed more during the February-March period while vegetables contributed more during the September/ October period. The vitamin A intake was higher in the later period as was found in the study in the Morogoro Region mentioned above (25).

What measures could be recommended in order to increase the vitamin A intake? An important characteristic of the vitamin A intake was the variation between children regardless whether they were cases or controls. This is for example clearly expressed by the fact that although the mean intake of vitamin A was higher for controls, a higher proportion of controls consumed less than half of the RDI when compared with cases. So, under fairly identical circumstances some children received an adequate amount of vitamin A, while others did not consume more than 20-60 μg RE/day. Therefore nutrition education, focussing on existing feeding practices and stressing the use of available foods as groundnuts, milk and vegetables (dried and fresh leafy vegetables and pumpkin squash) seems to have a first priority.

The results of this study in conjunction with the results obtained in the prevalence surveys (6), indicate that uncomplicated xerophthalmia due to a deficient intake of vitamin A, exists in Lusu Ward.

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REFERENCES

1 WHO. Control of vitamin A deficiency and xerophthalmia Report of joint WHO/UNICEF/USAID/Helen Keller International/IVACG meeting Techn Rep Series No. 672. Geneva: WHO, 1982.

2 Blankhart DM. Individual intake of food in young children in relation to malnutrition and night blindness. Trop Geogr Med 1967;19:144-53.

3 Tarwotjo I, Sommer A, Soegiharto T, Susanto D, Muhilal. Dietary practices and xerophthalmia among Indonesian children. Am J Clin Nutr 1982;35:574-81.

4 West KP, Chirambo M, Katz J, et al. Breastfeeding, weaning patterns, and the risk of xerophthalmia in Southern Malawi. Am J Clin Nutr 1986;44:690-7.

5 Van Steenbergen WM, Kusin JA, Voorhoeve AM, Jansen AAJ. Machakos Project Studies IX. Food intake, feeding habits and nutritional state of the Akamba infant and toddler. Trop Geogr Med 1978;30:505-22.

6 Pepping F, Hogeweg M, Mroso DM, West CE. A nutritional survey, with special reference to the prevalence of xerophthalmia in Tabora Region (West Tanzania) (submitted for publication).

7 Pepping F, Kavishe FP, Hackenitz EA, West CE. Prevalence of xerophthalmia in relation to nutrition and general health in preschool-age children in three regions in Tanzania (submitted for publication).

8 Bantje H. Birthweight distribution and antenatal care in Ikwiriri village, Tanzania. Trop Geogr Med 1982;34:213-23.

9 UNICEF. Analysis of the situation of children and women, volume 1 and 2 Government of the United Republic of Tanzania and United Nations Children's Fund (UNICEF). Dar es Salaam, 1985.

10 Marr JW. Individual dietary surveys: purposes and methods. World Rev Nutr Diet 1977;13:105-64.

11 Schultink JW, West CE, Pepping F. ß-carotene content of Tanzanian food stuffs determined by high performance liquid chromotagraphy. East Afr Med J 1987;64:368-71.

12 Pepping F, Vencken CMJ, West CE. Retinol and carotene content of foods consumed in Tanzania determined by high performance liquid chromatography (submitted for publication).

13 Speek AJ, Temalilwa CR, Schrijver J. Determination of ß-carotene content and vitamin A activity of vegetables by high performance liquid chromatography. Food Chemistry 1986;19:65-74.

14 FAO. Food composition table for use in Africa U.S. Department of Health Education and Welfare. Rome: FAO, 1968.

15 Van Poppel G. Menu-T, a program to calculate nutrient intakes TFNC Report No. 888. Dar es Salaam, Tanzania Food and Nutrition Centre, 1984.

16 WHO. Requirements of vitamin A, Thiamin, Riboflavin and Niacin. Report of a joint FAO/WHO Expert Group, FAO: Rome, 1956.

17 Mrisho F, Pepping F, Lukmanji Z. Proceedings of a national symposium for vitamin A deficiency, November 16-18 1981 Dar es Salaam, TFNC Report No. 735. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

18 Olson JA. Recommended dietary intakes (RDI) of vitamin A in humans. Am J Clin Nutr 1987;45:704-16.

19 TFNC. Nutrition specialist Recommended allowances Tanzania TFNC Report No. 282. Dar es Salaam: Tanzania Food and Nutrition Centre, 1974.

20 WHO. Energy and protein requirements Report of a joint FAO/WHO/UNU Expert Consultation Techn Rep Series No. 724. Geneva: WHO, 1985.

21 Maeda EE, Salunkhe DK. Retention of ascorbic acid and total carotene in solar dried vegetables. J Food Sci 1981;46:1288-90.

22 Burgess HJL, Maletnlema TN, Burgess AP. The nutritional status of young children in Hombolo, Tanzania. East Afr Med J 1968;45:605-12.

23 Bennet FJ, Mugalula-Mukiibi AA, Lutwama JSW, Nansubuga G. An inventory of Kiganda foods. Uganda Journal 1965;29:45-53.

24 Van Steenbergen WM, Kusin JA, Onchere SR. Machakos Project Studies VIII. Food resources and eating habits of the Akamba household. Trop Geogr Med 1978;30:393-413.

25 Lukmanji Z, Tanner M. Food consumption patterns in a rural Tanzania community (Kikwawila Village, Kilombero District, Morogoro Region) during lean and post-harvest season TFNC Report No. 940, Swiss Tropical Institute and Tanzania Food and Nutrition Centre, 1985.

26 Bos G. The role of vegetables in a Tanzanian dietary pattern A nutrition study in Ngara District, Tanzania. Department of Human Nutrition Report 83-08, Wageningen Agricultural University, 1982.

6. QUALITY CONTROL OF CLINICAL CHEMICAL ANALYSES IN RESEARCH ON VITAMIN A DEFICIENCY AND XEROPHTHALMIA

F. Pepping, A.E.M.F. Soffers and C.E. West

ABSTRACT

In order to achieve acceptable precision and accuracy of clinical chemical analyses, it is important to introduce and maintain adequate quality control procedures. The results of a quality control programme applied within a research programme on vitamin A deficiency and xerophthalmia showed that the total coefficients of variation obtained with pooled sera were 2.2% for retinol; 3.0% and 4.9% for albumin (two samples); 7.5% for prealbumin, and 4.6% and 5.9% for retinol-binding protein (two samples).

The accuracy of the retinol analysis was examined by inclusion of three external control sera with target values of 0.77, 1.47 and 2.44 µmol/l; the values obtained were 0.94, 1.60 and 2.27 µmol/l respectively.

INTRODUCTION

Quality control plays an essential role in ensuring that the results obtained from biomedical and industrial laboratories are accurate and precise.

As a result of the work of the International Vitamin A Consultative Group (IVACG) and the World Health Organization (WHO) a high degree of uniformity has been achieved in the classification of the eye lesions due to vitamin A deficiency and the interpretation of the proportion of children in a population with these lesions as indicators of public health status (1,2). IVACG has also published a manual on biochemical methodology for the assessment of vitamin A status (3). Although this manual deals extensively with quality control, it is unfortunate that the use of external reference standards has not been developed over the past five years.

In general, the biochemical methods used to assess nutritional status in vitamin A programmes are limited to the determination of retinol, ß-carotene and a number of proteins in serum. The proteins selected for analysis in serum are usually albumin, prealbumin (also called transthyretin),

retinol-binding protein (RBP) and transferrin. Only rarely, is it possible to analyse the vitamin A content of the liver, in which 90% of the body reserves are stored.

In quality control, there are two aspects which need to be considered. The first is the need to achieve "high reproducibility" or "precision". This can be achieved by using a pool serum which is often referred to as an "internal standard". Such a pool serum can be included in every analytical run and if the value obtained for the pool serum is outside well defined limits, often the mean value $\pm$ 2 SD, the results from that run are rejected. Since a high degree of reproducibility does not necessarily ensure that the results obtained represent the "true" value, the second aspect which is important is that of "accuracy" which is a measure of the deviation from what is regarded as the "true" value. However, there must be agreement on what the true value is and which method should be used to establish the true value. Once the reference method has been chosen, general agreement needs to be obtained on what the true value ("target value") for the content of the constituent under discussion is, and on the nature of the material which can be made available to various laboratories. Such material which would have an agreed content of a specific constituent can then be regarded as an "external standard".

In this article we report on the control system established to monitor the quality of biochemical analyses in a research programme on the magnitude and aetiology of xerophthalmia and post-measles blindness, carried out in Tanzania. Steps taken to ensure the quality of analyses for retinol and a number of proteins in serum are presented.

MATERIAL AND METHODS

Introduction

All chemicals were of analytical or chromatographic grade. Retinol was obtained from Fluka AG (CH 9470 Buchs, Switzerland) and the retinyl acetate solution was prepared from USP material (USPC Inc., Rockville, MD 20852, USA).

The monospecific antisera for radial immunodiffusion were obtained from Dakopatts a/s (DK-2600 Glostrup, Denmark). The commercial standards for albumin, prealbumin and retinol-binding protein were from Behringwerke AG (Marburg, FRG). For albumin and prealbumin, stabilized standardized human serum was used while for retinol-binding protein, freeze-dried standard material was reconstituted with destilled water before use.

Estimation of retinol

Since it is generally agreed that high performance liquid chromatography (HPLC) is the reference method, a short description of the method is given. Non-aqueous reversed-phase chromatography following deproteinization with ethanol and extraction with hexane is used in most instances (4,5), while some authors prefer deproteinization with acetonitrile (6).

The method used by us is essentially identical to that described by Driskell et al. (5). The modifications described by the same group of authors, using retinyl acetate as internal standard and ascorbic acid (0.1% w/v) to prevent degradation of retinol, were also applied (7). Serum (200 µl) was deproteinized with an equal volume of absolute ethanol containing a known amount of the internal standard, retinyl acetate and 0.1% (w/v) ascorbic acid. Hexane (700 µl) was added and the samples were mixed for 30 s. After centrifugation for 10 min. at 3200 g, the hexane layer was removed and the solvent evaporated under a stream of nitrogen. The residue was dissolved in 400 µl of ethanol and 50 µl of this solution was injected into the HPLC system.

Six retinol standards ranging from 0.28 - 2.79 µmol/l, were treated in an identical way as the serum samples. A standard curve was plotted with the concentration of retinol on the horizontal axis and the relative peak area of retinol and retinyl acetate on the vertical axis. An internal control was prepared from serum obtained from healthy volunteers. During the preparatory phase this internal control serum was analysed in triplicate in each run. A set of three external control sera was obtained from the Centers for Disease Control (Atlanta, GA 30333, USA). The retinol levels in the three samples were 0.77, 1.47 and 2.44 µmol/l. The external control sera were analysed in duplicate in 6 to 8 runs.

The HPLC system used was built up of a constant metering pump and a variable wavelength detector (LDC/Milton Roy, Riviera Beach, FL 33404, USA), with a Rheodyne (type 7010) injection valve and a 50 µl injection loop. The 10 µm C 18 reversed-phase column (250 x 4.6 mm i.d.) and guard column (75 x 2 mm i.d.) were obtained from Chrompack (Middelburg, The Netherlands). The sample in ethanol (50 µl) was eluted isocratically at a flow rate of 1.5 ml/min using a methanol:water mixture (96:4 v/v) as mobile phase and the absorbance was measured at 325 nm. The retention times for retinol and retinyl acetate were approximately 3.5 min. and 4.5 min. respectively.

Table 1: Total, within-run and between-run coefficient of variation for the analyses of retinol by HPLC and albumin, prealbumin and retinol-binding protein (RBP) by radial immunodiffusion

	Number of runs	Number of determinations	Coefficient of variation (%)			Mean value (μmol/l)*
			Within-run	Between-run	Total	
Retinol	13	40	1.56	1.50	2.16	1.79
Albumin, high	6	12	2.84	1.01	3.01	902
210783	12	24	3.09	3.85	4.93	726
Prealbumin, 210783	20	40	3.22	6.72	7.45	6.07
RBP, 210783	20	40	5.01	3.18	5.93	2.17
241285	20	40	3.56	2.97	4.63	2.32

* Mean values expressed in μmol/l: 1 μmol/l represents 28.5 μg retinol/100 ml, 0.061 g albumin/l, 55 μg prealbumin/ml and 21 μg RBP/ml.

Estimation of proteins

Albumin, prealbumin, and retinol-binding protein (RBP) were analysed using the radial immunodiffusion method as described by Mancini et al. (8). The procedure followed was essentially identical to the one described in the IVACG-manual (15). Four dilutions of a commercial standard were used to plot a standard curve and an internal control serum was analysed in duplicate in every run. Samples (4 μl) of serum or of the standards diluted with phosphate buffer (0.1 M, pH 7.4) in saline were applied to each well punched in the agarose gel.

RESULTS

The results obtained with the internal control sera are given in Table 1 for the four serum constituents under study. Within-run, between-run and total variation, expressed as coefficient of variation (CV), were calculated for the first series of runs in which each sample was analysed in duplicate. For retinol-binding protein and prealbumin the procedure was continued for 20 runs, while for retinol and albumin fewer runs were carried out. For both albumin and RBP, two pooled sera were used.

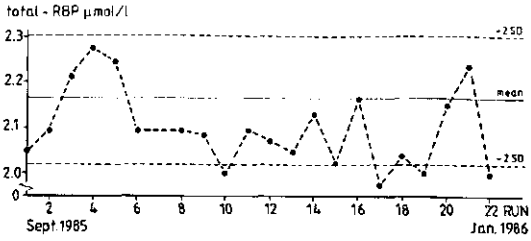

Figure 1: Serum concentration of total-RBP in pooled serum 210783 determined by radial immunodiffusion

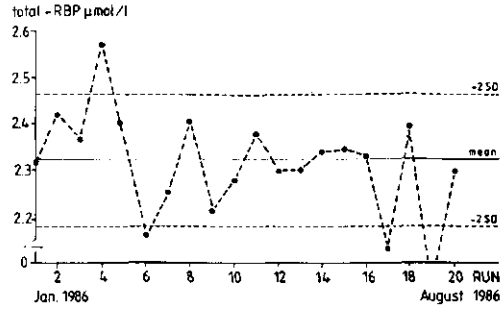

Figure 2: Serum concentration of total-RBP in pooled serum 241285 determined by radial immunodiffusion

The mean values resulting from this procedure are also given in Table 1. The results of the use of these mean values in the analyses carried out subsequently for RBP and retinol are given in Figures 1-3. In these figures the acceptable intervals of plus or minus two standard deviations are also given.

Generally between-run variation is greater than within-run variation. However, in our study this was only true for the second albumin pool and for prealbumin. The between-run variation was relatively high for prealbumin, while the within-run variation for the first RBP-pool was also high.

Table 2. Accuracy of serum retinol analysis estimated by the use of three external control sera

Retinol content of external control (µmol/l)		Number of runs	Number of determinations	Coefficient of variation (%)		
Target*	As measured			Within-run	Between-run	Total
A; 0.77	0.94	8	16	1.72	3.59	3.90
B; 1.47	1.60	6	12	1.50	2.32	2.76
C; 2.44	2.27	6	12	2.68	2.88	3.94

* Target values supplied by Centers for Disease Control, Atlanta GA, USA.

The results obtained from the external control sera analysed for their retinol content are presented in Table 2. The relationship between the observed and target value of the control sera is shown in Figure 4. The regression equation describing the calibration line between both determinations is: $y = 0.79x + 0.37$.

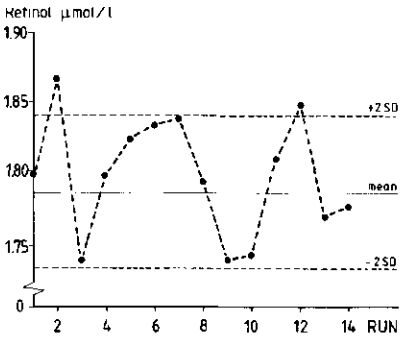

Figure 3: Serum concentration of retinol in pooled serum determined by HPLC

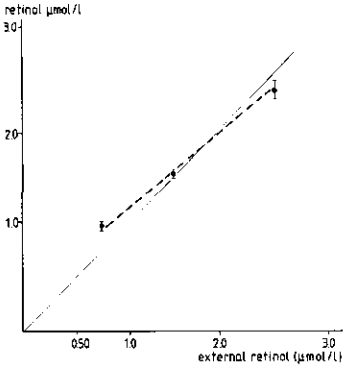

Figure 4: Relationship between observed serum concentration of retinol compared with target values. The dotted and solid lines represent the line of best fit and the ideal line respectively. The bars represent the SE

Strictly speaking this relationship can only be applied within the range covered by the external control sera (0.77 - 2.44 µmol/l). Comparison with the ideal line indicates that the method used in our laboratory slightly overestimates lower values and underestimates higher values (see Figure 4).

DISCUSSION

Taylor (9) stated a few years ago that the time was fast approaching when an analytical result would not be acceptable unless accompanied by a statement of its precision. Unfortunately it must be concluded that this time has not yet arrived in scientific publications dealing with vitamin A deficiency where quality control parameters are not often mentioned. This situation is quite different to research on serum total and HDL-cholesterol concentrations where for many years a quality control system coordinated by the Centers for Disease Control has been in operation (10). This has enabled not only the problem of precision, but moreover of accuracy to be examined (11).

For the analysis of retinol and of ß-carotene by HPLC in human serum, a within-run precision (coefficient of variation) of 2.6-4.8% and a between-run precision of 2.7-5.7% has been reported (6,12). In analysing retinyl esters in liver samples, Amédée-Manesme et al. (13) reported a within-day coefficient of variation of 2.6% and 3.1% and a between-day precision of 3.9% and 5.3%, for samples containing 1 and 15 µg, respectively. According to the within-run and between-run variation, our retinol analyses have a high degree of precision. The variation for the radial immunoassays is in general in accordance with the

goals set for this type of analyses (3). However, the rather high between-run variance for prealbumin needs further improvement.

The accuracy of our method for the analysis of serum retinol was found to be satisfactory. The total, between-run and within-run variation was below 4% for all three (external) control sera used. The subsequent use of an internal pooled serum in our routine analysis showed that it was necessary to repeat two runs. For the analysis of RBP it seemed necessary to replace the internal control serum (no. 210783) after a period of two and a half years by a new internal control serum (no. 241285) after three of the previous six runs had to be rejected.

Without general agreement upon a reference method for the analysis of the parameters discussed in this paper it is impossible to achieve progress in external standardization. Although the establishment of a reference laboratory was recommended at a meeting of IVACG in 1975 (1), very little progress has been made. Quality control sera have been produced on an ad hoc basis by the Centers for Disease Control (CDC) and the National Bureau of Standards (NBS, United States Department of Commerce, Gaithersburg, MD 20899, USA). Sera from CDC were used in the present study while sera from NBS have been analysed by some laboratories in the USA and Europe (14,15). These activities need to be expanded to make readily available supplies of sera with known ("target") concentrations of retinol, RBP (perhaps holo-RBP as well as total-RBP), prealbumin and ß-carotene. Special attention should be paid to providing sera with low levels of retinol and RBP (<0.35 μmol/l).

REFERENCES

1 IVACG. Guidelines for the eradication of vitamin A deficiency and xerophthalmia. Washington:The Nutrition Foundation, Inc., 1977.

2 WHO. Control of vitamin A deficiency and xerophthalmia Report of joint WHO/UNICEF/USAID/Helen Keller International/IVACG meeting Technical Report Series No. 672. Geneva: WHO, 1982.

3 Arroyave G, Chichester CO, Flores H, et al. Biochemical methodology for the assessment of vitamin A status. Washington: IVACG/The Nutrition Foundation, 1982.

4 Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr 1979;32:2143-9.

5 Driskell WJ, Neese JW, Bryant CC, Bashor MM. Measurement of vitamin A and vitamin E in human serum by high performance liquid chromatography. J Chrom 1982;231:439-44.

6 Nelis HJLF, De Roose J, Vandenbaviere H, De Leenheer AP. Nonaqueous reversed-phase liquid chromatography and fluorimetry compared for determination of retinol in serum. Clin Chem 1983;29:1431-4.

7 Driskell WJ, Bashor MM, Neese JW. Loss of vitamin A in long-term stored, frozen sera. Clin Chem Acta 1985;147:25-30.

8 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235-54.

9 Taylor IS. Analytical quality assurance in good laboratory practice. Chemistry in Australia 1983;50:82-6.

10 Lippel K, Ahmed S, Albers JJ, Bachorik P, Muesing R, Winn C. External quality control survey of cholesterol analyses performed by 12 lipid research clinics. Clin Chem 1978;24:1477-84.

11 Cooper GR. The World Health Organization-Center for Disease Control Lipid Standardization Program. In: Amido G, Van Kampen EJ, Rosalli SB, Rubin M, eds. Quality control in clinical chemistry. Berlin: De Gruyter, 1975:95-109.

12 Driskell WJ, Bashor MM, Neese JW. Beta-carotene determined in serum by Liquid Chromatography with an internal standard. Clin Chem 1983;29:1042-4.

13 Amédée-Manesme O, Furr HC, Olson JA. The correlation between liver vitamin A concentrations in micro-(needle biopsy) and macrosamples of human liver specimens obtained at autopsy. Am J Clin Nutr 1984;39:315-9.

14 Thurnham DI, Smith E, Flora PS. Measuring plasma carotenes in the British population Abstract Fifth European Nutrition Congress, Warsaw 20-23 May 1987, Federation of European Nutrition Societies, 1987:82.

15 Stacewisz-Sapuntzakis M, Bowen PE, Kikendall JW, Burgess M. Simultaneous determination of serum retinol and various carotenoids; their distribution in middle-aged men and women. J Micronut Anal 1987;3:27-45.

7. THE ROLE OF NUTRITIONAL STATUS WITH SPECIAL REFERENCE TO VITAMIN A IN THE DEVELOPMENT OF POST-MEASLES EYE LESIONS

I. NUTRITIONAL STATUS

F. Pepping, E.A. Hackenitz, and C.E. West.

ABSTRACT

A hospital-based study was carried out in Dar es Salaam on 665 children with measles and 176 controls. Nutritional status was assessed by anthropometric indices in more than 95% of the children in both groups and serum levels of retinol, retinol-binding protein (RBP), prealbumin and albumin were determined in 32% of the children with measles and 40% of the controls. Malnutrition, as assessed by a weight-for-height measurement of less than 80% of the reference standard, was observed in 39.8% of the children with measles. As children were admitted at different stages of measles, the relationship between nutritional status and stage of measles was examined. Weight-for-age and length-for-age were lower in children admitted at a later stage after eruption of the rash. Serum levels of all constituents studied were significantly lower in measles children than in controls and the reduction was most marked for retinol (56%). Deficient serum retinol levels were observed in 56.5% of the measles children and in 1.8% of the controls. Levels of albumin in serum were significantly lower in malnourished children (weight-for-age < 80%) with measles than in well nourished (≥ 80%) children with measles. After appearance of the measles rash levels in serum of albumin declined profoundly while there was also a decline in serum levels of retinol and RBP.

INTRODUCTION

The detrimental role of measles on childhood mortality and morbidity has been recognized for many years (1). Among the complications observed during measles are corneal eye lesions and the aetiology of these lesions is still under discussion. The role of vitamin A in this process has been highlighted by some authors (2,3), while other authors regard the eye lesions as an outcome of the measles infection itself (4). Secondary infections, for example herpes virus, have also been incriminated in this process (5,6). From studies carried

out in Africa and India it has been suggested (7) that the proportion of children with measles who develop serious eye lesions is higher, and that the lesions are more severe in Africa than in India (8-10).

A current hypothesis suggests that measles and malnutrition interact to lower the immunological resistance against secondary infection. This effect, together with the viral measles keratitis which may occur in every child, and the epithelial damage due to vitamin A deficiency, facilitate the invasion of bacteria and viruses. This would explain the occurrence of the so-called "post-measles eye lesions" often leading to partial or total blindness (10,11).

The role of measles as a cause of blindness in African children has been recently outlined by Foster and Sommer (12), who concluded that measles infection is the precipitating factor in the majority of bilateral corneal ulcers and resulting blindness. In community-based studies recently carried out in Tanzania we were able to confirm this finding (13).

We here present the results of hospital-based research carried out in and around Dar es Salaam, the capital of the United Republic of Tanzania. The main objective of this research was to investigate the role of nutritional status, specifically of vitamin A, in the development of eye lesions in children with measles. Anthropometric data and serum levels of retinol and three serum proteins in children with measles and their controls are presented in this article. In a second article, the relationship between eye lesions and other clinical complications observed will be discussed in relation to nutritional status (14).

BACKGROUND TO THE PRESENT STUDY

The present study formed part of a comprehensive programme on vitamin A deficiency, xerophthalmia and post-measles blindness established by Tanzania Food and Nutrition Centre. This programme was the outcome of a national meeting on vitamin A deficiency held in Dar es Salaam in 1981 (15).

In Tanzania, the incidence of measles follows a seasonal pattern, the peak season occuring at different times in various parts of the country. In the Dar es Salaam area, the incidence rises from September onwards and declines in February/March and, although the incidence is relatively low during the main rainy season (March-June), children may present with measles throughout the year. The City of Dar es Salaam, where the study was carried out comprises three districts, i.e. Temeke, Kinondoni and Ilala, each of which is served by a district hospital. Patients were recruited from two of the three district

hospitals (Temeke and Kinondoni). Other participating hospitals were Muhimbili Medical Centre (the university hospital which serves as a referral centre) and the Aga Khan Hospital, a private hospital serving the urban middle and upper class and providing medical care for employees and families of a number of industrial companies. A rural district hospital situated 30 km west of the capital (Kibaha Designated District Hospital) was also included in the study.

Data collection was carried out by a study team (see acknowledgements) which included an ophthalmologist (DMM), a dietician (AB, MN), either one or two medical laboratory technicians (VA, RK, AK, JM, GM, SD) and a nutritionist (EAH, FP). The team visited the participating hospitals every two to three days.

SUBJECTS AND METHODS

Two studies were carried out during periods when the prevalence of measles was high. A pilot study from December 1983 to March 1984 was followed by the main study which was carried out between October 1984 and March 1985. Permission to carry out the study was obtained from the Director of City Health Services in Dar es Salaam and the study design was approved by the hospital authorities concerned. Verbal consent of the mother or guardian was obtained in every case.

Hospitals. The pilot study was carried out in a single hospital (Temeke), while the main study was extended to the five hospitals mentioned earlier. Most of the measles cases examined were admitted to Temeke District Hospital (78.9%), while 7.8% were from Muhimbili Medical Centre, 7.1% from Kibaha, 6.5% from Mwananyamala Hospital (Kinondoni District), and 2.8% from the Aga Khan Hospital. Half of the children with measles studied came from two densely populated town quarters (Temeke and Tandika) where middle and lower class workers live. Control (i.e. measles-free) children were selected from the maternal and child health clinic at Temeke Hospital (82.4%), paediatric outpatients at Muhimbili Medical Centre (8.5%) and children attending a nutrition rehabilitation unit at Kibaha (9.1%). None of the controls had received a high dose of vitamin A as part of the treatment. Five children initially included in the control group were excluded from data analysis because they had suffered from measles within eight weeks before they were studied.

Clinical examination. Children with measles were enrolled in the study immediately after admission. The clinical examination was carried out by the

medical officer (usually a paediatrician) in charge of the measles ward. A questionnaire designed for this study included identity, age, anthropometry, feeding pattern, details of the clinical examination including complications, eye examination, collection of blood samples and treatment given. Of a total of 841 children studied, 665 (79.1%) had measles (cases), and data on weight were absent on 30 children (28 cases and two controls). All the questionnaires were scrutinized twice for completeness and inconsistencies and missing observations were taken from the hospital records. Children originally admitted with suspected measles but who failed to develop an unequivocal rash were excluded from the study. Standard treatment in the five hospitals varied little, and included prophylactic antibiotics, antimalarials and antipyretics. Food was supplied by the parents in most hospitals.

Anthropometry. Weight and length were measured using standard methods (16). Nutritional status was estimated in terms of deficit of weight-for-length, weight-for-age, and length-for-age. For weight-for-length, 80% of the reference standard was used as cut-off point to identify malnutrition (17), while for weight-for-age 90%, 75% and 60% of the reference standard (18) were used as cut-off points for diagnozing grade 1,2 and 3 malnutrition respectively (19); children with a weight-for-age greater than 90% of the reference were classified as normal. Data were expressed as proportion (percent) of the reference standard in each case.

Biochemical analysis. Blood samples were obtained from the anticubital or femoral vein. In order to ensure compliance from parents and professionals children below the age of six months (n=15), those who were very sick or malnourished, and those selected for blood transfusion or on an intravenous drip were excluded from blood sampling. Albumin, prealbumin and total retinol-binding protein (RBP) levels were determined by radial immuno-diffusion (20). Retinol was analysed in Wageningen by high performance liquid chromatography (HPLC) as decribed by Driskell et al. (21,22). Details of the analytical procedures and of the internal and external quality control applied are decribed elsewhere (23). Serum levels are expressed in S.I. units in which 1 μmol/l represents 28.5 μg retinol/100 ml, 21 μg RBP/ml, 55 μg prealbumin/ml and 0.061 g albumin/l.

Statistical analysis. Chi-square and Student's t-test were used for evaluation of differences between groups. Differences in biochemical parameters between measles children and controls were adjusted for age in a multiple regression model.

RESULTS

Description of subjects

The number of children enrolled in both studies, the sex and age distribution and the number on whom anthropometric measurements and blood sampling was carried out are presented in Table 1. The fatality rate among measles cases was at least 7.5% (47/624, nine children absconded and 32 were transferred to another hospital). The age profile of measles cases and controls and the proportion of children in each age group from whom blood samples were obtained are presented in Figure 1. Of the measles cases, 35.9% were below the age of 12 months against 44.0% of the controls while 18.0% of the cases and 8.6% of the controls were aged 36 months or above.

Table 1: Age, sex distribution and number of children for whom anthropometric data are available and from whom blood was collected during two studies on post-measles eye lesions in Dar es Salaam (December 1983-March 1985)

	Mean age (mo., range)	Number of children				
		Total	Boys	Girls	Anthropometric examination	Blood examination
Cases						
Pilot study	23 (4-108)	105	56	49	101	40
Main study	22 (2-102)	560	283	277	536	163
Total		665	339	326	637*	203
Controls	17 (2-88)	176	94	82	174	70

* The length of 57 children with measles and one without measles was not recorded.

Of those children with measles who were above the age of nine months (the recommended age for immunization in Tanzania), 31% were reported to have received measles vaccination compared with 68% of the control group. On admission 7.9% of the children were diagnozed as prodromal cases because of the appearance of Koplik's spots and 66.3% showed a maculopapular rash, while 21.3% showed a desquamating rash and in 4.6% of the children the skin showed dark dry spots ("black measles").

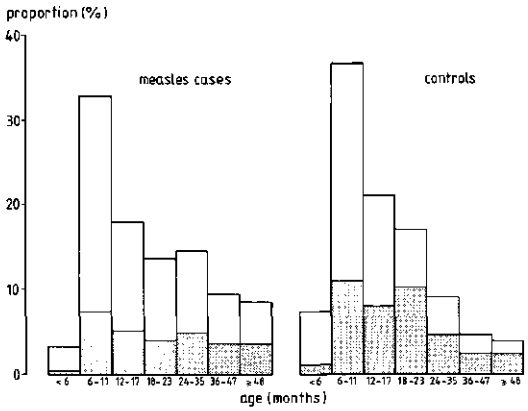

Figure 1: Age distribution of children with measles (cases) and controls. The shaded areas represent a subsample of children for which biochemical data are available

Table 2: Distribution according to age, of nutritional status expressed in terms of the proportion of standard weight-for-length (WL) in children with measles, and in controls

Age (months)	Distribution of nutritional status of children											
	Cases						Controls					
	< 80% WL		⩾ 80% WL		Total		< 80% WL		⩾ 80% WL		Total	
	n*	%	n	%	n	%	n	%	n	%	n	%
< 12	51	24.4+	158	75.6	209	36.0	4	5.3	72	94.7	76	43.9
12-23	89	49.7	90	50.3	179	30.9	3	4.5	63	95.5	66	38.2
24-35	40	46.0	47	54.0	87	15.0	2	12.5	14	87.5	16	9.2
⩾ 36	51	48.6	54	51.4	105	18.1	1	6.7	14	93.3	15	8.7
	231	39.8	349	60.2	580		10	5.8	163	94.2	173	

* n = Number of children and the proportion (%) in percent.
\+ Comparison of malnutrition below and above 12 months of age (X^2=31.4,p<0.001).

Feeding pattern and nutritional status

The younger age of the controls (see Table 1) is reflected in the higher proportion of the controls (73.5%) still being breastfed compared with the measles cases (61.7%). Among children under 24 months of age, the prevalence of breastfeeding was virtually identical in measles cases (88.4%) and controls (89.1%). In the same age group, 99.3% of the control children received solid foods compared with 86.8% of the measles cases. A change in the feeding pattern of the measles cases was reprted by 26% of the mothers. In most cases this

change meant that only water and/or other fluids were given.

Malnutrition (<80% of the reference weight-for-length) was observed in 39.8% of the children with measles against 5.8% among the controls (Table 2). Malnutrition in children with measles was more prevalent in children 12 months of age and above compared with those aged less than 12 months ($p<0.001$). Using weight-for-age as indicator of malnutrition, moderate and severe malnutrition (grade 2 and 3) were identified in 43.3% and 10.2% of the measles cases, and in 17.2% and 4.6% of the controls, respectively.

Children were admitted at different stages of measles infection. The relationship between nutritional status expressed as the proportion of the respective reference standards and stage of measles, indicated by the number of days since the appearance of the rash, is illustrated in Figure 2. Weight-for-age fluctuated more than the other indices and there was a significant difference in the mean weight-for-age and length-for-age when children admitted within six days after the eruption of rash were compared with those children admitted later ($p<0.01$). There was no influence of age on this difference.

When data were analysed according to hospital, the nutritional status of children with measles studied at the Aga Khan Hospital was better than of those studied at the other hospitals. Those studied at Muhimbili Medical Centre were in the least favorable condition. The proportion (mean±SD, %) of the reference weight-for-length was 88.7±11 (n=19), 82.8±10 (n=523) and 76.8±15 (n=38) for the Aga Khan Hospital, Temeke/Mwananyamala/Kibaha Hospitals, and Muhimbili Medical Centre, respectively.

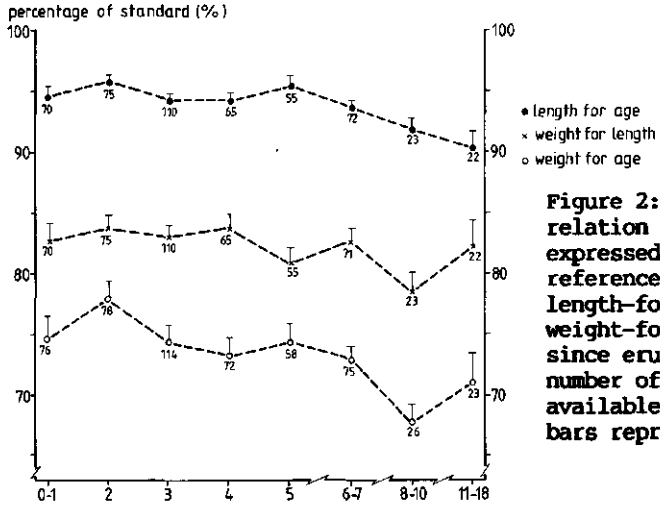

Figure 2: Nutritional status in relation to stage of measles expressed as proportion (%) of the reference standards for length-for-age, weight-for-length and weight-for-age, and number of days since eruption of measles rash. The number of children for whom data are available is indicated. The vertical bars represent the SE.

Biochemical determinations

Blood samples were taken for biochemical analyses from 32% of the children with measles and from 40% of the controls for whom data on nutritional status were available. The nutritional status of the children with measles from whom a blood sample was taken did not differ from those for whom no sample was taken. Those in the control group from whom blood was sampled had a lower mean weight-for-length compared with those not sampled ($p<0.01$).

The mean serum levels of the four biochemical parameters studied according to age and nutritional status are shown in Table 3. The serum levels of retinol, RBP, prealbumin and albumin were significantly lower in measles cases than in controls ($p<0.001$) and these differences were very similar after adjustment for age in a multiple regression model. The reduction of serum levels associated with measles varied from 16% for albumin, 38% for prealbumin and RBP, and up to 56% for retinol. In the measles group, retinol and albumin levels were lower in children older than 24 months compared with levels in younger children ($p<0.05$). No influence of age on serum levels was found in the control group. Serum retinol levels generally regarded as deficient (< 0.35 μmol/l or 10 μg/100 ml) were observed in a much larger proportion (56.5%, 96/170) of children with measles than in controls (1.8%, 1/55). Extremely low serum retinol levels (< 0.17 μmol/l) were observed in 14.7% (25/170) of the measles patients and in none of the controls. Low albumin levels (< 500 μmol/l or 30.5 g/dl) were recorded in 64.6% of the measles cases against 25% among the controls.

Serum albumin, RBP and retinol levels were lower in measles patients with a deficit in weight-for-length (wasting). After adjustment for age, the difference between wasted and non-wasted children was 43 μmol/l ($p=0.01$) for albumin, 0.07 μmol/l ($p=0.08$) for RBP, and 0.02 μmol/l ($p=0.33$) for retinol, respectively. There was no effect modification of age.

Blood samples were collected immediately after admission, and samples from half of the cases were collected within four days of the appearance of rash. The relationship between serum levels of retinol and serum proteins and the time elapsed since eruption of the rash is illustrated in Figure 3. Albumin levels showed a steady and continued decline as characterized by the regression equation: $y = -10.8x + 532$ (where y is the albumin level in μmol/l and x is the number of days after the appearance of the rash, n=127; significance of b, $p<0.01$). Serum retinol, RBP and prealbumin levels fell in the first days after

Table 3: Serum concentration of retinol and serum proteins in measles children according to age and nutritional status, and in controls

Age (months)	Concentration in serum (mean ± SE, µmol/l)			
	Cases*			Controls
	< 80% WL	⩾ 80% WL	Total	Total
Retinol				
< 12	0.38±0.05 (9)	0.47±0.05 (32)	0.44±0.04 (43)	0.85±0.08 (18)
12-23	0.38±0.05 (26)	0.36±0.05 (19)	0.36±0.03 (53)	0.79±0.04 (24)
24-35	0.31±0.05 (14)	0.34±0.06 (17)	0.33±0.04 (31)	0.83±0.10 (7)
⩾ 36	0.30±0.04 (18)	0.30±0.04 (20)	0.30±0.03 (43)	0.83±0.11 (6)
Total	0.34±0.03 (67)	0.38±0.03 (88)	0.36±0.02(170)+	0.82±0.04 (55)
Retinol-binding protein				
< 12	0.57±0.06 (12)	0.71±0.07 (29)	0.67±0.05 (43)	0.91±0.10 (12)
12-23	0.65±0.07 (21)	0.67±0.05 (21)	0.61±0.04 (50)	1.12±0.07 (28)
24-35	0.60±0.06 (10)	0.67±0.09 (13)	0.64±0.06 (23)	1.07±0.12 (6)
⩾ 36	0.57±0.06 (13)	0.64±0.08 (18)	0.60±0.05 (36)	0.96±0.10 (8)
Total	0.60±0.03 (56)	0.68±0.04 (81)	0.63±0.02(152)	1.04±0.05 (54)
Prealbumin				
< 12	1.7±0.1 (12)	2.0±0.2 (34)	1.9±0.1 (48)	3.2±0.3 (20)
12-23	1.8±0.1 (26)	1.7±0.1 (22)	1.8±0.2 (56)	3.0±0.2 (31)
24-35	2.0±0.2 (14)	1.9±0.1 (15)	1.9±0.2 (29)	3.0±0.3 (8)
⩾ 36	1.9±0.3 (18)	1.9±0.1 (21)	1.8±0.2 (44)	3.5±0.4 (7)
Total	1.9±0.1 (70)	1.9±0.1 (92)	1.9±0.1 (177)	3.1±0.1 (66)
Albumin				
< 12	468±26 (12)	497±16 (28)	489±13 (42)	573±29 (11)
12-23	481±20 (21)	509±27 (20)	486±15 (49)	566±19 (28)
24-35	428±30 (10)	451±24 (13)	441±18 (23)	526±71 (5)
⩾ 36	400±27 (11)	503±31 (17)	458±20 (33)	563±43 (7)
Total	452±12 (54)	494±12 (78)	472±8 (147)+	563±15 (51)

* Nutritional status is expressed in terms of the proportion of standard weight-for-length (WL). The number of children studied is given in parentheses. No data on weight or length were available for 15 children.

\+ $p<0.05$ for comparison between serum levels in children above and below 24 months of age.

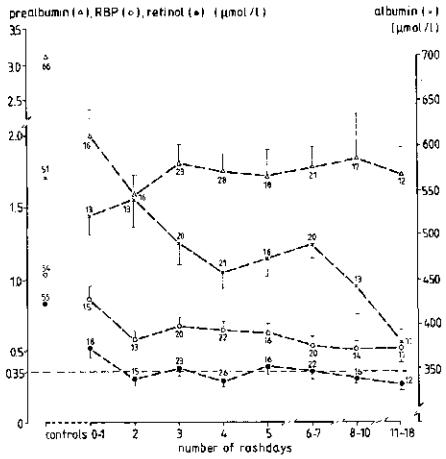

Figure 3: Serum concentration of retinol, RBP, prealbumin and albumin in relation to time elapsed (in days) since eruption of measles rash. The number of children for which data are available is indicated. The vertical bars represent the SE

the appearance of the rash. Samples collected within two days of the outbreak of the rash showed higher levels for retinol and RBP than those collected later ($p<0.01$).

The molar ratio (mean±SD) of retinol:RBP was 0.57±0.24 (n=137) for measles cases and 0.76±0.20 (n=42) for the controls. Thus, in both groups there was a molar excess of RBP and the magnitude of this excess of RBP as represented by a lower ratio of retinol to RBP was greater in children with measles than in control children. The ratio was not significantly lower in malnourished children than in well nourished children.

DISCUSSION

We have presented cross-sectional data on the nutritional status of children hospitalised with measles. Difficulties in interpretation of such data are acknowledged but longitudinal studies on children with measles (24-26) and particularly those including biochemical investigations before and after infection are rare (10).

Our data suggest that measles in Dar es Salaam is seen in very young children. Of the 665 children with measles in Dar es Salaam, 35.9% contracted the disease before the age of 12 months. It is recognized that children contract measles at a younger age in areas with high population density compared with rural, less densely populated areas (27). In two other studies carried out recently in Tanzania outside the capital city, the proportion of children with measles below the age of 12 months was lower: 20.6% of 188 children studied at Mvumi (28) which is in a rural area and 25.7% of 913 children studied at the regional hospital in Mbeya (29) which serves both a large urban area and the

surrounding rural area.

Because the children in the control group were derived mainly from children attending a MCH clinic, the proportion of children vaccinated against measles, 68%, cannot be regarded as representative of the overall measles vaccination coverage in Dar es Salaam and surrounding areas. Vaccination against measles did not by any means convey total protection against infection. In fact, 31% of the children with measles had a record of vaccination against measles, which calls in question the effectiveness of measles vaccination in these children.

Of the children with measles, 39.8% were malnourished (WL <80%). However in order to enable a comparison with other studies, weight-for-age should be used as indicator of nutritional status. In our studies, 10.2% of the children with measles were clinically severely malnourished (WA < 60%). This proportion was slightly less than that reported by Barclay et al. (13.9%, ref 28) and practically identical to that observed by Burgess et al. (10.9%, ref 29).

In India, Reddy et al. (10) observed an average weight loss of 0.4 kg in 142 children with measles and an increase from 5.2% to 13.0% in the prevalence of grade 3 malnutrition in 307 children. However the effect of dehydration, and the resulting weight loss, on assessment of nutritional status based on weight-for-age or weight-for-length cannot be ignored in the severely ill child (30).

When the Waterlow nutritional classification (17) was applied to our anthropometric data, 18.5% of the measles cases were significantly stunted. Of these children the majority (10% of the total) had an acceptable weight-for-length ($\geq$ 80%) while the remainder (8.5% of the total) were also wasted. The proportion of stunted children was similar in the controls (18.5%) but only 2.3% were also wasted while 16.2% were stunted only. The control group was on average younger than the measles group. Nevertheless nutritional status was poorer in measles children compared with age-matched controls. When our data are compared with other community data in Tanzania and taking into account the rather young age of our study population, it seems reasonable to assume that the children studied here were similar in pre-measles nutritional status to the general population (13). By inference this would also be true for the subsample for whom biochemical data were available.

The nutritional status of the children varied between the hospitals. The relatively better condition of the children from the Aga Khan Hospital is probably related to higher social class but it was not possible to test this statistically. As Muhimbili Medical Centre is a referral hospital, it tends to attract children with more severe medical problems which probably explains why the children admitted there had a relatively poorer nutritional status.

Levels of retinol and serum proteins in the control children were generally satisfactory. One boy in the control group did have a serum retinol level indicating vitamin A deficiency (0.32 μmol/l). He was aged eight months, not malnourished, still being breastfed, and had no complications and an adequate serum albumin level (623 μmol/l). His prealbumin level was rather low (1.75 μmol/l).

The effect of measles on biochemical parameters could be considered in two ways: as differences between children with measles and controls or as changes over time after appearance of the measles rash. Infection with measles was associated with a fall in the mean level in serum of retinol and the three serum proteins studied. This confirms the results of previous studies (10,11).

In his study in Kenya (4), Dekkers observed low serum values for albumin in the children with measles and with severe malnutrition, while in this study and in the work carried out in Hyderabad (8,10) serum albumin levels in severely malnourished children with measles were not much lower than those in children with measles and a more adequate nutritional status. It may well be that the population studied by Dekkers had a high proportion of children with chronic malnutrition characterized by low levels of serum albumin.

No explanation can be provided for the lower levels of albumin and retinol in serum of children 24 months of age and older compared with younger children. The difference was only seen in the children with measles.

Cross-sectional and longitudinal data collected in a community-based study in India (10) indicated that measles depressed retinol levels by about 0.21 μmol/l (6 μg/100 ml) while in this study, the decline was on average 0.46 μmol/l. The fact that mild xerophthalmia was highly prevalent in the Indian community studied, may explain this difference. As shown earlier by Inua et al. (11), these effects are greater than those produced by malnutrition alone.

The change in serum concentration of retinol and the three serum proteins following the appearance of the measles rash can be interpreted as a time course and the levels in control children could be regarded as pre-infection levels (Figure 3). From such an interpretation it would appear that the decline in levels of retinol, RBP and prealbumin is over within two days while the decline in the level of albumin in serum continues for the entire period for which data are presented. Such results were not found by Inua et al. (11) but there were fewer observations in that study. Prealbumin and RBP can be regarded as negative acute phase proteins, the synthesis of which is rapidly reduced by the measles infection. Albumin has a much slower metabolic turnover and therefore its concentration in serum reacts more slowly to changes in its rate of synthesis or

removal from the circulation. In children with measles, there was a higher molar excess of RBP associated with retinol than in the children without measles. It is difficult to interpret this observation although it may represent on increased uptake of retinol by target tissues. The work of James et al. (31), indicated that the release of retinol from the liver following the injection of a water-miscible preparation of retinyl palmitate was not inhibited by measles. However further understanding of the basic biochemical processes involved requires further study possibly involving the use of animal models. The additional effect of deteriorating nutritional status must be taken into consideration when interpreting these findings. Our anthropometric data demonstrate a gradual deterioration in nutritional status in the immediate post-measles period (evidenced by a fall in mean weight-for-age). A further complicating factor is likely to be that children with pre-existing stunting were more heavily represented among the children admitted more than five days after eruption of the measles rash (i.e. post-measles debility).

We have demonstrated that age, nutritional status and stage of measles all exert an effect on serum levels of retinol and serum proteins. In an accompanying article, we show that these factors also play a role in the appearance of the cornea (14). Conflicting accounts in the literature on the effects of measles on nutritional status may be explained by differences in age distribution, severity of malnutrition and, most importantly, the stage of measles at which children were studied. This is especially so for data on serum levels of albumin but is also true to a lesser extent for retinol, RBP and prealbumin levels.

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REFERENCES

1 Morley DC. Severe measles in the tropics. Br Med J 1969;i:297-300.

2 Franken S. Measles and xerophthalmia in East Africa. Trop Geogr Med 1974;26: 39-44.

3 Sauter JJM. Xerophthalmia and measles in Kenya. Groningen: Drukkerij van Denderen, 1976.

4 Dekkers NWHM. The cornea in measles. Den Haag: Junk Publishers, 1981.

5 Whittle HC, Sandford-Smith J, Kogbe OI, Dossetor J, Duggan MB. Severe ulcerative herpes of mouth and eyes following measles. Trans Roy Soc Med Hyg 1979;73:66-9.

6 Foster A, Sommer A. Corneal ulceration, measles, and childhood blindness in Tanzania. Br J Ophthalmol 1987;71:331-43.

7 Pepping F, Hackenitz EA, West CE, Duggan MB, Franken S. Relationship between measles, malnutrition and blindness: a prospective study in Indian children. Letter to the editor. Am J Clin Nutr 1987 (in press).

8 Bhaskaram P, Reddy V, Raj S, Bhatnagar RC. Effect of measles on the nutritional status of preschool children. J Trop Med Hyg 1984;87:21-5.

9 Bhaskaram P, Mathur R, Rao V, et al. Pathogenesis of corneal lesions in measles. Hum Nutr:Clin Nutr 1986;40C:197-204.

10 Reddy V, Bhaskaram P, Raghurumulu N, et al. Relationship between measles, malnutrition, and blindness: a prospective study in Indian children. Am J Clin Nutr 1986;44:924-30.

11 Inua M, Duggan MB, West CE, et al. The role of vitamin A, malnutrition and measles in post-measles corneal ulceration in children in Northern Nigeria. Ann Trop Paediatr 1983;3:181-91.

12 Foster A, Sommer A. Childhood blindness from corneal ulceration in Africa: causes, prevention and treatment. Bull Wrld Hlth Org 1986;64:619-23.

13 Pepping F, Hogeweg M, Mroso DM, West CE. A nutritional survey, with special reference to the prevalence of xerophthalmia in Tabora Region (West Tanzania) (submitted for publication).

14 Pepping F, Hackenitz EA, Mroso DM, Franken S, West CE. The role of nutritional status with special reference to vitamin A in the development of post-measles eye lesions II. Eye lesions, and other clinical complications in relation to nutritional status (submitted for publication).

15 Mrisho F, Pepping F, Lukmanji Z. Proceedings of a national symposium for vitamin A deficiency, November 16-18 1981 Dar es Salaam, TFNC Report No. 735. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

16 Jellife DB. The assessment of the nutritional status of the community. Geneva: WHO, 1968.

17 Waterlow JC. The presentation of height and weight data for comparing the nutritional status of groups of children under the age of 10 years. Bull Wrld Hlth Org 1977;35:489-98.

18 WHO. Measuring change in nutritional status. Geneva: WHO, 1983.

19 Gomez F, Galvan RR, Frenk S, Cravioto J, Chavez R, Vasquez J. Mortality in second and third degree malnutrition. J Trop Pediat 1956;2:77-83.

20 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235-54.

21 Driskell WJ, Neese JW, Bryant CC, Bashor MM. Measurement of vitamin A and vitamin E in human serum by high-performance liquid chromatography. J Chrom 1982;231:439-44.

22 Driskell WJ, Bashor MM, Neese JW. Loss of vitamin A in long-term stored, frozen sera. Clin Chem Acta 1985;147:25-30.

23 Pepping F, Soffers AEMF, West CE. Quality control of clinical chemical analyses in research on vitamin A deficiency and xerophthalmia (submitted for publication).

24 Voorhoeve AM, Muller AS, Schulpen TWJ, Gemert W, Valkenburg HA, Ensering HE. Machakos Project Studies III. The epidemiology of measles. Trop Geogr Med 1977;29:428-40.

25 Smedman L, Lindeberg A, Jeppsson O, Zetterstrom R. Nutritional status and measles: a community study in Guinea-Bissau. Ann Trop Paediatr 1983;3:169-76.

26 Koster FT, Curlin GC, Aziz KMA, Haque A. Synergistic impact of measles and diarrhoea on nutrition and mortality in Bangladesh. Bull Wrld Hlth Org 1981;59:901-8.

27 Loening WEK, Coovadia HM. Age-specific occurrence rates of measles in urban, peri-urban, and rural environments: implications for time of vaccinations. Lancet 1983;ii:324-6.

28 Barclay AJG, Foster A, Sommer A. Vitamin A supplements and mortality related to measles: a randomised clinical trial. Br Med J 1987;294:294-6.

29 Burgess W, Mduma B, Josephson GV. Measles in Mbeya, Tanzania 1981-1983. J Trop Pediat 1986;32:148-53.

30 Duggan MB, Milner RDG. Composition of weight gain by Kenyan children during recovery from measles. Hum Nutr:Clin Nutr 1986;40C:173-83.

31 James HO, West CE, Duggan MB, Ngwa M. A controlled study of the effect of injected water-miscible retinyl palmitate on plasma concentration of retinol and retinol-binding protein in Northern Nigeria. Acta Paediatr Scand 1984;73:22-8.

8. THE ROLE OF NUTRITIONAL STATUS WITH SPECIAL REFERENCE TO VITAMIN A IN THE DEVELOPMENT OF POST-MEASLES EYE LESIONS

II. EYE LESIONS AND OTHER CLINICAL COMPLICATIONS IN RELATION TO NUTRITIONAL STATUS

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ABSTRACT

Eye lesions and other clinical complications were studied in 841 children of whom 665 were hospitalised because of measles. Respiratory infections and/or diarrhoea were observed in two thirds of the children with measles. Those children who died while in hospital for treatment for measles were younger and had a lower weight-for-age and length-for-age, and also lower levels in serum of retinol, retinol-binding protein (RBP) and prealbumin. Corneal ulceration/ keratomalacia was observed in 3.3% (19/583) of the children with measles while 50% percent of the children with measles did not show any lesions during the period of observation. Children with corneal xerosis and/or ulceration had significantly lower levels in serum of retinol, RBP and prealbumin than did children with measles but without eye lesions.

INTRODUCTION

Measles has been implicated as a major cause of blindness among children in Africa (1). Research on post-measles eye lesions has not yet resulted in agreement about their aetiology which is possibly multifactorial (2-4), as confirmed by the results from a recent study in Tanzania (5). Of a total of 48 measles-associated corneal ulcerations, 24 (50%) were attributed to vitamin A deficiency, ten (21%) to an infection with herpes simplex virus, eight (17%) to the use of traditional eye medicines, and six (12%) to a confluent measles keratitis (5).

We have earlier reported on the results of a hospital-based study in Tanzania on the aetiology of post-measles blindness with respect to nutritional status of children with measles (6). Since nutritional status, as assessed by anthropometry and the concentration of serum albumin, deteriorated following measles, this would suggest that the malnutrition observed in children with measles was directly related, at least partly, to the measles infection and its

complications. The concentration of retinol, retinol-binding protein (RBP) and prealbumin also declined after the appearance of the measles rash. This reduction took place within two days as opposed to that of albumin which continued to decline for up to at least two weeks. Serum levels of retinol regarded as deficient (< 0.35 μmol/l or 10 μg/100 ml) were observed in 56.5% of the children with measles.

We here report on the eye lesions and other clinical complications observed in children with measles. Relationships between eye lesions, complications, anthropometric indices and serum levels of retinol and serum proteins are discussed.

METHODS

Details of the study design and of the analytical procedures used have been described earlier (6). During two periods (December 1983 - March 1984 and October 1984 - March 1985), 665 children with measles admitted to five hospitals in and around Dar es Salaam in the United Republic of Tanzania, were studied. Measles-free control children (n=176) were selected from a maternal and child health (MCH) unit, from paediatric outpatients clinics and from a nutrition rehabilitation unit. High doses of vitamin A had not been administered to any of these children. Severe (< 60%) and moderate (60-75% of the reference weight-for-age) malnutrition was observed in 10.2% and 43.3% of the measles patients and in 4.6% and 17.2% of the controls respectively. Using weight-for-length as indicator of malnutrition, 39.8% of the measles children showed a serious deficit in weight-for-length (< 80% of the reference standard).

A protocol for the diagnosis of the eye lesions was compiled by one of the authors (SF) for use by all investigators participating in the study. Although six investigators were involved, all eye examinations during the pilot study and 80% of those during the main study were carried out by one ophthalmologist (DMM). Examination of the eyes was carried out with a hand torch or illuminated magnifier (X5, International Centre for Eye Health, London, England) and fluorescein filter paper strips were used to stain the ocular surface. For a review of some of the ocular signs and complications in measles, readers are referred to the work of Dekkers (7).

The ophthalmological lesions listed on a precoded questionnaire were: confluent measles keratitis (MK), superficial punctate keratitis (SPK), broken tearfilm (BT), absence of watery tears (NWT), pigmented lateral triangle (PLT),

conjunctival xerosis (X1A), Bitot's spots (X1B), corneal xerosis and corneal ulceration/keratomalcia. This diagnostic classification was chosen in order to combine the standard WHO xerophthalmia classification (8) with an ordered classification of eye lesions known to be associated with measles (7). This is not to presuppose that post-measles eye lesions inevitably progress to keratomalacia (i.e. that there is a causal link between the first and second classification). It did however enable us to document progress of lesions in those children in whom repeated eye examination was possible. Furthermore, it reduced the likelihood of under-reporting of "precursor" lesions which might be of ophthalmological significance.

The occurrence of measles keratitis has been extensively discussed by Dekkers (7). The proportion of children seen with measles keratitis seems to vary with the time when the child is examined and, as a result, higher prevalence rates have been recorded in longitudinal studies (70%) than during cross-sectional studies (around 30%, see ref 7). Superficial punctate keratitis may result from corneal xerosis, it may be a form of measles keratitis, or it may be due to some other cause. In case of SPK, inflammation was located in the epithelium and was only seen in small "discrete patches" (9), in contrast to MK where the inflammation was confluent. The eyes of children with measles often remain dry because of lack of mucous production with rapid breaking up of the tearfilm within a few seconds. Break-up time as described by Sauter (10), for the diagnosis of xerophthalmia, was not used as a criterion in this study. The production of watery tearfluid may be absent even when the child cries. Absence of corneal wetting can occur without clinically recognizable keratinization of epithelial tissue but can also be observed in xerophthalmia. Pigmentation may accumulate in the temporal conjunctiva and is quite common in African children as described by McLaren (11), and by Sauter who observed a higher frequency in malnourished children (10). In the present studies pigmentation was diagnozed as described by Sauter (10). The last four lesions are included in the WHO classification scheme for xerophthalmic eye lesions (8). We have not used the terms X2 and X3 for corneal xerosis and corneal ulceration/ keratomalacia respectively because this would presuppose prime involvement of vitamin A deficiency in these conditions.

The eyes were re-examined at intervals of two to three days throughout the hospital admission. The only exception to this practice was in the first week of the pilot study and during a two-week period in the main study when pressure on hospital beds during an epidemic neccessitated early discharge. In total, 583 children with measles were enrolled in the ophthalmological examinations

and a second eye examination was carried out on 242 children with measles (44%): eyes were examined three or more times in 97 (17%) children. The eyes of 87 children (82 cases and five controls) were not examined.

As recommended by WHO (8), high-dose vitamin A capsules (55 mg retinyl palmitate equivalent to 200,000 I.U. vitamin A; 40 I.U. vitamin E) were administered routinely to all children with measles in the hospitals participating in this study. The routine treatment with vitamin A was modified so that children with severe measles keratitis, corneal xerosis or corneal ulceration on admisssion received immediate treatment with vitamin A and those with other lesions at discharge. Furthermore eye ointment (chloramphenicol) was applied to the eyes of those children with severe eye lesions and eye pads were applied to the eyes of some children during the acute phase of the ulceration.

RESULTS

Morbidity and mortality

Respiratory tract infections (62.9%) and diarrhoea (65.3%) were the most common complications recorded, being present simultaneously in 32.2% of the children with measles. Conjunctivitis associated with measles was observed in 20% of the children, stomatitis in 10% of the children and encephalitis was observed in two children. Malaria, anaemia and oedema were seen in less than 10% of the measles patients. Among the controls, diarrhoea was noted in 5.5% of the children and this was accompanied by a depression of retinol levels by 15%, of RBP levels by 28%, and of prealbumin levels by 11%.

In children with measles, serum concentrations of retinol and prealbumin were observed to be slightly lower in children with associated diarrhoea and/or respiratory infections. When these data were further analysed it became evident that children with upper respiratory tract infections, viz. bronchitis, laryngitis and laryngo-tracheo-bronchitis had lower levels of retinol (-18%), RBP (-11%), prealbumin (-13%) and albumin (-2%) than children with pneumonia. The levels were also lower in this group than in those children without respiratory infections and diarrhoea.

Of the children with measles, 7.5% (47/624) died. As seen from Table 1, these children were younger than survivors discharged from hospital after clinical improvement. The anthropometric indices and levels of retinol and serum proteins of the two groups are presented in Table 1. All parameters except weight-for-length and serum albumin were significantly lower in the children who died.

Table 1: Age, anthropometric indices and serum levels of retinol and serum proteins (mean ± SD) of the children with measles who died in hospital, and of those who were discharged after improvement

	Discharged		Died	
	n*	mean ± SD	n	mean ± SD
Total	577		47	
Age (months)	534	22.7 ± 18	42	15.8 ± 13§
Weight-for-age (%)+	534	74.9 ± 12	42	67.9 ± 11¶
Length-for-age (%)	495	94.4 ± 6	30	92.8 ± 5|
Weight-for-length (%)	493	83.2 ± 10	30	81.6 ± 8
Serum retinol (μmol/l)	150	0.38 ± 0.23	8	0.24 ± 0.15|
Serum RBP (μmol/l)	142	0.66 ± 0.30	7	0.37 ± 0.15¶
Serum prealbumin (μmol/l)	161	2.0 ± 0.8	8	1.4 ± 0.6|
Serum albumin (μmol/l)	104	482 ± 104	7	449 ± 109

* n = Number of children.
\+ Expressed as percentage of the NCHS reference standards (12).
Statistical comparison with discharged children: | $p<0.05$, § $p<0.01$ and ¶ $p<0.001$.

Eye lesions

Eye lesions seen during the pilot and main study are illustrated in Table 2. When multiple lesions were observed these were recorded separately. During the pilot study the reporting system for eye lesions was cross checked and this indicated under-reporting of keratitis and of absence of tear production, and misclassification of severe keratitis as corneal xerosis. In the main study, pigmentation of the lateral part of the conjunctiva (PLT) was seen in 3.8% of the cases and 9.4% of the controls (16/171, data not shown). Apart from conjunctival pigmentation and a single case with a Bitot's spot (X1B), no other eye lesions were seen in the control group. Nine (56%) of the control children with PLT had been selected from the nutrition rehabilitation unit and the nutritional status of these children indicated that all, except one, were chronically malnourished.

Xerophthalmia-related lesions. Corneal xerosis without ulceration was seen in 2.7% (13/479) of the children with measles during the main study. Corneal ulceration/keratomalacia was observed in four children during the pilot study (3.8%) and in 15 children during the main study (3.1%). In two thirds of the

Table 2: Number and type of eye lesions observed in hospitalised children with measles, all lesions recorded separately

Type of eye lesion	Pilot study N/n*	%	Main study N/n	%
Number of children examined	104		479	
Children without eye lesions	57	54.8	230	48.0
Pigmented lateral triangle (PLT)	n.r.		18/18	3.8
Xerophthalmia-associated lesions				
-Corneal ulceration/keratomalacia	4/1	3.8	15/6	3.1
-Corneal xerosis	23/14+	22.1	21/16	4.4
-Conjunctival xerosis (X1A)	n.r.		4/4	0.8
-Bitot's spots (X1B)	0		1/0	0.2
Measles-associated lesions				
-Measles keratitis (MK)	25	24.1	136/120	28.4
-Superficial punctate keratitis (SPK)			25/21	5.2
-Broken tear film (BT)	n.r.		10/9	2.1
-Absence of watery tears	n.r.		118/118	26.7

* N indicates the total number of lesions recorded and n gives the number of children in which the lesion was bilateral, the proportion (%) indicates those with bilateral lesions, and n.r. indicates not registered.
\+ Includes an overreporting for corneal xerosis and underreporting for measles keratitis/superficial punctate keratitis.

children with corneal ulceration/keratomalacia, the lesions were already present on admission, while in five children they appeared during the hospital stay and were preceded by xerosis in three children. Mortality was 37% (7/19) in children with corneal ulceration and 13% (4/30) in children with corneal xerosis in the absence of corneal ulceration. Bilateral corneal ulceration with or without xerosis was seen in seven of the 19 cases (37%) and 70% of the ulcers recorded were located in the lower part of the cornea. Confluent measles keratitis (MK) was observed in 25% of the children with ulceration, and watery tears were absent (NWT) in 38% of these children. Thirteen children with corneal ulceration were observed after the administration of vitamin A and the eye lesions healed in seven (54%) of them. There was no apparent change in three children and the ulceration progressed in the remaining three children. In one of the 19 cases, who had a unilateral corneal ulcer, this was clinically diagnozed as due to herpes, although the ulcer was preceded by measles keratitis and mild xerosis. The serum retinol level in this child was low (0.10 μmol/l). Absence of tears was observed in 77% of the children with corneal

xerosis and the absence of tears was sometimes preceded and was sometimes simultaneous with the xerosis. Confluent measles keratitis was present in 50% of the cases with corneal xerosis. Of the 13 children with corneal xerosis who were re-examined, nine showed a positive response to vitamin A treatment. The lesions observed in four children with corneal ulceration/keratomalacia are presented in Appendix II (photographs no. 9-12), and detailed drawings of size and localization of the lesions recorded in 16 children are presented in Appendix III. Examination of these data indicates that in four out of sixteen children there is a possible involvement of measles keratitis in the development of the ulceration. The four children include the child in which herpes simplex virus is also regarded as having a role.

Conjunctival xerosis (n=4) and Bitot's spots (n=1) were rarely seen in children with measles. The child with a unilateral Bitot's spot was aged 5½ years and clinically well nourished.

Measles-associated lesions. During the main study, measles keratitis was observed in 28.4% and absence of watery tears in 26.7% of the children. These findings became evident on average 5.1 and 4.5 days after the appearance of the rash respectively, and were bilateral in nearly all cases. Further analysis of the children enrolled in the main study and excluding children with corneal xerosis/ulceration and PLT demonstrated that of the 103 children without watery tears, confluent measles keratitis was present in 43 (41.7%) and superficial punctate keratitis in 24 (23.3%) children. This shows that superficial keratitis was not recorded as a single lesion. Broken tear film was observed in a further 10 children and of these, six also had measles keratitis.

As mentioned earlier, the occurrence of measles-associated lesions may depend on the time elapsed after the eruption of rash. The sequence of the occurrence of keratitis, absence of tears and corneal xerosis/ulceration as observed during the main study are presented in Figure 1. The results of these mixed cross-sectional and longitudinal observations show a decline in the prevalence of keratitis with respect to time after eruption of rash but even in those children who presented late (Days 11-18), there was a relatively high proportion with keratitis (24%). Except for the high proportion found on the day after the measles rash appeared (Day 1), the prevalence of absence of tears showed less fluctuation than for keratitis and showed an increase between Day 3 and Days 6/7. The increase of the proportion of children seen with corneal xerosis/ulceration shows that these children are admitted rather late during the course of the illness (i.e. on Days 6 and 7). Of the 242 children of whom the eyes could be examined on more than one occasion during the main study, 93 (38.4%)

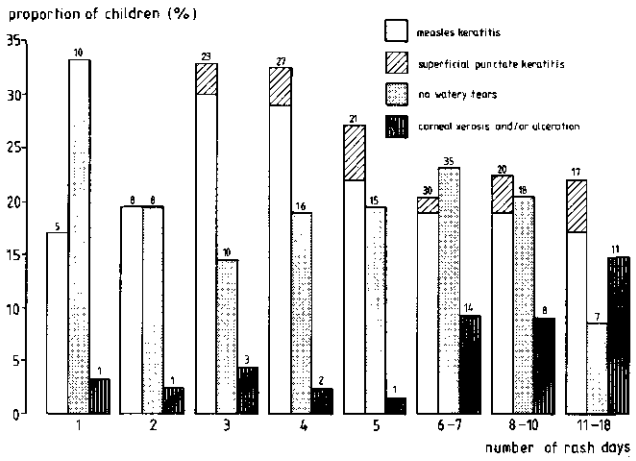

Figure 1: Proportion of children in the main post-measles blindness study with confluent and superficial punctate keratitis, absence of tears and corneal xerosis/ulceration in relation to the time elapsed since eruption of rash (cross-sectional and longitudinal data). The number of children with the various lesions is given.

Table 3: Results of longitudinal observations in children with measles in main post-measles blindness study

	Type of eye lesion*		
	Total	MK	NWT
Total observations	479	136	118
Children examined once	237	44	38
Children examined twice or more	242	92	80
Xerophthalmia-associated lesions/pigmentation	42	12	21
No lesions	93	-	-
Children with lesions	107	80	59
- Lesions unchanged during admission		22 (28%)	16 (27%)
- Lesions healed during admission		32 (40%)	29 (49%)
- Lesions progress to MK		3 (4%)	-
- Lesions progress to NWT		-	3 (5%)
- Lesions developed during admission		23 (29%)	11 (19%)

* MK = measles keratitis and NWT = no watery tears.

showed no lesions at any time during admission. Of the remaining 149 children, xerophthalmia-associated lesions and/or pigmentation were observed in 42 children, and thus 107 children with only measles-associated lesions were recorded. Fifty two children showed these lesions on admission and in about half of this group the lesions healed before discharge. Fifty seven children developed the lesions during admission and of these only 9 (16%) healed before discharge (see Table 3). Measles keratitis healed before discharge in 40% of the children while absence of tear production was at discharge no longer recorded in 49% of the children. In 14% (34/242) of the children examined on two or more occasions MK and NWT were recorded simultaneous.

Eye lesions and nutritional status

Of those children with corneal ulceration and/or corneal xerosis, 30.6% (15/49) were well nourished (weight-for-age $\geq$ 75%) compared with 46.6% (266/571) of the children with measles without corneal eye lesions (X^2=4.7, $p<0.05$). Using the 80% weight-for-length as a cut-off point for malnutrition, 48.9% (22/45) of the children with corneal xerosis and ulceration were malnourished, compared with 40.2% (211/525) of those not showing eye lesions of this type. The nutritional status of the children with bilateral ulceration (n=7) was less than that of those with a unilateral ulceration (n=12): 63% compared with 73% WA respectively.

Mean serum levels and mean anthropometric indices for the children showing various types of eye lesions are presented in Table 4. In this table, single and multiple lesions are combined and the children are classified according to the most serious lesions recorded, assuming corneal xerosis and/or ulceration to be more serious than the measles-associated lesions. Children with measles with pigmentation were classified separately regardless of other eye lesions noted. This group of children showed remarkably low serum levels (except for prealbumin) and although the number of control children with pigmentation and from whom blood was sampled was small (n=5), these children also showed lower levels in serum of retinol and the three serum proteins studied, and lower anthropometric indices than the control children without pigmentation (data of the control group not shown). Mean levels of retinol and the three serum proteins in the serum of children not showing eye lesions, either at one or more eye examinations, did not differ from those identified with measles-associated lesions. The levels, except for albumin, were significantly lower, for children with corneal ulceration and/or xerosis except, when compared with children without eye lesions or with measles-associated eye lesions. Of the

Table 4: Serum concentrations of retinol, RBP, prealbumin and albumin and anthropometric indices in measles patients showing various types of eye lesions*

	Type of eye lesions+			
	No eye lesions	Xerophthalmia associated	Measles associated	PLT
Serum retinol	0.38±0.02 (89)	0.23±0.05 (19)§	0.39±0.03 (48)	0.31±0.08 (6)
Serum RBP	0.67±0.03 (81)	0.47±0.07 (11)|	0.62±0.04 (48)	0.49±0.11 (6)
Serum prealbumin	1.95±0.08 (91)	1.47±0.12 (18)|	1.80±0.08 (55)	2.93±1.06 (5)
Serum albumin	473±11 (78)	449±52 (10)	488±14 (47)	378±46 (6)
Weight-for-length(%)	82.5±1 (90)	78.2±3 (18)	82.2±1 (52)	80.5±2 (4)
Weight-for-age (%)	73.9±1 (92)	71.3±3 (19)	75.1±2 (56)	72.7±3 (6)
Length-for-age (%)	94.6±1 (90)	93.2±1 (18)	95.4±1 (52)	95.0±4 (4)

* Results are expressed as mean±SE with the number of children given in parentheses. All values for serum constituents are given in μmol/l and the anthropometric indices are given as the proportion of the reference standard (12).
\+ Xerophthalmia-associated lesions include corneal xerosis and corneal ulceration; measles-associated eye lesions include measles keratitis, superficial punctate keratitis, no watery tears and broken tear film; and PLT refers to pigemented lateral triangle.
Statistical comparison with children with no eye lesions, | $p<0.05$, and § $p<0.01$.

children with corneal ulceration and/or xerosis, 89.4% (17/19) had biochemical evidence of hypovitaminosis A, i.e. deficient retinol levels, against 53.7% (79/147) of the children without such lesions. Serum retinol levels for both groups were 0.23±0.05 μmol/l (mean±SE; or 6.6 μg/100 ml, n=19) and 0.38±0.02 μmol/l (n=147) respectively ($p<0.05$).

DISCUSSION

Respiratory infection and diarrhoea were observed in the majority of the measles patients. Since these are secondary complications which are recorded somewhat subjectively proportions reported vary widely not only because of inter-observer variation but also because of other factors such as duration of hospital admission. It is therefore not surprising that the figures given for these complications vary widely (13,14).

As indicated earlier, the case fatality rate of 7.5% recorded here should be regarded as a conservative estimate (6). Evidence is accumulating that mortality from measles in children in East Africa is higher than that recorded in India. A

mortality rate of 1.4% was recorded in a hospital-based study in which 18% of the children were severely malnourished (13) and no deaths were recorded among 318 children in a community where xerophthalmia was a problem as indicated by a prevalence of Bitot's spots of 2.8% (4). In a community-based study carried out in Guinea-Bissau (15), factors related to crowding and the family structure were thought to play a more important role than pre-existing malnutrition in determining the outcome of measles. Nieburg and Dibley (16) in reviewing the risk factors for fatal measles concluded that under different circumstances, the various risk factors may play roles of varying importance and that the relationship between measles outcome, pre-measles nutritional status and other risk factors is not yet known. In the present study not only weight-for-age but also length-for-age was lower in those children who died suggesting that malnutrition predisposes to increased mortality from measles.

Of a total of 583 measles patients for whom ophthalmological data are available, 19 children had corneal ulceration (3.3%) and 13 out of 479 children (2.7%, main study only) had definite corneal xerosis. Foster and Sommer (5) reviewed a number of studies carried out in Africa and their own observations in 48 children with measles-associated corneal ulceration, and concluded that the proportion of hospitalised children with measles who developed corneal ulceration was 4%. Dekkers (7) described in detail epithelial keratitis associated with measles which in 4% of the children progressed to macro erosions which healed without leaving any corneal scars. In their study of 125 children with measles, Bhaskaram et al. (17) observed coarse fluorescein-positive lesions in 50% of the children. Fine punctate lesions were observed in a further 15% of the children, but most (all except two) of these lesions were self limiting and disappeared spontaneously.

In our studies, all children with corneal ulceration had a defínite loss of stromal thickness and these lesions can therefore be qualified as corneal ulceration/keratomalacia. A white conjunctiva, as illustrated in photographs 10 and 11 (see Appendix II) often accompanied these lesions and indicates an absence of inflammatory reaction. Sauter (10) observed a striking inflammatory response in 45 healthy measles children while this was absent in malnourished measles children. We did not investigate systematically the relationship between nutritional status and inflammatory response in the children with corneal ulceration. Others (see ref 5), have explained the absence of early clinical signs of xerophthalmia (night blindness and Bitot's spots) simply as a result of the accompanying inflammation which could cause the conjunctival xerosis or Bitot's spots to disappear. In our children such mild signs of xerophthalmia

were rarely seen but no effort was made to diagnoze night blindness.

The majority of children with xerosis (70%), and more than half of the children with corneal ulceration, responded rapidly to treatment with vitamin A (see for example children no. T82 and W04 in Appendix III). On the other hand, a slow response was also seen in some children (see for example child no. T266 who was severely malnourished, WA 57%). In 75% of the children with ulceration, the lesion was present on admission, i.e. within 5-7 days after eruption of the rash. Burgess et al. (14), who studied 900 children with measles in Southern Tanzania and observed an almost identical proportion of children with severe malnutrition as we did, attributed the absence of eye lesions to administration of vitamin A. Our data suggest that a higher proportion of children will be found to have post-measles eye lesions when positively sought by careful ophthalmological examination and this seems to have been confirmed by other reports (18). Since most patients who develop corneal ulceration present with the condition, administration of vitamin A to such children can only be used in a curative and not a prophylactic manner.

Although in the present study, herpes simplex virus was suspected to play a causative role in post-measles blindness in only one out of 19 children (5%), we do not want to underestimate the role that this virus may play (5,19). Facilities were not available to enable us to carry out studies to confirm whether or not the virus or other pathogenic organisms was present in the corneal lesions.

The serum levels of retinol, prealbumin and RBP were significantly lower in children with corneal ulceration and/or xerosis than in measles children without such lesions. Other investigators such as Laditan and Fafunso in Nigeria (20), Sauter in Kenya (10), and Reddy et al. in India (4) did not observe a significant difference in serum retinol levels between measles children with and without corneal ulceration. The mean difference in serum retinol levels between such children in the present study was 0.15 μmol/l despite that the levels were already low in the unaffected children (0.38 μmol/l). Foster and Sommer (5) recorded a mean serum retinol level of 0.24 μmol/l (6.8 μg/100 ml) for eight xerophthalmic children with post-measles corneal ulceration and this was significantly lower than that for 24 controls (0.36 μmol/l; 10.2 μg/100 ml) matched for age, sex and the presence of measles. In the children with corneal ulceration and/or xerosis prealbumin and RBP levels also were reduced, not only when compared with the controls but also with those children not suffering from corneal ulceration and/or xerosis. This indicates that the availability of transport proteins is even more reduced in children with eye lesions than in

analogous children without eye lesions.

The major question to be answered is whether or not eye lesions, manifesting either as corneal xerosis/ulceration or as measles keratitis/absence of tears, occur in children well nourished with respect to protein and energy and to vitamin A. Protein-energy malnutrition can be assessed by anthropometric indices and by serum protein levels. The consistent steady decline in serum albumin levels observed following the appearance of measles rash and before blood samples could be taken, renders albumin unsuitable as an indicator of pre-measles nutritional status. Since serum albumin levels in children (both measles cases and controls) with pigmentation were extremely low, it is tempting to suggest a relationship between protein-energy malnutrition and pigmentation. The interpretation of serum levels of albumin and other proteins and anthropometric indices in relation to measles-associated eye lesions remains extremely difficult. Similar to Dekkers (7) and Bhaskaram et al. (17), we were not able to demonstrate any relationship between either serum levels and anthropometric parameters and the presence of such eye lesions. We acknowledge that the limited period in which we were able to study the children in our study may reduce the chance of identifying factors associated with the occurrence of such lesions. In spite of the weight loss due to measles, just over 50% of the children with corneal xerosis/ulceration were able to maintain a weight-for-length which was equal to or above 80% of the reference. However, serum levels of albumin fell below adequate levels. The high proportion of children with corneal xerosis who showed a confluent measles keratitis might indicate a possible relationship between both lesions. Furthermore most of these children responded rapidly to vitamin A, which is not surprising as their serum retinol levels were low. Unfortunately, we have no information about the levels prior to the onset of measles in children who develop eye lesions and those who do not. We do know that in the first few days after the appearance of the rash, serum retinol levels fall sharply. It would be easy to accept that the vitamin A status prior to the onset of measles of the children who did develop xerophthalmic lesions was inferior to that of the children who did not. However, we cannot prove this and it may well be that other factors predispose children with measles to the development of post-measles corneal ulcers. The fact that children with corneal ulceration did present with lesions on admission also made it difficult to investigate the role of measles keratitis in the process of ulceration in these children. Foster and Sommer (5) described the ulceration developing from measles superficial punctate keratitis as round, epithelial and usually localized in the center of the cornea. As indicated earlier we noticed

an involvement of measles keratitis in 25% of the children on whom detailed information was available. In conclusion, we feel that our data suggest a major role of vitamin A in the development of corneal xerosis and corneal ulceration, as evidenced by both low serum levels of retinol and by a rapid response to treatment with vitamin A. The contributing role of herpes simplex virus and measles keratitis was also recorded but data do not allow a definite conclusion about the relative importance of these factors. Among the children with corneal ulceration, no evidence was found of the use of traditional eye medicines.

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REFERENCES

1 Foster A, Sommer A. Childhood blindness from corneal ulceration in Africa: causes, prevention and treatment. Bull Wrld Hlth Org 1986;64:619-23.

2 Inua M, Duggan MB, West CE, et al. The role of vitamin A, malnutrition and measles in post-measles corneal ulceration in children in Northern Nigeria. Ann Trop Paediatr 1983;3:181-91.

3 Bhaskaram P, Madhusudan J, Radhrakrishna KV, Raj S. Immunological response to measles vaccination in poor communities. Hum Nutr:Clin Nutr 1986;40C:295-9.

4 Reddy V, Bhaskaram P, Raghurumulu N, et al. Relationship between measles, malnutrition, and blindness: a prospective study in Indian children. Am J Clin Nutr 1986;44:924-30.

5 Foster A, Sommer A. Corneal ulceration, measles, and childhood blindness in Tanzania. Br J Ophthalmol 1987;71:331-43.

6 Pepping F, Hackenitz EA, West CE. The role of nutritional status with special reference to vitamin A in the development of post-measles eye lesions I. Nutritional status (submitted for publication).

7 Dekkers NWHM. The cornea in measles. Den Haag: Junk Publishers, 1981.

8 WHO. Control of vitamin A deficiency and xerophthalmia Report of joint WHO/UNICEF/USAID/Helen Keller International/IVACG meeting Techn Report Series No. 672. Geneva: WHO, 1982.

9 Sandford-Smith J. Eye diseases in hot climates. Bristol: Wright, 1986.

10 Sauter JJM. Xerophthalmia and measles in Kenya. Groningen: Drukkerij van Denderen, 1976.

11 McLaren DS. Malnutrition and the eye. New York: Academic Press, 1963.

12 WHO. Measuring change in nutritional status. Geneva: WHO, 1983.

13 Bhaskaram P, Reddy V, Raj S, Bhatnagar RC. Effect of measles on the nutritional status of preschool children. J Trop Med Hyg 1984;87:21-5.

14 Burgess W, Mduma B, Josephson GV. Measles in Mbeya, Tanzania 1981-1983. J Trop Pediat 1986;32:148-53.

15 Smedman L, Lindeberg A, Jeppsson O, Zetterstrom R. Nutritional status and measles: a community study in Guinea-Bissau. Ann Trop Paediatr 1983; 3:169-76.

16 Nieburg P, Dibley MJ, Risk factors for fatal measles infections. Int J Epid 1986;15:309-11.

17 Bhaskaram P, Mathur R, Rao V, et al. Pathogenesis of corneal lesions in measles. Hum Nutr:Clin Nutr 1986;40C:197-204.

18 Lindtjørn B. Severe measles in the Gardulla area of Southwest Ethiopia. J Trop Pediatr;32:234-9.

19 Whittle HC, Sandford-Smith J, Kogbe OI, Dossetor J, Duggan MB. Severe ulcerative herpes of mouth and eye following measles. Trans Roy Soc Trop Med 1979;73:66-9.

20 Laditan AAO, Fafunso M. Serum levels of vitamin A, beta-carotene and albumin in children with measles. East Afr Med J 1981;58:51-5.

9. GENERAL DISCUSSION

INTRODUCTION

The major aim of the research activities described in this thesis was to contribute to the background information required for planning and implementation of a national programme for the prevention of nutritional blindness. Much progress has been made since 1980 (1-4), and this has led to formulation of a national programme on the control of vitamin A deficiency (see Chapter 1).

In this chapter, the inter-relationship between the various research projects reported in the previous chapters are discussed in the context of available information on the magnitude and severity of xerophthalmia and post-measles blindness. The solution of the problems of xerophthalmia and vitamin A deficiency depends partly on the formulation and implementation of a policy at the national level in the nutrition, health and agricultural spheres. Thus the role and function of various organizations in Tanzania with respect to xerophthalmia and vitamin A deficiency are discussed. Constraints faced during the execution of the field and laboratory work are also discussed.

NUTRITION, HEALTH AND AGRICULTURE IN TANZANIA IN RELATION TO VITAMIN A DEFICIENCY

Nutrition

The fieldwork described in this thesis formed part of the ongoing research activities of Tanzania Food and Nutrition Centre (TFNC). This centre was founded in 1973 and became operational in 1975. After a period of growth, it achieved its full complement of over one hundred staff about 10 years ago. The centre developed out of the Nutrition Unit of the Ministry of Health which was established in 1947 when the first full-time nutrition officer was employed (5). In the period 1974-78, the activities of the centre were mainly focussed on data collection and on food and nutrition planning at various levels. In 1980, the centre organized a meeting in which a draft food and nutrition policy for Tanzania was discussed (5).

As the national reference centre on food and nutrition issues, TFNC has taken the initiative to organize the series of meetings in cooperation with other parties (see Chapter 1) which resulted ultimately in the formulation of the

national programme for the control of vitamin A deficiency and xerophthalmia (see above and reference 6).

Health

The programme on vitamin A deficiency and xerophthalmia at TFNC is now being continued under the five-year Prevention of Blindness Programme (1986-1990) established by the National Prevention of Blindness Committee (NPBCT). The NPBCT was established some 15 years ago and reactivated in 1983. It is chaired by the Director of Preventive Services, one of the three directorates within the Ministry of Health. In recent years, annual meetings have been held with the corresponding respective national committees from Kenya and Tanzania and these have been attended on a number of occasions by delegates from Uganda.

Training of health personel forms an important part of the national prevention of blindness programme. Compared with many countries, Tanzania has put much emphasis on training of ophthalmological staff (7). In addition to ophthalmologists working mainly at referral and regional hospitals, about twenty Assistant Medical Officers (AMO) Ophthalmology and sixty eyenurses had been trained by the end of 1985. Furthermore there are training programmes for ophthalmic technicians and specific programmes for the training of AMOs in cataract surgery. As would be expected, the Assistant Medical Officers and the eye nurses are the key persons at the district level in the organization of any action to combat xerophthalmia. On the other hand, it should be realized that most of the time of these workers will be spent on routine curative hospital work and not on preventive activities.

Agriculture

In order to overcome vitamin A deficiency, it is necessary to increase the production and consumption of foods rich in (pro)vitamin A. These tasks require an input from the agricultural sector. The Expert Committee Meeting (3), made detailed recommendations on a number of activities regarded as essential in combatting vitamin A deficiency. These were:

- promotion of the production and consumption of red palm oil which is a rich source of provitamin A,
- research on the carotenoid content of indigenous vegetables, and
- research on the suitability of fish oil for human consumption.

No recent information is available on the country-wide production of red palm oil. Reasons given for the assumed decreased production over the last fifteen years include: lack of good high-yielding hybrid varieties, unfavorable weather

conditions, laborious manual processing methods, and poor management of the plantations (2,4,8). Other reasons given are that palm oil is not seen as a cash crop, increased production of palm wine, lack of an infrastructure for marketing of the oil, and the influence of villagization. The process of villagization and its impact on agriculture and health have been summarized in a UNICEF report (9). Increased production of palm oil cannot be achieved by simple means and cannot be expected without general improvement of the agricultural production in Tanzania and improvement of marketing facilities. It will involve principally an increase in the area of oil palms under cultivation. This should be encouraged not only in Kigoma Region where most of the oil palms are now cultivated but also in other suitable areas, as outlined by Liwenga (see ref 4).

As a short-term measure, the introduction of improved methods for extraction of palm oil from the crop now available has been suggested (4). At present, the extraction rate is between 50 and 60% of the available oil. With the use of improved hand presses or screw presses, it is possible to increase the extraction rate to 90%, thus resulting in a considerable increase of the amount of oil obtained from the same harvest. Preliminary discussions by TFNC with interested parties have indicated that improved handscrew presses such as those produced and installed by the Royal Tropical Institute (Amsterdam) in a number of countries (10), can be manufactured by the Institute of Product Innovation (IPI) of the University of Dar es Salaam. A number of national and international organizations have shown interest in starting or increasing their work on oil extraction from palm and other oil seeds such as sunflower.

In cooperation with the Crop Science Department of the Faculty of Agriculture, Forestry and Veterinary Sciences of the University of Dar es Salaam in Morogoro, now the Sokoine University of Agriculture, a study was started in 1983 on the carotenoid content of a number of indigenous vegetables. As claimed by several participants during the meeting in 1981, many varieties or species of vegetables had almost disappeared. Reference was made to varieties of species of Amaranthus (mchicha) and Corchorus (mlenda) and also to Solanum nigrum (mnafu) and Gyandropsis gyandra (mgani) (2). After designing a research project, work commenced in September 1983 and two batches of vegetables harvested from the glasshouses in Morogoro were taken to TFNC for analysis. Transfer of the horticulturist responsible for the work in Morogoro and breakdown of the high performance liquid chromatography (HPLC) at TFNC resulted in the project being terminated prematurely.

In 1981 no detailed recommendations could be given on the promotion of fish oil for human consumption. This was because fish from Lake Victoria were

suspected of being contaminated with pesticides. Recent analysis of oil prepared from fat of Nile perch (Lates niloticus) and Haplochomis spp did not confirm the suspected contamination although the number of samples examined was not large and cannot be regarded as being representative of the situation around the whole lake (11). Fish oil cannot be compared as a source of vitamin A with fish liver oil, which contains far more vitamin A. However, more extensive production of fish oil could help to reduce the shortage of fat and also contribute to the vitamin A intake of many people in Tanzania.

In 1987, a report was prepared by an FAO consultant outlining a policy and programme for increasing the availability of (pro)vitamin A in Tanzania (12). Particular attention was paid to the possible role of FAO within the framework of their ten-year action programme for the prevention and control of vitamin A deficiency. In order to increase the involvement of the agricultural sector, the establishment was recommended of an implementation committee which would be chaired by the Ministry of Agriculture with TFNC responsible for the secretariat. It was emphasized that, for the coming years, a more prevention-oriented strategy was desirable with more emphasis being placed on food production and utilisation rather than on nutritional blindness per se.

THE ANALYSIS OF FOOD AND SERUM SAMPLES

As part of the research work on vitamin A deficiency carried out in collaboration with TFNC, support was given to the further development of the TFNC laboratory. Three medical laboratory technicians from Tanzania, including two from TFNC, participated in an eight-week upgrading course for laboratory technicians held in 1983 in Wageningen. The course focussed on the analysis of vitamin A, ß-carotene and serum proteins by methods applicable in moderately equipped laboratories. Furthermore much work was carried out during the initial phase of the project on the development of a food composition table (see Appendix IV, and ref 13-16).

Analysis of serum constituents

The manual on biochemical methods published by the International Vitamin A Consultative Group (IVACG) provides detailed instructions for preparation and storage of blood samples (17). Regarding collection of samples, Mejía et al. (18) investigated the effect of ingesting a breakfast rich in vitamin A (337 μg retinol equivalents) on postprandial serum concentrations of retinol, retinol-binding protein (RBP) and carotenoids in children. Up to four hours

after consumption of the breakfast, postprandial concentrations of the constituents mentioned above were not increased significantly. Under field conditions, as experienced during the prevalence surveys and the hospital-based studies described in the Chapters 2,3, 7 and 8 of this thesis, it was not always possible to examine children under fasting conditions. The study of Mejía et al. (18) has shown that under field conditions, blood samples can safely be collected throughout the morning without regard to whether or not or when a breakfast was consumed.

The same group of research workers (19) investigated the influence of holding blood prior to separation of the serum from the clot containing the red blood cells. Serum retinol and RBP were stable at 4°C and at room temperature even when the serum was separated from the clot 24 hours after blood had been collected. The results of both experiments described above are in agreement with the findings of Sinaga, who also investigated the influence of sub-optimal storage conditions (20). He reported a limited effect of repeated freezing and thawing on serum retinol levels as long as samples were protected from air and light. In the studies described in this thesis, serum was separated always within four to six hours after collection, and blood samples were stored immediately after collection in the dark.

The introduction of HPLC has improved the quality of the analysis of retinol in serum. Problems such as those encountered with corrosive reagents and also those arising from the incomplete separation of retinol from its esters have been largely overcome. An overview of existing HPLC methods and the characteristics of various systems available has been prepared by Lambert et al. (21). The volume of serum required for one single analysis has been reduced to 0.1-0.2 ml. Recently a method was described in which only 5 μl of serum was required for the analysis of retinol by HPLC on a Polygosil 60-5, 5 μm column using fluorimetric detection (22). Application of such micro methods would allow the analysis of retinol in fingerprick serum samples. In fingerprick samples from the present studies, it was only possible to analyse serum proteins including retinol-binding protein and not retinol.

As emphasized in Chapter 6, much more can be done on external standardization of retinol analyses. We acknowledge the urgent need for reference sera with low levels of retinol. The range covered by the three calibration sera used in Chapter 4 did encompass the values found during the prevalence surveys (Chapter 3), and those of the control group in the post-measles blindness study. However, the serum retinol levels of the children with measles were clearly not covered by this range.

Although ß-carotene was not measured in the serum samples collected during the experiments described in this thesis, this constituent is often measured in combination with retinol. Recent investigations have shown that carotenoids dissolved in petroleum ether are not as sensitive to light as previously thought (23). It was reported that when only carotenoids are to be measured, serum specimens can be kept either under refrigeration or at room temperature for two to three days. Specimens stored at -20°C lose significant amounts of carotenoids within weeks. Thus samples of serum should be stored at -70°C when carotenoids are to be measured.

Observations which have indicated that carotenoids and/or vitamin A may protect against the development of some forms of cancer (24) have created considerable interest in the analysis of sera which have been stored for long periods of time. Much of our knowledge on the effect of storage conditions on the estimation of vitamin A has come from the analysis of such sera (25). The observation, that retinol analyses in sera collected many years previously were less reproducible, prompted a search for techniques to overcome this problem. Addition of ascorbic acid prior to analysis improved the reproducibility of the determination of retinol. The stability of vitamin A in sera stored at -20°C for five to eight years was satisfactory as the concentration was 310 μg/l in fresh serum and 300 μg/l after storage (26). Retinol was analysed prior to storage by the trifluoracetic acid method and after storage by HPLC.

An alternative method for estimating retinol in serum is to measure the concentration of retinol bound to retinol-binding protein (RBP): i.e., to measure the concentration of holo-RBP. This requires the separation of holo-RBP from apo-RBP and the subsequent measurement of the amount of holo-RBP present. The earliest method developed was based on the separation of holo-RBP and apo-RBP on a polyacrylamide gel which was then scanned fluorimetrically to quantitate the holo-RBP present (27). In a more recent method, quantitifation is achieved by electro-immunoassay (17) and this method has been tested extensively in our laboratory. Using pooled serum prepared from healthy volunteers, reasonable results could be obtained although the "floating" cones which formed in the agarose gel during the rocket electrophoresis reduced the reproducibility. However, this problem could be overcome by modifying the buffers in the electrophoresis system (J. Glover, pers comm). Instead of using barbital buffer (pH 8.6) in both compartments, a Tris/HCL buffer (pH 8.1) is used in the anodic compartment and a Tris/glycine buffer (pH 8.9) in the cathode compartment. These changes did provide significantly better peak areas. However, one major problem in the analysis of sera with low levels of retinol remained.

The fluorescence observed in the polyacrylamide gel under UV light produced by the retinol present in the sample, was far too low to make a reliable estimate of where holo-RBP was located in the gel. A solution was sought in increasing the amount of serum loaded on the gel and by using a 10% gel instead of a 5% gel. However, with the alternative procedure, the coefficient of variation increased from 10.2% to 13.2% (n=10) and the recovery of holo-RBP decreased by 10% (28). Thus a decision was made not to use the method in the present series of studies and it would appear that the method will be of limited use for sera with low levels of retinol.

Analysis of food samples

A number of methodological aspects concerning the estimation of retinol, ß-carotene and other provitamin A carotenoids has been discussed in Chapter 4. In their extensive review on the chromatographic separation of vitamin A-active compounds in foods, Lambert et al. (21) examined the importance of the proportion of total carotenoids as α- or ß-carotene in estimating the vitamin activity in foods. Generally, α- and ß-carotene are responsible for about 90% of the vitamin A activity, except in citrus fruits which contain relatively large amounts of cryptoxanthin which, like α-carotene, has half the vitamin A activity of ß-carotene. However, this should not be used as a justification for assigning 50% of the vitamin A activity of ß-carotene to the fraction of "other carotenoids" (total carotenoid content measured spectrophotometrically at 450 nm minus the ß-carotene content, see Chapter 4). Many carotenoids included in this fraction such as lutein in vegetables, lycopene in tomatoes and zeaxanthin in maize have no vitamin A activity. The lutein content of various vegetables has been reported recently (29). Large-scale re-analysis of foods included in existing food tables will be necessary. An example of such an effort are the recent analyses carried out in 50 vegetables from Thailand (30).

The use of an internal standard for the determination of ß-carotene in food samples has not yet been established as common practice. Through assistance provided by Dr G. Beecher (Nutrient Composition Laboratory, United States Department of Agriculture, Beltsville MD, USA), we were able to evaluate the suitability of a synthetic carotenoid with 45 carbon atoms (nonapreno-ß-carotene) for this purpose. Dried homogenized leafy vegetables have also been examined for possible use as an external reference material and research on both aspects is still in progress.

THE MAGNITUDE AND SEVERITY OF XEROPHTHALMIA AND POST-MEASLES BLINDNESS

Since publication in 1982 of the WHO report on the control of vitamin A deficiency and xerophthalmia which made no specific mention of the situation in Tanzania, much has changed. The meeting held in Dar es Salaam in November 1981 (2), sparked off a substantial amount of work. Thus, it can now be asked to what extent do these data answer the questions posed in 1981 and what information is still lacking.

Data collection between 1982 and 1986 in Tanzania

A summary of the data on the prevalence of xerophthalmia in Tanzania collected between 1982 and 1986 is given in Table 1. The data on 38,429 children distributed across 12 of the 21 regions of the country (see map on page 18) include those collected in the hospital-based surveillance study conducted by Assistant Medical Officers (AMO) Ophtholmology (40%) and those collected during prevalence surveys (60%).

Apart from data on measles patients collected within the AMO study, information on post-measles eye complications was collected in three prospective hospital-based studies. These studies, reported in Chapters 7 and 8, in papers by Foster and coworkers (31-33), and by Scudder and Makupa (4,33) are summarized in Table 2. In addition, the results of a further study by Foster et al. of 130 children with corneal ulceration of which 48 were post-measles cases, were discussed in Chapters 1 and 8 (34).

Magnitude and severity of xerophthalmia

The extent of mild forms of xerophthalmia observed in the hospital-based studies (which do not as such generate prevalence data, see also Chapter 1) and the community-based surveys with all age groups combined were similar in both groups of studies. However, corneal ulceration was more often recorded in the hospital-based surveillance programme but this is not surprising as many children would have been hospitalised because of the ulceration.

As outlined in Chapter 2, the hospitals in Ilembula and Iringa (both in Iringa Region), recorded more children with xerophthalmia than did other hospitals participating in this programme. In addition, children with Bitot's spots were observed in Tanga and Mbeya Hospitals during the first year and at Dodoma Hospital during the second year of the programme. However the proportion of hospitalised children enrolled in the study with xerophthalmia from these three hospitals was low: 0.25%, 0.19% and 0.25%, respectively. Night blindness

Table 1: Summary of data collected between 1982 and 1986 on the existence of xerophthalmia in Tanzania

Source (ref)	Location	Number of children examined	Xerophthalmia rates*
AMO surveillance	15 hospitals		XN 0.18%; X1B 0.15%;
programme (31)+	in 11 regions	17,006	X2 0.73%.
Prevalence surveys,			
see Chapters 2 and 3	Mbeya Rural District	188	
	Iringa Region		
	Phase one	5,975	X1A 0.13%; X1B 0.15%;
	Phase two	1,331	X2 0.04%; X3 0.02%;
	Phase three	1,049	XS 0.14%.
	Tabora Region		
	Phase one	5,266	
	Phase two	3,177	
	Kagera Region	4,437	
Grand total		38,429	

* For a description of the various stages of xerophthalmia see Chapter 1.
\+ Hospital-based surveillance programme conducted by Assistant Medical Officers Ophthalmology: total number of children examined was 20,861 of whom 3,855 were suffering from measles.

Table 2: Summary of data on the existence of post-measles eye lesions in hospitalised children collected in Tanzania between 1982 and 1986 within the national programme on post-measles blindness

Location (ref)	Measles patients		Corneal lesions	
	Total	Ophthalmological examinations	Xerosis n (%)*	Ulceration n (%)
KCMC, Moshi (4,33)|	213	150	4 (2.6%)	2 (1.3%)
Mvumi Hospital, Dodoma (4)	193	180	-	7 (3.9%)
Dar es Salaam				
- pilot (4,35)	105	104	-	4 (3.8%)
- main (35)	560	479	13 (2.7%)	15 (3.1%)
AMO surveillance				
programme (31)+	3,855	3,855	-	165 (4.3%)

* n = Number of children with proportion (%) in parentheses.
\+ See similar footnote in Table 1.
\| KCMC = Kilimanjaro Christian Medical College.

(XN) was reported much more during the second year of the programme (0.33%, 28/8,364) than during the first year (0.07%, 6/8,642) and was only recorded in meaningful numbers from the hospitals in Arusha, Dodoma and Tanga. This also reflects the difficulties met in diagnozing XN as outlined in Chapters 2 and 3. The number of corneal scars recorded was rather similar in both years: 0.66% and 0.56% in the first and second year respectively for unilateral scars, and 0.12% in both years for bilateral scars. The highest proportion of children with corneal scars (see Chapter 2) was observed in Ilembula (9.8%), in Iringa (3.4%) and in Singida (1.1%, 26/2,429).

The problem of diagnozing conjunctival xerosis (X1A) has been discussed in Chapters 1 and 2. Under field conditions, where it is not possible to investigate the results of treatment, it may be difficult to diagnoze corneal xerosis. During a supplementation trial carried out in North Sumatra (Indonesia), "conjunctival (X1A) and corneal xerosis (X2) were excluded as being potentially less reliable" (36). It is therefore not surprizing that although much has been done to standardize the diagnosis of xerophthalmia, not all symptoms are easily diagnozed.

An overall prevalence of 0.02% for corneal ulceration/keratomalacia (X3) and of 0.14% for xerophthalmic scars (XS) recorded during the prevalence surveys are both in excess of the WHO criteria (see Table 1 of Chapter 1). During the initial survey in Iringa Region, some overreporting of corneal scars may have taken place. However, even during the surveys in Tabora Region when conservative estimates were made of the prevalence of xerophthalmic scars (XS), the prevalence was nearly identical to (0.04%, 1986) or exceeded (0.08%, 1985) the WHO criterion of 0.05% for XS.

In the large-scale investigations in Indonesia, corneal scars (XS) were observed twice as often as corneal xerosis and corneal ulceration (37). In 18,660 children in Bangladesh, scars were seen 2½ times as often as X2/X3 (38). Our data are more in line with the results of these surveys, than with the results of a survey carried out in Malawi in which corneal scars were observed ten times more often than corneal xerosis/ulceration (39). Out of a total of 76 scars, 44 were reported as non-xerophthalmic scars. The survey in Malawi was in an area known for its high proportion of blind people and this might explain the differences partly.

The findings presented in Chapter 5 concerning the intake of vitamin A among children with and without xerophthalmia confirm a low intake generally as well as a wide variation in intake between individuals. However, such studies in which the intake of the majority of cases and controls does not reach the

recommended intake of vitamin A call the appropriateness of the recommended daily intake (RDI) into question. In Chapter 5, it has been proposed that the WHO/FAO standards should be used in Tanzania instead of the RDI established for the country. Olson (40) reviewed the arguments which formed the basis for the current RDI. The report of a joint FAO/WHO Expert Group set up in 1985 to review the requirements for vitamin A and other vitamins is still awaited (41). The new recommendations expressed in terms of safe levels of intake are expected, at least for adults, to be lower than the current recommendations for most countries.

Guidelines have been suggested for making a rough estimate, based on prevalence data, of the number of children who lose their sight because of vitamin A deficiency (42). Using the data presented for Tabora Region, and assuming this region is representative of Tanzania as a whole, about 5,000 children will develop xerophthalmic corneal scars annually in Tanzania. This figure is a little higher than that suggested earlier (33). However, in the absence of incidence data, it is not possible to provide a more reliable estimate of the number of children developing eye lesions each year as a result of xerophthalmia.

Combining the data of the prevalence surveys presented in this thesis with other data available, it can be concluded that xerophthalmia exists in certain pockets (see Appendix I for list of villages studied) which may comprise one village (Ilula) or two or more villages (Kitenga and Kijombe; Mfyome and Itagutwa, and the villages in Lusu ward) and that, in general, xerophthalmia may be a threat in the drier Central Highlands covering parts of Dodoma, Iringa, Singida, Shinyanga and Tabora Regions. Xerophthalmia was also present in areas like the southern part of Kagera Region while also in the northern part of this region xerophthalmia has been recorded (E.A. Hackenitz, pers obs). No data are available from Southern Tanzania (Ruvuma, Mtwara and Lindi Regions), and Western Tanzania (Rukwa and Kigoma Regions).

Blindness due to measles

Combining the results presented in Table 2, the proportion of measles patients developing corneal ulceration was 4%. As procedures for admitting patients with measles to hospital will differ between hospitals, it is difficult to compare the severity of post-measles eye lesions recorded in various hospitals. However, as explained in Chapter 8, it is unlikely that the routine administration of oral high doses of vitamin A to children admitted to hospital in Mbeya could explain why no corneal lesions were observed among 900 measles

patients in Mbeya while such lesions were observed among measles patients admitted elsewhere. It is difficult to relate differences in the prevalence of post-measles corneal eye lesions observed in Mbeya (43) and in Moshi, Dodoma (Mvumi) and Dar es Salaam (Table 2) with nutritional status. Both Mbeya and Moshi would appear to have more food available and a more pleasant climate than do Dar es Salaam and Dodoma. In addition, there were no clear differences between nutritional status of the children admitted to hospital in Moshi and Dar es Salaam.

The question can now be asked as to how many children develop, each year following measles, loss of visual acuity. The number of children suffering from measles each year was estimated a number of years ago to be 600,000 (see Chapter 1). Efforts to increase vaccination coverage in recent years may have had some effect in reducing the incidence of measles and it may well be that the annual incidence has been reduced to 400,000. If it is assumed that the proportion of children with measles who will develop corneal ulcers is 4% as has been found in the hospital-based studies (Table 2), this would give rise to 16,000 new cases each year of whom 25-30% are likely to die before recovering from measles. Of the survivors, about 50%, 6,000 are likely to have permanent visual impairment. This figure possibly should be regarded as a maximum incidence rate of post-measles blindness as it is based on a prevalence of 4% of corneal ulceration in children developing measles.

Future activities

Surveys on xerophthalmia. It is envisaged that data collection on the prevalence of xerophthalmia will continue as part of ongoing nutrition programmes. For 1987 large scale surveys are planned for Mtwara Region. As outlined by Kusin et al. (44), the guidelines establised for prevalence surveys (see for example ref 42, 45) fullfil scientific criteria but are unrealistic when financial resources are limited. Therefore, ophthalmological examinations should be included, where possible, in nutritional surveillance programmes as was done in Iringa and Kagera Regions (Chapter 2). It should be investigated whether it is possible to include blindness prevention (as primary eye care) as part of a primary health care component in rural development programmes including those receiving multilateral or bilateral support. Good results have been achieved with this in Dodoma Region by the programmes organized by Mvumi Hospital and Dodoma Regional Hospital and as part of the Kongwa Primary Eye Care Project (see ref 33). In addition, more attention will need to be paid to other consequences of vitamin A deficiency and measles such as the increased risk of mortality and morbidity

from diarrhoea and pneumonia.

Availability of high-dose capsules. High-dose capsules of vitamin A have not always been available (2). Hopefully, the inclusion of these capsules in the Essential Drug Programme can help to overcome this problem. As recommended at the 1985 meeting, about one million capsules per year would be required to provide capsules to children with measles, with xerophthalmia, or severly malnourished. It seems to be entirely unnecessary, as was suggested by a UNICEF consultant on the basis of the results and recommendations of the 1985 meeting, to provide two to three capsules each year to all preschool-age children in Tanzania (46). Such a policy would require over 14 million capsules each year which is unnecessary and unrealistic. It will be necessary, however, to stress the importance of reporting children with a measles rash to a dispensary or health centre as soon as possible after the appearance of the rash. Then such children will be able to receive vitamin A as early as possible during the course of the illness. This message will have to be incorporated in the information given to all MCH-attendants.

Training. Through the series of meetings outlined in Chapter 1, more people have become aware of the xerophthalmia/post-measles blindness problem in Tanzania. Ophthalmological staff, including eye nurses, attended the meetings and/or participated in surveys and became much more aware of the whole issue and were able to record more patients with xerophthalmic eye lesions. Therefore it is necessary to continue organizing meetings, no longer at the national level but at the level of two to three regions. Suitable teaching material are available for this purpose. This will allow the identification and treatment of cases to be extended and a start to be made on preventive measures. Mild xerophthalmia (notably Bitot's spots) is not recognized by many health workers at the lower levels, such as MCH nurses and the Rural Medical Aids, despite that vitamin A deficiency is included in their training curricula.

Research. Research in Tanzania should now be directed more towards that required for prevention programmes. This would include work on the (pro)vitamin A content of foods not only as fresh products but also after drying preservation by other techniques, and preparation for consumption. TFNC is capable of such work as it has built up a good research capacity but it is disappointing to note that little bilateral or multilateral support has been available to support its research even though vitamin A deficiency has been identified by WHO, FAO and UNICEF as a subject for a special ten-year programme. In addition to research in direct support of prevention programmes, more research still needs to be carried out on the fundamental mechanisms involved in the aetiology of lesions resulting

from infection with measles and the lack of vitamin A. Often such fundamental work will involve the use of animal models.

Agricultural projects. A number of background activities have already been carried out. These included investigation of the pesticide content of fish oil prepared from fish caught in Lake Victoria and this has been shown not to be alarming. The decline in production of red palm oil has also been investigated and suggestions have been made for further action. In the FAO consultants report (12), a number of suggestions have been made for further activities on the agricultural front. However, it will be necessary to involve agriculturists and other non-medical personnel in the planning of programmes to combat vitamin A deficiency.

REFERENCES

1 TFNC. Vitamin A deficiency in Tanzania, Report of a National Seminar Dar es Salaam, TFNC Report No. 650. Dar es Salaam: Tanzania Food and Nutrition Centre, 1981.

2 Mrisho F, Pepping F, Lukmanji Z. Proceedings of a national symposium for vitamin A deficiency, November 16-18 1981 Dar es Salaam, TFNC Report No. 735. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

3 TFNC. Upungufu wa vitamin A Tanzania, Expert Committee Report, TFNC Report No. 718. Dar es Salaam: Tanzania Food and Nutrition Centre, 1982.

4 Kisanga P, Pepping F, Kavishe FP. Proceedings of a workshop on the control of vitamin A deficiency and xerophthalmia in Tanzania held at the Salvation Army in Dar es Salaam on 9th-11th September 1985, TFNC Report No. 980. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

5 TFNC. The food and nutrition policy for Tanzania, First National Food and Nutrition Conference 3-5 September Moshi, 1980 TFNC Report No. 483. Dar es Salaam: Tanzania Food and Nutrition Centre, 1980.

6 TFNC. A national programme on the control of vitamin A deficiency in Tanzania. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

7 Kinabo N. Eye diseases and services in Tanzania. Soc Sci Med 1983;17:1767-72.

8 Temalilwa CR, Sangana LH. Report on the present status of red palm oil production in Kigoma District, TFNC Rep No. 930. Dar es Salaam: Tanzania Food and Nutrition Centre, 1985.

9 UNICEF. Analysis of the situation of woman and children, volume 1 and 2 Government of the United Republic Of Tanzania and United Nations Children's Fund. Dar es Salaam, 1985

10 Merx RJHM. Introduction of a handoperated system for sunflower seed processing in the Ipuli Parish at Tabora (Tanzania). Amsterdam: Royal Tropical Institute, 1985.

11 Vencken, CMJ. De aanbeveling van visolie (Lates niloticus en Haplochromis) als energie- en vitamine A-bron in Tanzania. Wageningen: Department of Human Nutrition, 1987.

12 Van der Haar F. Ten years United Nations action programme Prevention and control of vitamin A deficiency in Tanzania: tentative FAO elements. Wageningen: ICFSN, 1986.

13 Scholte I. The development of a food composition table for use in a research project on vitamin A deficiency in Tanzania. Wageningen: Department of Human Nutrition Report No. 84-15, 1984

14 West CE, (ed.) Food composition table for use in a research programme on vitamin A deficiency in Tanzania. Interim edition. Department of Human Nutrition, Agricultural University Wageningen (The Netherlands), 1984.

15 Schultink JW. Food composition table for use in a research programme on vitamin A deficiency in Tanzania: report of work to improve the quality of data on vitamin A and provitamin A through analysis of selected foods and a search of the literature. Wageningen: Department of Human Nutrition, 1984.

16 Schultink JW, West CE, Pepping F. ß-carotene content of Tanzanian foodstuffs determined by high performance liquid chromatography. East Afr Med J 1987;64:368-71.

17 Arroyave G, Chichester CO, Flores H, et al. Biochemical methodology for the assessment of vitamin A status. Washington: IVACG/The Nutrition Foundation, 1982.

18 Mejía LA, Pineda O, Noreiga JF, Benitez J, Falla G. Significance of postprandial blood concentrations of retinol, retinol-binding protein, and carotenoids when assessing the vitamin A status of children. Am J Clin Nutr 1984;39:62-5.

19 Mejía LA, Arroyave G. Determination of vitamin A in blood. Some practical considerations on the time of collection of the specimens and the stability of the vitamin. Am J Clin Nutr 1983;37:147-51.

20 Sinaga HSRP. Vitamin A and protein status of preschool children in Suka village, North Sumatra. PhD thesis University of Amsterdam, 1981.

21 Lambert WE, Nelis HJ, De Ruyter MGM, De Leenheer AP. Vitamin A: Retinol, carotenoids, and related compounds. In: De Leenheer AP, Lambert WE, De Ruyter MGM, eds. Modern chromatographic analysis of the vitamins. New York/Basel: Marcel Dekker, 1986:1-72.

22 Speek AJ, Wongkham C, Limratana N, Saowakontha S, Schreurs WPH. Microdetermination of vitamin A in human plasma using high performance liquid chromatography with fluorescence detection. J Chrom 1986;382:284-9.

23 Mathews-Roth MM, Stampfer MJ. Some factors affecting determination of carotenoids in serum. Clin Chem 1984;30:459-61.

24 Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature 1981;290:201-8.

25 Driskell WJ, Bashor MM, Neese JW. Loss of vitamin A in long-term stored, frozen sera. Clin Chem Acta 1985;147:25-30.

26 Driskell WJ, Lackey AD, Hewet JS, Bashor MM. Stability of vitamin A in frozen sera. Clin Chem 1985;:871-2.

27 Glover J, Moxley L, Muhilal H, Weston S. Micro-method for fluorimetric assay of Retinol-binding protein in blood plasma. Clin Chem Acta 1974;50:371-80.

28 Gijbels M. Bepalen van holo- en apo-RBP in serum van kinderen uit Tanzania met behulp van poly-acrylamidegelelektroforese en immuno-elektroforese. Wageningen, Department of Human Nutrition, 1987.

29 Ramos DMR, Rodriquez-Amaya DB. Determination of the vitamin A value of common Brazilian leafy vegetables. J Micronut Anal 1987;3:147-55.

30 Speek AJ, Speek-Saichua S, Schreurs WHP. Total carotenoid and ß-carotene content of Thai vegetables and the effect of processing. Food Chemistry 1987 (in press).

31 Foster A, Kavishe F, Sommer A, Taylor HR. A simple surveillance system for xerophthalmia and childhood corneal ulceration. Bull Wrld Hlth Org 1986;64:725-8.

32 Barclay AJG, Foster A, Sommer A. Vitamin A supplements and mortality related to measles: a randomised clinical trial. Br Med J 1987;294:294-6.

33 Foster A, ed. Focus on blindness in Africa, Proceedings of the sub-regional prevention of blindness seminar for East and Central Africa, Moshi, Tanzania, Feb 13-18 1984. Moshi: Africa Region Medical Office of Christian Blind Mission International, 1984.

34 Foster A, Sommer A. Corneal ulceration, measles, and childhood blindness in Tanzania. Br J Ophthalmol 1987;71:331-43.

35 Pepping F, Hackenitz EA, Mroso DM, Franken S, West CE. The role of nutritional status with special reference to vitamin A in the development of post measles eye lesions II. Eye lesions, and other clinical complications in relation to nutritional status (submitted for publication).

36 Sommer A, Tarwotjo I, Djunaedi E, et al. Impact of vitamin A supplementation of childhood mortality A randomised controlled community trial. Lancet 1986;i:1169-73.

37 Sommer A. Nutritional blindness: Xerophthalmia and keratomalacia. New York: Oxford University Press, 1982.

38 Cohen NC, Mitra M, Sprague J, Islam S, Leemhuis-de Regt E, Jalil M. Impact of massive dosis of vitamin A on nutritional blindness in Bangladesh. Am J Clin Nutr 1987;45:970-6.

39 Tielsch JM, West KP, Katz J, et al. Prevalence and severity of xerophthalmia in Southern Malawi. Am J Epid 1986;124:561-8.

40 Olson JA. Recommended dietary intakes (RDI) of vitamin A in humans. Am J Clin Nutr 1987;45:704-16.

41 Pitt GA. The proposed new FAO/WHO recommendations for vitamin A requirements. Abstract 7th Fat Soluble Vitamins Meeting Leeds England, 1987.

42 Sommer A. Field guide to the detection and control of xerophthalmia 2nd ed. Geneva: WHO, 1982.

43 Burgess W, Mduma B, Josephson GV. Measles in Mbeya, Tanzania 1981-1983. J Trop Pediat 1986;32:148-53.

44 Kusin JA, Sinaga HSRP, Marpaung AM. Xerophthalmia in North Sumatra. Trop Geogr Med 1977;29:41-6.

45 Tielsch JM. A generalized xerophthalmia survey package. Baltimore: International Center for Epedemiological and Preventive Ophthalmology, 1984.

46 Eastman S. Joint WHO/UNICEF Nutrition Support Programme Vitamin A deficiency, xerophthalmia and nutritional blindness in JNSP countries: a review and recommendations, New York: 1986.

SUMMARY

Vitamin A deficiency is responsible for eye lesions especially in children. Involvement of the retina results in night blindness which is readily reversible when sufficient vitamin A is supplied. However involvement of the conjunctiva and cornea produces first a dry appearance of the exposed surface of the eye (xerophthalmia) and ultimately corneal ulceration (keratomalacia) which may result in permanent loss of vision in the affected eye. Eye lesions which develop in children following measles are somewhat similar to the corneal lesions produced by vitamin A deficiency. The aim of the studies described in this thesis was to investigate eye lesions as a result of either vitamin A deficiency or measles. The work was carried out as an integral part of the research programme of the Tanzania Food and Nutrition Centre in Dar es Salaam.

In Chapter 1, the role of vitamin A in metabolism, and the classification of the various stages of xerophthalmia are outlined briefly. After an explanation of the concept of "post-measles blindness", a literature review is presented on the prevalence of xerophthalmia in Tanzania and of post-measles blindness throughout East Africa. Recent developments in our knowledge of the influence of vitamin A status on morbidity and mortality are then discussed. This is followed by an outline of the work carried out in Tanzania between 1981 and 1986, of which the work in this thesis forms a part, on vitamin A deficiency, xerophthalmia and post-measles blindness.

In Chapters 2 and 3, the results of a number of studies carried out in order to estimate the prevalence of xerophthalmia are presented. A total of 21,423 children were examined of which 5,457 children were included in three follow-up surveys. Apart from the prevalence of xerophthalmia, the nutritional status of the children based on standard anthropometric criteria such as weight-for-age, length-for-age and weight-for-length was examined. Fingerprick blood samples were collected to examine the prevalence of malaria, packed cell volume and levels of haemoglobin and serum proteins including retinol-binding protein (RBP) and prealbumin. Information on morbidity and status of vaccination against a number of diseases was obtained by administering a short questionnaire. The most important findings were as follows.

- Xerophthalmia was present in a number of villages or clusters of villages in Tabora, Iringa and Kagera Regions. Hence xerophthalmia should be regarded as a public health problem in a number of areas albeit of restrictive size.

- Bitot's spots (X1B) were predominantly found in boys and most cases were above the age of six years. Of the children with Bitot's spots one third was chronically malnourished and 50% responded positively to treatment with vitamin A.
- Half of the corneal scars could be attributed to measles, while only a minority could be attributed to vitamin A deficiency.
- Chronic malnutrition was found to increase with age and, in Tabora Region, chronic and acute malnutrition were observed in about 25% of the children. In the eight districts surveyed in Mbeya, Iringa and Kagera Regions, the prevalence of severe malnutrition ranged from 1.1% to 8.3% in children below the age of five years. Moderate malnutrition was observed in 41.1% to 51.8% of the underfive population. The prevalence of malnutrition was higher in Kagera than in other regions.

The content of α- and ß-carotene (provitamin A carotenoids) and of retinol in a number of foods is presented in Chapter 4. Dried leafy vegetables still contain considerable amounts of ß-carotene while staple foods, such as sorghum and millet contain practically no vitamin A. The analytical values obtained in the present studies by high performance liquid chromatography (HPLC), differ from previous values particularly when the proportion of vitamin A activity provided by α- and ß-carotene is low. The data obtained have been incorporated into a food composition table created for the research described in this thesis.

Results on the food intake of 26 children in Tabora Region, nine of whom had xerophthalmia (Bitot's spots, X1B), are presented in Chapter 5. Data were collected over two periods of two days on food prepared by the family and on food intake of each child. Energy intake was found to be low while protein intake appeared to be adequate although the protein came mainly from vegetable sources. Vitamin A intake was lower (although not significantly) in the children with xerophthalmia and this difference could be attributed to a lower intake of retinol. Nutrition education, based on increased adoption of existing good feeding habits would appear to be the method of choice for improving the nutritional status, including that of vitamin A, of the children.

Chapter 6 deals in detail with the clinical chemical analyses which were carried out in the course of the work. Special attention is given to the quality control measures taken. In each analytical series, carried out by either radial immunodiffusion for the analyses of albumin, prealbumin or RBP, or by HPLC for the determination of retinol, a pooled serum was analysed as

part of the internal quality control programme. When the results obtained with this pooled serum did not meet previously established criteria the results obtained in the series were rejected. Studies in which external reference standards were used indicated that our analyses of retinol produced a slight overestimation for lower values and a slight underestimation for higher values. The necessity of external standardization of analyses is emphasized.

The research carried out on the aetiology of post-measles blindness is described in Chapters 7 and 8. A total of 841 children, including 665 with measles, were examined in a cross-sectional study in four hospitals in Dar es Salaam and in one hospital 30 km west of Dar es Salaam. The eyes of half the children were examined on two or more occasions. Some children contracted measles at a very young age (3 months) and two thirds of the children with measles studied were below the age of 24 months. Malnutrition was predominantly of an acute form, characterized by a deficit in weight-for-length: 39.8% of the children with measles were below 80% of the reference standard. Mortality among measles patients while in hospital was 7.5%. All serum parameters estimated (retinol, RBP, prealbumin and albumin) were significantly depressed in children with measles. Although it is known that serum levels fall as a result of infection, it is alarming that 15% of the children with measles had extremely low serum retinol levels (< 0.17 μmol/l). In children free of acute infection, a level of 0.35 μmol/l of retinol is regarded as a level below which xerophthalmic eye lesions are often seen. Serious eye lesions, such as corneal xerosis and corneal ulceration, which can easily lead to permanent reduction of vision were found in 5% of the children. Recovery from corneal xerosis was in most cases (70%) successful. Of the children with corneal ulceration, 25% developed the condition after admission. The mortality rate in children with corneal ulceration was 37%. Serum retinol levels in children with corneal xerosis and ulceration were significantly lower (0.23$\pm$0.05 μmol/l; mean$\pm$SE, n=19) than in children with measles without eye lesions (0.38$\pm$0.02 μmol/l, n=89). Confluent measles keratitis and the absence of watery tears were often found together in the eye lesions which arose as a complication of measles. In the longitudinal ophthalmological investigations spontaneous healing was observed within four days of the first observation in at least one third of the lesions.

In the final chapter (Chapter 9), the results of the research on vitamin A deficiency in Tanzania between 1983 and 1986, are discussed. The role of various organizations and progress made in Tanzania in combatting xerophthalmia and vitamin A deficiency are outlined. Methodological problems

associated with the collection and storage of serum samples and of the determination of holo-RBP and carotenoids are discussed. The results of the prevalence surveys are summarized and related to previous work which has been carried out.

The data obtained do not allow a conclusion about the number of children surviving each year with permanent eye lesions due to xerophthalmia. However, further sensitization of health staff will be necessary to locate other foci with a high prevalence of xerophthalmia and to ensure proper treatment.

Assuming that 4% of all children with measles develop corneal ulceration, it is estimated that 6,000 children would develop permanent eye lesions due to measles annually. However, as the proportion of 4% is based on hospitalised children, the actual number developing post-measles corneal scarring may be lower. The last chapter of the thesis closes with a discussion of recommendations for actvities which possibly could reduce xerophthalmia and post-measles eye lesions.

SAMENVATTING

Vitamine A deficiëntie is verantwoordelijk voor oogafwijkingen welke vooral gezien worden bij kinderen. Aantasting van het netvlies (retina) kan leiden tot nachtblindheid wat snel geneest wanneer voldoende vitamine A wordt verstrekt. Echter aantasting van het oogbindvlies (conjunctiva) en van het hoornvlies (cornea) veroorzaken eerst een droog uiterlijk van het oog (xerophthalmie) en uiteindelijk een ulcus corneae (keratomalacie) wat kan resulteren in een permanent verlies van het gezichtsvermogen in het aangetaste oog. De oogproblemen waargenomen bij kinderen met mazelen vertonen overeenkomst met de oogproblemen als gevolg van een vitamine A tekort. Deze beide vormen van oogafwijkingen, enerzijds ten gevolge van een vitamine A tekort, en anderzijds de problemen gerelateerd aan mazelen (post-measles blindness), vormen het studieobject van dit proefschrift. Het onderzoek beschreven in dit proefschrift is uitgevoerd als een integraal onderdeel van het onderzoeksprogramma van het Nationale Tanzaniaanse Voedingsinstituut (TFNC).

In hoofdstuk 1 wordt in het kort ingegaan op de rol van vitamine A, in het metabolisme en op de classificatie van de verschillende stadia van xerophthalmie. Na een uitleg van het begrip "post-measles blindness" wordt een overzicht gegeven van de literatuurgegevens over het voorkomen van xerophthalmie in Tanzania en van post-measles blindness in Oost Afrika. Na bespreking van recente ontwikkelingen betreffende de invloed van vitamine A status op morbiditeit en mortaliteit, wordt een overzicht gegeven van het onderzoek dat tussen 1981 en 1986 in Tanzania uitgevoerd is op het gebied van vitamine A deficiëntie, xerophthalmie en post-measles blindness, waarvan het werk beschreven in dit proefschrift een onderdeel uitmaakt.

In de hoofdstukken 2 en 3 worden een aantal onderzoeken naar de prevalentie van xerophthalmie besproken. In totaal werden 21.423 kinderen onderzocht, waarvan 5.457 kinderen in drie vervolgonderzoeken.

Naast de prevalentie van xerophthalmie werd de voedingstoestand onderzocht aan de hand van een aantal anthropometrische parameters, te weten gewicht voor leeftijd, lengte voor leeftijd en gewicht voor lengte.
Vingerprik-bloedmonsters werden verzameld ter bestudering van o.a. het voorkomen van malaria, de haematokrietwaarde en het haemoglobinegehalte en de niveaus van serumeiwitten: retinol-bindingseiwit (RBP) en prealbumine.
Gegevens over morbiditeit en de vaccinatie tegen een aantal kinderziekten werden verkregen door het afnemen van een korte vragenlijst.

De belangrijkste uitkomsten van deze prevalentiestudies waren als volgt:

- xerophthalmie komt voor in een aantal dorpen of groepen van dorpen in de regio's Tabora, Iringa en Kagera en derhalve dient xerophthalmie in een aantal gebieden, beperkt van omvang, als een volksgezondheidsprobleem te worden beschouwd;
- Bitotse vlekken (X1B) werden voornamelijk waargenomen bij jongens en bij kinderen boven de leeftijd van zes jaar. Van de groep kinderen met Bitotse vlekken was ruim 30% chronisch ondervoed en reageerde 50% positief op behandeling met vitamine A;
- corneale littekens waren in ongeveer de helft van de gevallen een gevolg van mazelen en slechts in een minderheid van de gevallen een gevolg van xerophthalmie (XS);
- vooral chronische ondervoeding nam toe met het stijgen van de leeftijd. In de regio Tabora werden chronische en acute ondervoeding waargenomen in een kwart van de kinderen onder de leeftijd van zes jaar. In de acht districten onderzocht in de regio's Mbeya, Iringa en Kagera werd ernstige ondervoeding waargenomen variërend van 1,1% tot 8,3% per district bij kinderen beneden de leeftijd van vijf jaar. De situatie in Kagera was ongunstig in vergelijking met de andere regio's.

Het gehalte aan α- en ß-caroteen, welke ook wel aangeduid worden als provitamine A, en retinol in een aantal voedingsmiddelen wordt gepresenteerd in Hoofdstuk 4. Gedroogde bladgroenten bevatten nog een aanzienlijke hoeveelheid ß-caroteen. Basisvoedsel zoals sorghum en gierst bevat nagenoeg geen vitamine A activiteit. De analysecijfers, verkregen door middel van hoge druk vloeistofchromatografie (HPLC) wijken meer af van bestaande waarden naarmate het deel van de vitamine A activiteit geleverd door α- en/of ß-caroteen afneemt. De verkregen waarden zijn ingepast in een voedingsmiddelentabel, opgezet voor het onderzoek beschreven in dit proefschrift.

De voedselopname van 26 kinderen, waarvan negen met xerophthalmie (Bitotse vlekken), in Tabora regio wordt besproken in Hoofdstuk 5. Gedurende vier dagen, verdeeld in twee perioden van twee dagen, werd de voedselbereiding in het betreffende gezin en de voedselopname van het individuele kind gemeten. De energieopname was laag terwijl de eiwitopname adequaat was, hoewel voornamelijk bestaande uit plantaardig eiwit. De vitamine A opname was lager (alhoewel niet significant) voor de kinderen met xerophthalmie in vergelijking met de kinderen zonder xerophthalmie en dit verschil werd veroorzaakt door een lagere retinolopname in de eerste groep. Voedingsvoorlichting uitgaande van een verdere toepassing van bestaande goede voedingsgewoonten, lijkt de eerst aangewezen methode ter verbetering van de voedingstoestand, met inbegrip van

de vitamine A status, van de kinderen.

In Hoofdstuk 6 wordt nader ingegaan op de klinisch-chemische analyses zoals uitgevoerd gedurende het project. Speciale aandacht wordt besteed aan de toegepaste kwaliteitscontrole. In elke serie bepalingen, hetzij uitgevoerd m.b.v. radiale immunodiffusie voor de bepaling van albumine, prealbumine of retinol-bindingseiwit of m.b.v. HPLC voor de bepaling van retinol, werd een poolserum geanalyseerd als onderdeel van de interne kwaliteitscontrole. Indien dit poolserum een uitkomst gaf welke niet voldeed aan vooraf vastgestelde eisen, werden de resultaten verkregen in de betreffende serie verworpen. Uit experimenten uitgevoerd met extern referentiemateriaal, bleek dat er in onze retinolanalyses sprake was van een geringe overschatting van lage waarden en een geringe onderschatting van hoge waarden. Voorts wordt ingegaan op de noodzaak van een uitbouw van het gebruik van extern referentiemateriaal.

Het uitgevoerde onderzoek naar de etiologie van de zogenaamde "post-measles blindness" wordt beschreven in de Hoofdstukken 7 en 8. In totaal werden in een dwars-doorsnede onderzoek 841 kinderen onderzocht, waarvan 665 met mazelen. Dit onderzoek is uitgevoerd in vier ziekenhuizen in Dar es Salaam en één ziekenhuis 30 km ten westen van deze stad. Herhaald oogonderzoek werd uitgevoerd bij de helft van de kinderen. Mazelen komt in Dar es Salaam al op zeer jonge leeftijd (3 maanden!) voor en tweederde van de onderzochte kinderen was jonger dan twee jaar. De waargenomen ondervoeding bij de kinderen met mazelen was voornamelijk van acute aard, gekarakteriseerd door o.a. een laag gewicht voor lengte, waarbij ondervoeding bij 39,8% van de onderzochte populatie werd waargenomen. De sterfte tijdens de periode van ziekenhuisopname bedroeg ruim 7%. Alle gemeten serumwaarden (retinol, retinol-bindingseiwit, prealbumine en albumine) waren sterk verlaagd in de kinderen met mazelen. Alhoewel het bekend is dat serumwaarden dalen als gevolg van infectie is het feit dat 15% van de kinderen een extreem laag serumretinolgehalte (< 0,17 μmol/l) vertoonde zorgwekkend. In kinderen vrij van acute infectieziekten wordt een ondergrens van 0,35 μmol/l retinol aangehouden als grenswaarde waar beneden xerophthalmia veelvuldig optreedt. Ernstige oogaandoeningen, te weten xerosis corneae en ulcus corneae welke gemakkelijk kunnen leiden tot permanente oogafwijkingen, kwamen voor bij ruim 5% van de kinderen. Het herstel van de kinderen met xerosis corneae verliep in de meeste gevallen (70%) gunstig. Van de waargenomen ulcera ontwikkelde 25% zich gedurende opname. De mortaliteit bij de kinderen met ulcus corneae was 37%. Serum-retinolwaarden in kinderen met xerosis en/of ulcus corneae [0,23$\pm$0,05 μmol/l (gemiddelde $\pm$ standaardfout), n=19] waren significant lager in vergelijking

met kinderen met mazelen zonder oogaandoeningen 0,38±0,02 µmol/l (n=89). Wat betreft de oogaandoeningen welke voorkomen als een complicatie van de mazelen bleek dat mazelen keratitis en de afwezigheid van traanvloeistof veelvuldig in combinatie voorkwamen. Uit het longitudinale oogonderzoek bleek dat in tenminste één derde van de gevallen deze aandoeningen binnen vier dagen na de eerste observatie verdwenen.

In het slothoofdstuk (Hoofdstuk 9) wordt ingegaan op de bereikte resultaten in Tanzania tussen 1983 en 1986 van het onderzoek naar en de preventie van vitamine A deficiëntie. De plaats van verschillende organisaties in het kader van maatregelen ter bestrijding van xerophthalmie en vitamine A deficiëntie worden toegelicht. Er wordt ingegaan op methodologische aspecten van de afname en opslag van serummonsters, van de analyse van holo-RBP en van de bepaling van carotenen. De resultaten van het prevalentieonderzoek worden samengevat en gerelateerd aan andere beschikbare gegevens. De resultaten van het onderzoek naar het voorkomen van oogaandoeningen tijdens of na mazelen worden vergeleken met recente gegevens eveneens uit Tanzania. De verkregen gegevens staan geen conclusie toe over het aantal kinderen wat elk jaar permanent oogletsel oploopt als gevolg van xerophthalmie. Echter een verdergaande bewustwording bij personeel in de gezondheidszorg is nodig om andere gebieden met een hoge prevalentie aan xerophthalmie te localiseren en een goede behandeling te garanderen.

Wanneer er van uitgegaan wordt dat 4% van alle kinderen met mazelen een ulcus corneae ontwikkelt, resulteert dit tot een schatting van 6.000 kinderen met permanent oogletsel als gevolg van mazelen per jaar. Daar echter deze 4% gebaseerd is op gehospitaliseerde kinderen ligt het werkelijke aantal waarschijnlijk lager. Tot slot worden een aantal activiteiten besproken welke mogelijk in de toekomst kunnen bijdragen aan een vermindering van de prevalentie van xerophthalmie en van oogaandoeningen na mazelen.

A P P E N D I C E S

APPENDIX I

LIST OF VILLAGES STUDIED IN THE PREVALENCE SURVEYS ON XEROPHTHALMIA WHICH WERE ORGANIZED BY TANZANIA FOOD AND NUTRITION CENTRE BETWEEN 1983 AND 1986 RESULTS OF THE SURVEYS ARE PRESENTED IN CHAPTERS 2 AND 3

Survey date	Village	Number of children*
MBEYA REGION		
Mbeya Rural District		
18.11.83	Ilembo	27
19–20.11.83	Santilya	129
23.11.83	Ijombe	32
IRINGA REGION		
Iringa Rural District Pawaga Division		
28.03.84	Mkombilenga	72+
29.11.84	"	81|
19.04.85	"	93¶
29.03.84	Magozi	90+
29.11.84	"	136|
19.04.85	"	47¶
30.03.84	Luganga	106+
28.11.84	Kisanga	68|
Iringa Rural District Kalenga Division		
27.03.84	Mangalali	239+
27.11.84	"	93|
6.04.84	Nyamihuu	78
26.11.85	"	204|
20.04.85	"	172¶
31.03.84	Mfyome	209
30.11.84	"	211|
25.04.85	"	158¶
3.04.84	Itagutwa	190
3.12.84	"	164|
24.04.85	"	147¶
2.04.84	Ihemu	104
5–10.03.84	Ilula	670§
Iringa Rural District Mlolo Division		
4.04.84	Ngenza	246
5.04.84	Nyabula	266
IRANGA REGION		
Makete District Lupalilo Division		
4.06.84	Tandara	129+
5.06.84	Ihele	97+
6.06.84	Mago	117+
7.06.84	Malembuli	101+
IRINGA REGION		
Mufindi District Ifwagi Division		
14.05.84	Kitelewasi	112
15.05.84	Luganga	124
16.05.84	Ludilo	186
17.05.84	Mkonge	218
18.05.84	Ihefu (Sao Hill)	135
19.05.84	Ikanga	180
21.05.84	Kitiru	112
22.05.84	Wami	113
7.11.84	Ikongozi	270
IRINGA REGION		
Njombe District Wanging'ombe Division		
9.04.84	Wangutwa	132
10.04.84	Kijombe	328|
23.11.84	"	160|
22.04.85	"	216¶
11.04.84	Igelehedza	103
12.04.84	Ilewavila	103
13.04.84	Kitenge	215|
24.11.84	"	204|
23.04.85	"	216¶
16.04.84	Itandula	106+
17.04.84	Wanging'ombe	170+
IRINGA REGION		
Ludewa District Lugarawa Division		
24.05.84	Lugarawa	275
25.05.84	Lipangala	195
26.05.84	Mkongobaki	154
27.05.84	Kiyombo	302
28.05.84	Amani	267
31.05.84	Manga	225
1.06.84	Madiru	359
2.06.84	Madope	104

Survey date	Village	Number of children
TABORA REGION		
Tabora Rural District		
18.03.85	Ndono	284
19.03.85	Ufuluma	389
20.03.85	Nsololo	349
21.03.85	Kabila	215
TABORA REGION		
Urambo District		
22.03.85	Vumilia	362
23.03.85	Igagala	237
25.03.85	Ulyankulu	76
TABORA REGION		
Nzega District		
27.03.85	Isanzu	576
28.03.85	Mwaluzwilo	787
10.02.86	"	387§§
11.02.86	Bujulu	193§§
12.02.86	Ifumba	349§§
13.02.86	Mwakabasa	407§§
14.02.86	Mwasala	712§§
29.03.85	Isagehe	335
30.03.85	Ndekeli	923
TABORA REGION		
Igunga District		
1.04.85	Ussongo	217
2.04.85	Mwamashimba	245
3.04.85	Itumba/Lugubu	188
17.02.86	" "	419§§
18.02.85	Mgazi	172§§
19.02.85	Chagana	205§§
4.04.85	Igunga	79

Survey date	Village	Number of children
KAGERA REGION		
Biharamulo District Nyarubungo Division		
17.07.85	Kikomakoma	184
18.07.85	Kigoma	186
19.07.85	Kabindi	327
20.07.85	Katoke	216
22.07.85	Myamahanga	193
23.07.85	Luziba Rugando	377
24.07.85	Bisibo/Bisota	259
25.07.85	Biharamulo Town	57
Biharamulo District Lusahunga Division		
26.07.85	Ntumagu	239
27.07.85	Nyamigere	310
29.07.85	Nyakuhara	137
30.07.85	Lusahunga	258
KAGERA REGION		
Ngara District Bushubi Division		
31.07.85	Murusagamba	215
1.08.85	Ntanga	178
2.08.85	Keza	221
3.08.85	Nyamahwa	221
5.08.85	Kanyinya	231
6.08.85	Bukiriro	265
7.08.85	Rwinyana	285
8.08.85	Muyenzi	233

* Number of children examined includes children of all ages.
\+ The results of these villages are not included in the total number of eye examinations because no ophthalmological staff was available.
| Villages surveyed during first follow-up in Iringa Region.
¶ Villages surveyed during second follow-up in Iringa Region.
§ Village where study on risk factors of pregnancy was carried out.
§§ Villages surveyed during follow-up in Tabora Region.

APPENDIX II

PHOTOGRAPHS OF EYE LESIONS OBSERVED DURING THE STUDY

1: Bitot's spot (X1B) in the right eye of a six year old boy.

2: Bitot's spot in the left eye of a four year old boy.

3: Bitot's spot in the right eye of a four year old boy with night blindness, clearly showing a very dark pigmentation of the conjunctiva.

ALL CHILDREN PRESENTED IN PHOTOGRAPH 1-3 HAD BILATERAL BITOT'S SPOTS.

4: Bilateral Bitot's spots (X1B) in an eight year old boy.

5: Bilateral corneal scars (XS) in a three year old boy with a history of diarrhoea, vomiting and traditional medicine applied to the eyes because of eye problems. There was no history of measles. The child is visually handicapped grade II or III.

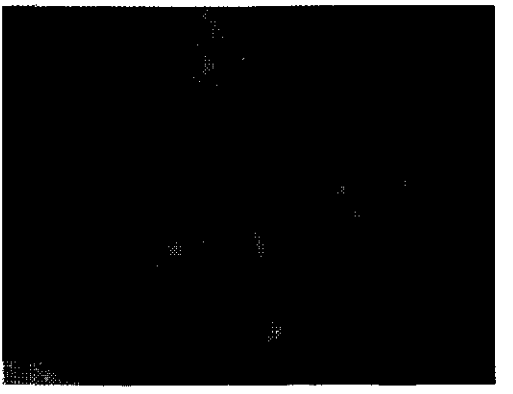
6: Corneal scar in the right eye of a six year old girl after measles. Her younger sister (4½ years old) showed small bilateral corneal scarring which, according to their mother, occurred during the same measles epidemic.

(Photographs no. 2,3,4 and 5 were taken by Margreet Hogeweg)

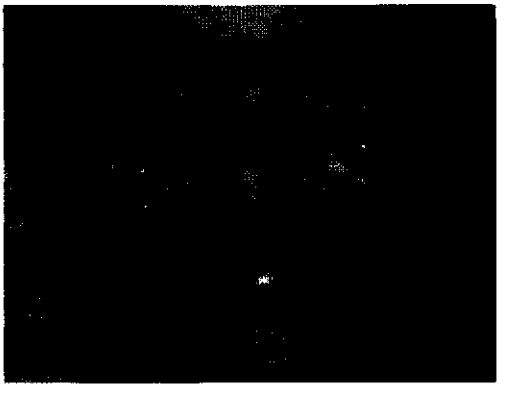
1

2

3



4



5

6

7: Phthisis bulbi in left eye of a five year old boy who developed the eye lesion at the age of two years following measles.

8: Ulcer/keratitis in a child with measles.

9: Loss of corneal epithelium with beginning keratomalacia in the lateral part of the cornea in a child with measles.

10: Keratomalacia in the right eye of a girl aged 17 months with measles, the girl died 15 days after the appearance of the rash.

11: Keratomalacia with deep ulcer in the left eye of a girl aged 22 months admitted with measles. Note the white appearance of the conjunctiva with no signs of an inflammatory reaction.

12: Perforating keratomalacia in right eye of a boy aged 18 months. The boy died nine days after the appearance of measles rash.

(Photographs no. 7 and 8 were taken by Margreet Hogeweg and no. 10 by Erica A. Hackenitz)

7

8

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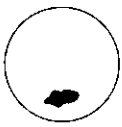
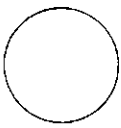
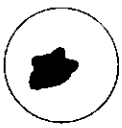
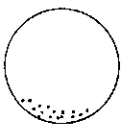
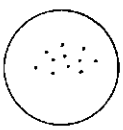
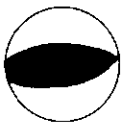
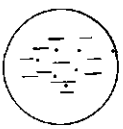
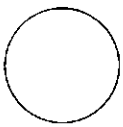
12

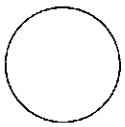
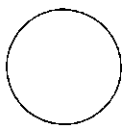
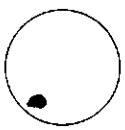
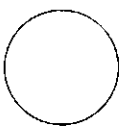
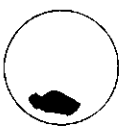
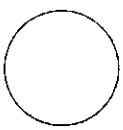
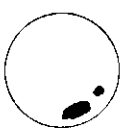
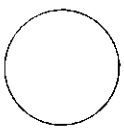
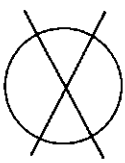
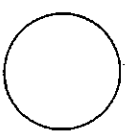
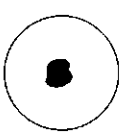
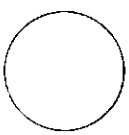
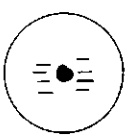
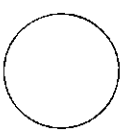
APPENDIX III

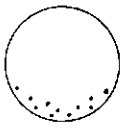
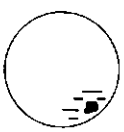
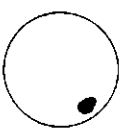
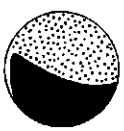
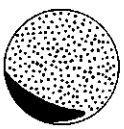
DRAWING OF THE EYE LESIONS AND SOME CHARACTERISTICS OF CHILDREN WITH CORNEAL ULCERATION/KERATOMALACIA FOLLOWING MEASLES

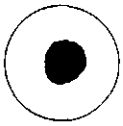
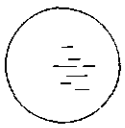
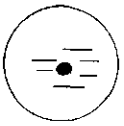
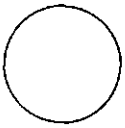
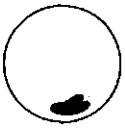
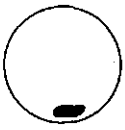
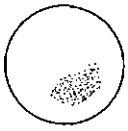
= ulceration; = corneal xerosis; = receding or beginning corneal infiltration

RE = right eye, LE = left eye; R + indicates the number of days after the outbreak of measles rash; WL, WA and LA indicate the proportion of reference weight-for-length, weight-for-age and length-for-age standards respectively.

Child code	RE	LE	Characteristics
T 58 R + 6			Boy; 15 months old; died on R+8; WL 80%, WA 77%, LA 97%; no abnormalities in LE and corneal xerosis RE (not shown)
T 75 R + 5			Girl; 9 months old; WL 76%, WA 76%, LA 102%; stomatitis, bronchitis, diarrhoea; measles keratitis RE; no follow up; vitamin A given on R+5
T 82 R + 7			Girl; 8 months old, incipient keratomalacia (LE); WL 78%, WA 70%, LA 96%; confluent measles rash, severe laryngitis, otitis, diarrhoea, vitamin A given on R+7 and R+8
R + 11			RE corneal xerosis, RE + LE no watery tears
R + 14			child discharged on R+14; RE clear, LE remains with scar

Child code	RE	LE	Characteristics
T 98 R +8			Girl; 32 months old; WL 89%, WA 72%, LA 87%; RE measles keratitis, LE corneal xerosis on R+3 (not shown) with also no watery tears RE + LE; vitamin A on R+3 and R+7
T 136 R + 8			Girl; 22 months old; WL 81%, WA 71%, LA 91%; RE and LE showed pigmented lateral triangle; no follow-up
T 167 R + ?			Boy; 22 months old; post-measles case; WL 72%, WA 60%, LA 89%; fever, pneumonia; retinol 0.18, albumin 434, prealbumin 1.31 and RBP 0.60 (μmol/l), no other eye lesions
			lesion one week later; ulcer healed slowly
T 226 R + 7			Girl; 17 months old; RE and LE corneal xerosis after measles keratitis; WL 74%, WA 72%, LA 96%; vitamin A given but RE deteriorated further; child died after transfer from Temeke Hospital to Muhimbili Hospital on R+15
R + 13			LE still showing xerosis, RE phthisis bulbi
T 231 R +6			Boy; 16 months old; child was discharged too early; WL 77%, WA 74%, LA 98%; no watery tears in RE and LE
R + 8			

Child code			Characteristics
	RE	LE	
T 237 R +13			Boy; 6 months old; first discharged at R+10 without eye lesions; WL 92%, WA 77%, LA 93%; re-admitted with post-measles complications (pneumonia and severe desquamation); prealbumin 1.47 and RBP 0.37 (µmol/l), child died on R+18
T 266 R + 6			Girl; 54 months old; WA 57%; corneal xerosis LE (not shown); retinol 0.28, albumin 438, prealbumin 1.00 and RBP 0.38 (µmol/l); vitamin A given on R+6
R + 8			
R + 15			corneal scar RE + LE
T 423 R + ?			Girl; 32 months old; died on day of admission; WA 42% (from hospital records), bronchopneumonia and anaemia; RE + LE no watery tears
K 46 R + 6			Boy; 16 months old; child died on day R+9; WA 65%; pneumonia; maculopapillar rash; ulcer in RE perforated; vitamin A given on R+3

Child code	RE	LE	Characteristics
W 4 R + 6			Boy; 18 months old; WL 85%, WA 77%, LA 95%; subcutaneous emphysema, retinol 0.15, albumin 830, prealbumin 1.75 and RBP 0.33 (μmol/l); received vitamin A on day of admission and next day
R + 15			child was discharged, both corneas healing with still small ulcer in LE
P 88 R + 13			Girl; 18 months; WL 83%, WA 80%, HA 98%, Superficial keratitis on R + 3 in both eyes, on R + 6 a dull cornea with mild xerosis in RE (not shown); on R + 15 ulcer had receded; prealbumin 0.89, retinol 0.10 (μmol/l); herpatic ulcer? vitamin A given on R+12
P 66 R + 10			Girl; 24 months; desquamating rash; Wl 69%, WA 57%, LA 88%; in RE lesion progressed to descemetolece; vitamin A given on R+10 and R+11
P 38 R + 7			Boy; 26 months; WL 82%, WA 72%, LA 95%; no follow-up

Note: The routine treatment of these children included the oral administration of vitamin A (200,000 I.U.) and the application of eye ointment (chloramphenicol). Pads were applied to the eyes of some children during the acute phase of ulceration.

APPENDIX IV

LIST OF FOODS INCLUDED IN FOOD COMPOSITION TABLE DEVELOPED FOR USE IN THE RESEARCH PROJECT ON VITAMIN A DEFICIENCY IN TANZANIA

English name	Swahili name	Scientific name
Cereals and grain products		
Maize, immature on cob, fresh	Mahindi mabichi	Zea mays
Maize, whole kernel, white, dried	Mahindi	"
Maize, whole kernel, yellow, dried	"	"
Maize, white, toasted		"
Maize flour, 60-80% extraction, white	Sembe	
Maize flour, 96% extraction, white	Unga wa mahindi	
Maize meal	Dona	
Millet finger, whole grain	Ulezi	Eleusine spp.
Millet finger, flour	Unga wa ulezi	"
Millet bullrush	Uwele	Pennisetum typhoides
Rice, lightly milled, parboiled	Mchele uliotokoswa	Oryza sativa
Rice, milled, polished	Mchele mweupe	"
Sorghum, whole grain	Mtama	Sorghum spp.
Sorghum, flour	Unga wa mtama	
Wheat whole parboiled	Ngano	Triticum vulgare
Wheat flour, 85% extraction	Unga wa ngano	
Wheat flour, 70% extraction	Unga wa ngano	
White bread	Mkate	
Brown bread	Mkate	
Cakes (from rice flour)	Vitumbua	
Cakes	Mandazi	
Biscuit		
Chapati	Chapati	
Starchy roots, tubers and fruit		
Breadfruit pulp, raw	Stafeli	Artocarpis communis
Cassava, bitter, fresh	Mhogo mbichi	Manihot esculenta
Cassava, meal	Unga wa mhogo	
Plantain, ripe, raw	Ndizi za kupika	Musa paradisiaca
Potato, raw	Viazi ulaya	Solanum tuberosum
Sweet potato, raw, yellow variety	Viazi vitamu	Ipomoea batatas
Sweet potato, raw, pale variety	Viazi vitamu	"
Taro/cocoyam, raw	Magimbi	Colocasia spp
Turnip/swede, root, raw	Figili	Brassica rapa
Yam, fresh	Viazi vikuu	Dioscora spp
Yam, flour	Unga wa viazi vikuu	
Grain legumes and legume products		
Beans/peas, fresh, shelled	Maharagwe ya njegere	Phaseolus spp
	mbichi baada ya kumenywa	Vigna spp
Beans, dried	Maharagwe	
Beans, green in pod, raw	Maharagwe mabichi bila kumenywa	
Bonavist/hyacinth bean, dried	Fiwi	Lablab niger
Chickpea, whole seeds, raw	Dengu	Cicer arietinum
Cowpea, dried	Kunde	Vigna spp
Cowpea, young green pods, raw	Kunde	
Kidney bean, red, dried	Maharagwe	Phaseolus vulgaris
Lentil, dried	Adesi	Lens esculenta
Mung bean, green, dried	Choroko Vigna radiata	Phaseolus aureus

English name	Swahili name	Scientific name
Mung bean, black, dried	Choroko nyeusi	Phaseolus mungo Vigna mungo
Pea, dried	Njegere	Pisum sativum
Pigeon pea, dried	Mbaazi	Cajanus cajan
Soya bean, dried	Soya	Glycine max
Velvet bean, dried	Upupu	Stizolobium deeringianum
Nuts and seeds		
Bambara groundnut, fresh	Njugu mawe	Voandezia subterranea
Cashew nut, dried	Korosno	Anacardium occidentale
Cocunut, immature kernel, fresh	Datu	Cocos nucifera
Cocunut, mature kernel, fresh	Nazi kavu	
Groundnut, dry	Karanga kavu	Arachis hypogaea
Melon seeds, without coat	Mbegu za tikiti	Citrullis vulgaris
Pumpkin seeds, without coat	Mbegu za mboga	Cucurbita spp Telfairia spp
Sunflower seeds, without coat	Mbegu za alizeti	Helianthus annuus
Vegetables and vegetable products		
Amaranth, leaves, raw	Mchicha	Amaranthus spp
Amaranth, leaves, cooked		
Bamboo shoots, raw	Kilele cha mwanzi	Bambusa spp
Baobab, leaves, raw	Majani ya ubuyu	Adansonia spp
Bean sprouts, raw	Maharage yaliootsehwa	Phaseolus/Vigna
Carrots, raw	Karoti	Daucus carota
Cassava, leaves, raw	Kisamvu	Manihot esculenta
Cauliflower, raw		Brassica oleracea
Cucumber, raw	Tango	Cucumis sativus
Cowpea, leaves, raw	Mkunde	Vigna unguiculata
Egg plant, raw	Biringanya	Solanum melongena
Hare's lettuce, raw	Mchunga	Sonchus spp
Leaves, pale green		
Leaves, medium green		
Leaves, dark green		
Lettuce, raw	Saladi	Lactuca sativa
Mushrooms, fresh	Uyoga	Agaricus spp
Okra, pods, raw	Bamia	Hibiscus esculentus
Okra, leaves, raw	Majani ya bamia	
Onion, shalot, raw	Vitunguu	Allium ascalonicum
Peppers, sweet green, raw	Pilipili mbichi na zilizoiva	Capsicum annuum
Peppers, sweet red, raw		
Pepper, leaves, raw	Majani ya mpilipili	Piper nigrum
Pumpkin, squash, raw	Boga	Cucurbita spp
Pumpkin, leaves, raw	Majani ya Mboga	
Pumpkin, leaves, dried		
Sweet potato, leaves, raw	Matembele mabichi	Ipomoea batatas
Taro, leaves, raw	Magimbi	Colocasia esculenta
Tomatoes	Nyanya na maganda	Lycopersicom esc
Turnip, leaves, raw	Majani ya figiri	Brassica rapa
Fruit		
Avocado	Parachichi	Persea americana
Baobab	Mbuyu	Adansonia digitata

English name	Swahili name	Scientific name
Banana	Ndizi mbivu	Musa sapientum
Citrus, orange/tangerine	Machungwa na chenza	Citrus sinensis C. aurantium C. reticulata
Citrus, grapefruit/pummelo	Balungi	Citrus grandis C. paradisi
Citrus, lemon/lime	Limao	Citrus aurantifolia C. limon
Dates, dried	Tende kavu	Phoenix dactylifera
Groundcherry/cape gooseberry	Zabibu mwitu	Physalis peruviana
Guava	Mapera	Psidium guajava
Mango, ripe, wihout skin	Embe	Magnifera indica
Mango, unripe, without skin	Embe	
Papaya	Papai	Carica papaya
Pineapple, fresh	Nanasi	Ananus comosus
Pomegranate	Komamanga	Punica granatum
Tamarind, dried	Ukwaju	Tamarindus indica
Tree tomato	Nyanya mshumaa	Cyphomandra betacea
Watermelon	Tikiti	Citrullus vulgaris
Sugars and syrups		
Soft drinks, commercial		
Sugar cane	Maji ya mwua	Saccharum officinarum
Sugar	Sukari nyeupe	
Meat, poultry and eggs		
Bacon, fat, whole side	Nyama ya nguruwe iliyonona	
Beef, moderately fat	Nyama ya ng'ombe ya kawaida iliyonona	
Egg, hen	Mayai ya kuku	
Goat, moderately fat	Nyama ya mbuzi	
Heart, beef	Moyo	
Kidney, beef	Mafigo	
Liver, beef	Maini	
Mutton, moderately fat	Nyama ya kondoo	
Pork, moderately fat	Nyama ya nguruwe	
Poultry, chicken	Nyama ya kuku	
Turtle	Kasa	
Fish and fish products		
Crustaceans (crab, lobster)	Kaa ya pwani	
Fish, dried	Samaki wakavu	
Fish, average filet	Samaki wa maji baridi mnofu	
Small dried fish	Dagaa	
Milk and milk products		
Milk, cow, whole	Maziwa ya ng'ombe yaliyo na mafuta	
Milk, cow, skimmed	Maziwa ya ng'ombe yaliyoondolewa mafuta	
Milk powder, cow, whole	Maziwa ya ng'ombe ya unga	
Milk, goat	Maziwa ya mbuzi	
Buttermilk		
Bebelac, no 1		
Bebelac, acidified		
Cerelac		
Lactogen		

English name	Swahili name	Scientific name
Totolac		
Vitilac		
Oils and fats		
Beef suet		
Butter, from cow's milk	Siagi	
Coconut oil		
Fish liver oil	Mafuta ya samaki	
Ghee, clarified butter	Samli	
Lard/animal fats	Mafuta na nguruwe na ya wanyama	
Margarine, fortified	Margarine	
Red palm oil, fresh	Mafuta ya mawese	
Red palm oil, stale		
Salad oil		
Sunflower oil	Mafuta ya alizeti	
Vegetable oils	Mafuta ya mimea	
Other		
Beer, local	Chibuku	
Beer, local	Pombe	
Caterpillars	Kiwavi	
Coconut milk	Tui la nazi	
Termites, fresh	Kumbikumbi	
Yeast, baker's, dry	Hamira	

Note: The initial version of the food composition table was used in association with a programme developed for an Apple microcomputer by Dr A.B. Cramwinckel. The most recent version can be used with the MicroNap data base access software developed by Dr G.P. Sevenhuysen on an IBM microcomputer. A poster version of the table has also been prepared.

CURRICULUM VITAE

Freerk Pepping werd op 24 juli 1953 geboren te Gieten. In 1969 behaalde hij het MULO-B diploma aan de openbare MULO te Gieten en in 1972 het HBS-B diploma aan het Dr Nassaucollege (tot 1971 de Rijks HBS) te Assen. In datzelfde jaar begon hij te studeren aan de Landbouwhogeschool te Wageningen. Van mei 1973 tot september 1974 vervulde hij zijn militaire dienstplicht. In maart 1981 slaagde hij voor het doctoraalexamen met als hoofdvak Voedingsleer en als bijvakken Biochemie en Medische Parasitologie (K.U. Nijmegen).
Van augustus 1981 tot en met april 1983 werkte hij voor de vakgroep Humane Voeding en de International Course in Food Science and Nutrition aan een aantal opdrachten in Wageningen, Tanzania en Senegal.
Per 1 mei 1983 trad hij in dienst van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek (ZWO) en verrichtte met financiële steun van de Stichting voor Wetenschappelijk Onderzoek van de Tropen (WOTRO) en van het Nationale Tanzaniaanse Voedingsinstituut het in dit proefschrift beschreven onderzoek.