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VITAMIN A SUPPLEMENTATION: METHODOLOGIES FOR FIELD TRIALS

Subcommittee on Vitamin A Deficiency
Prevention and Control
Committee on International Nutrition Programs
Food and Nutrition Board
Commission on Life Sciences
National Research Council (U.S.)

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Cover Photo: A Bangladeshi child receives a vitamin A capsule from a health worker through the government's nutrition program. The research reviewed by the subcommittee will be integrated with the government vitamin A deficiency control program. Photograph: UNICEF/Shamsuz Zaman.

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PREFACE

The Office of Nutrition in the U.S. Agency for International Development (USAID) plans to sponsor field trials to determine the impact of vitamin A supplementation on child mortality. In August 1985, that office asked the National Research Council (NRC) to provide guidance on the planning of these trials and eventually to evaluate their results. In subsequent discussions with the Committee on International Nutrition Programs (CINP), it was decided that a review of the designs of these studies prior to their implementation would also be beneficial.

To accomplish these tasks, the Subcommittee on Vitamin A Deficiency Prevention and Control was formed in March 1986 under the auspices of the CINP within the Food and Nutrition Board (FNB) of the NRC's Commission on Life Sciences. In August 1986, the subcommittee sponsored a workshop to examine the protocols for these studies and their methodological implications. The workshop participants included representatives from study teams in the United States and abroad as well as members of the subcommittee.

The first chapter is an executive summary, which highlights the major findings of the subcommittee. The second reviews the purpose and scope of the workshop. In Chapter 3, the subcommittee reviews and critiques the research that initially stimulated USAID's interest in this subject, namely, the vitamin A supplementation studies conducted by Sommer and his colleagues (1986) in Indonesia. In the fourth chapter, the subcommittee reviews the protocols for four proposed studies to be undertaken in the Philippines, Bangladesh, and the Sudan under USAID sponsorship. Three of these studies focus primarily on mortality; one is for an in-depth examination of morbidity in a smaller sample in the Philippines. In this chapter, the subcommittee also examines studies planned in India and The Gambia, which

were briefly discussed at the workshop. Chapter 5 addresses the major methodological issues relating to these studies, including the appropriateness of the study designs, choice of population, methods of measurement, and ethical considerations. The final chapter contains the subcommittee's conclusions regarding expected outcomes of the studies and study design and measurement. Although this report takes the study designs presented at the workshop as its point of departure, the subcommittee recognizes that as the investigators undertake the next step of implementing the studies, many aspects of the proposed designs may be modified or even the choice of country may be changed.

This report is confined to the material presented at the workshop. It does not attempt to provide overall directions for research to examine the impacts of vitamin A on morbidity and mortality. The subcommittee will address these broader issues in a future report.

The ethics of controlled trials proved to be the most difficult topic, and considerable time was devoted to it in the subcommittee's deliberations. Because agreement could not ultimately be reached, two subcommittee members, Lincoln Chen and Frank Chytil, wrote minority reports. This diversity of opinion should not come as a great surprise, since the ethics of controlled trials involve many complex issues. An example is the recent controversy in England over the need for controlled trials to investigate the role of folic acid in the prevention of anencephaly and spina bifida (Beardsley, 1983).

The subcommittee is grateful to members of CINP and the FNB for their helpful comments on drafts of the report. It also acknowledges the interest and support of Martin J. Forman, Nicolaas Luykx, and Samuel Kahn of USAID's Office of Nutrition. The subcommittee thanks Virginia Hight Laukaran, the FNB program officer for the study, for her timely and effective assistance. Her ingenuity and diligence greatly facilitated the work of the subcommittee. Thanks are also extended to Sushma Palmer, Director of the FNB; to Frances Peter, Deputy Director of the FNB, who edited the report; to Beth Hamill, FNB research assistant who validated the references; Maureen Sullivan who assisted with preparations for the workshop; and to Susan Barron and Janie Marshall of the FNB staff

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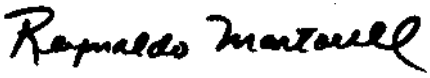

Reynaldo Martorell
Chairman
Subcommittee on Vitamin A
Deficiency Prevention and
Control

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Chapter 1

EXECUTIVE SUMMARY

This report is based on the outcome of a workshop held in August 1986 to review the protocols for three proposed field trials on child mortality and one on morbidity to study the impact of vitamin A supplementation. Recent studies in Indonesia by Sommer et al. (1983, 1986) have suggested that vitamin A supplementation may substantially reduce child mortality in populations with a high prevalence of mild and moderate vitamin A deficiency. In a randomized trial of massive doses of vitamin A given every 6 months to children 1 to 7 years of age, villages assigned to treatment experienced a 34% reduction in mortality compared with untreated villages. Under the sponsorship of the U.S. Agency for International Development, replications of this research in the form of large-scale community trials will be undertaken to validate the findings and learn the extent to which they might be generalized. The purpose of the workshop was primarily to review all relevant aspects of the proposed study designs and methods of measurement and to provide guidance to improve the research protocols. A secondary purpose was to develop recommendations that would be useful to other investigators who may be interested in undertaking field studies of vitamin A supplementation.

The subcommittee believes that the question of whether increased vitamin A intake will reduce child mortality rates deserves further research, given the policy implications for child survival programs. Three proposed studies of mortality involving population-based samples of approximately 30,000 to 40,000 children and one smaller intensive study of morbidity in approximately 600 children were reviewed by the subcommittee. The proposed mortality studies involved randomization of

villages (in Bangladesh and the Philippines) or households (in the Sudan) to treatment and control groups. The baseline data collection, duration of the intervention phase, frequency of vitamin A administration, eligibility criteria, and methods of assessment vary among the studies.

The sample size estimates in the proposed mortality studies are based on the assumption of a 20% to 25% reduction in child mortality, which was based on that reported in the Indonesian study. The subcommittee recognized that in populations with different levels and patterns of mortality and prevalences of vitamin A deficiency, mortality declines of this magnitude may not occur. Although even a much smaller reduction in mortality could have important public health implications, the subcommittee concluded that the sample size projections were reasonable in light of the resource constraints. For this reason, among others, the subcommittee recommended that the effect of vitamin A supplements on morbidity also be tested. Morbidity studies would offer greater statistical power than mortality studies; they should reinforce the results of mortality studies and provide useful information in their own right.

The subcommittee carefully considered the ethical issues that are relevant to the proposed studies. It concluded that each of the protocols should identify and treat children exhibiting mild to moderate as well as severe ocular signs or symptoms of vitamin A deficiency and exclude them from the study. Furthermore, existing knowledge does not unequivocally establish the beneficial effects of vitamin A in reducing mortality, nor does it exclude possible adverse effects. The subcommittee concluded that there is no ethical barrier to randomized controlled studies and that a double-blind design is preferred, although observational designs would also be useful where it is not possible to carry out experimental studies. The subcommittee also recommended that children in the control groups should derive a direct benefit from participating in the studies; for example, both treatment and control villages could be immunized according to established protocols.

The subcommittee made the following recommendations for design of the studies.

- The preferred duration of the studies is at least 2 years and should be not less than 1 year.

- Baseline studies should include ascertainment of mortality and also an adequate assessment of vitamin A nutriture, including measurements of serum vitamin A levels and some other indicators, such as presence of xerophthalmia. Standardized baseline measurements will facilitate comparisons among the studies and the interpretation of these comparisons.

- Morbidity and mortality should be carefully ascertained at baseline and during the course of follow-up.

- Randomization of villages rather than households or individuals is an acceptable procedure. Although it will increase the sample size requirement, it will greatly simplify the field work.

- Stratified randomization is not essential in the mortality studies, since stratified analysis after simple randomization will serve the purpose.

The subcommittee recognized that the tasks that lie ahead for the investigators, both in the United States and abroad, have great importance for policies to improve child survival in developing countries. While the studies are in progress, the subcommittee has planned to review the mechanisms by which vitamin A exerts its effects on infections. At the completion of the planned field studies, the subcommittee will once again convene to review and evaluate the results.

Chapter 2

INTRODUCTION

This report summarizes the outcome and recommendations of a workshop held August 17-18, 1986, at the National Academy of Sciences in Washington, D.C. (See Appendix A.) The goal of the workshop was to provide scientific and technical guidance on the design and methods of several field trials planned by the U.S. Agency for International Development (USAID) to determine the impact of vitamin A supplementation on morbidity and mortality in developing countries. USAID's Office of Nutrition requested that the Food and Nutrition Board's (FNB) Committee on International Nutrition Programs form a subcommittee to plan and sponsor this workshop and in the future to provide other guidance relating to its support of research on vitamin A and child survival, including an independent assessment of the final results of the field trials, which are expected to be completed by 1989.

Workshop participants included subcommittee members, field trial investigators from institutions abroad and in the United States, and other experts in the field of vitamin A supplementation trials (see Appendix B). The workshop was scheduled as early in the development of the USAID-sponsored studies as possible to provide a critical review of the design and methods of each study before the field work begins and to foster an informal collaborative network among investigators interested in studies of the impact of vitamin A on child survival.

The subcommittee decided that the workshop should focus on the identification of methods that can be applied in a uniform manner in the different study sites. Such uniformity would avoid differences in outcome attributable entirely to differences in method and would allow one to search for other explanations in the event that different results were obtained despite the use of

standard procedures. The subcommittee also emphasized the need to characterize local conditions, including the level of vitamin A deficiency and the prevalence of infectious diseases, and considered what types of data would be useful in this regard.

The importance of vitamin A deficiency as a cause of blindness in the developing world is well established. Xerophthalmia, keratomalacia, and blindness are widely recognized problems, and programs to prevent and treat vitamin A deficiency have been developed for this reason in many countries. There is compelling evidence that vitamin A serves dual functions: as the chromophore of the retina (which is responsible for light perception) and, equally important, proper differentiation of epithelial cells.

It was only recently that possible effects of vitamin A on the mortality of children without clinical evidence of deficiency was recognized. (See Appendix C for a discussion of research on the role of vitamin A in infection.) Studies in Indonesia suggested an association between vitamin A deficiency and mortality (Sommer et al., 1983) in children with signs no more severe than the World Health Organization's categories of XN (night blindness) and X1B (Bitot's spots) (WHO, 1976). In a subsequent community trial, children living in Indonesian villages without a program of vitamin A supplementation were found to have a mortality rate 35% greater than that in control villages with a program (Sommer et al., 1986). The results of these studies were presented at the workshop; their implications for the proposed research are discussed in Chapter 3.

The field trials proposed by USAID's Office of Nutrition are intended to test the hypothesis suggested by these studies. Protocols for three provisionally selected sites (the Philippines, Bangladesh, and the Sudan) were presented at the workshop. In the Philippines, USAID intends to support two separate studies to be undertaken by two different research groups. One is a community trial focusing on mortality; the other is a clinical trial to study morbidity. Only one study is under consideration for Bangladesh and for the Sudan. Studies to test the hypothesis are also being undertaken by different groups of investigators with other independent sources of funding. Representatives of two of these groups discussed their projects at the workshop: investigators from the National Institute of Nutrition in Hyderabad, India, which will conduct

research in Andhra Pradesh, and one investigator from the London School of Hygiene and Tropical Medicine, which will undertake a study in The Gambia.

The workshop began with presentations of protocols for each of the USAID-sponsored studies by representatives of the research groups. These were followed by a formal discussion of the methodological issues by a member of the subcommittee and then by informal open discussion. The Indian and Gambian studies were then described and discussed more briefly. Next, the participants focused on design issues, such as methods of randomization, the use of double-blind techniques, and interventions that might serve as an appropriate control for vitamin A supplementation.

Measurements, such as baseline enumeration, assessment of vitamin A status, and ascertainment of morbidity and mortality outcomes, were considered in detail. The subcommittee also reviewed key design issues that might affect the interpretation of results and discussed possibilities for standardizing methodologies among the studies. It recognizes that each proposal reviewed at the workshop is still under development and will be modified before the study is initiated. The conclusions and recommendations in the final section of this report are based on these open and formal workshop discussions along with further discussion by the subcommittee in executive session after the workshop.

Chapter 3

THE ACEH MORTALITY STUDIES

Few studies have created as much interest in the international health and nutrition community in recent years as the vitamin A supplementation trials conducted by Alfred Sommer and colleagues in Aceh, Indonesia (Sommer et al., 1986). If the results are validated through further research, vitamin A supplementation could become a cost-effective means of improving child survival. Because of the policy implications of Sommer's findings, however, all aspects of the study, including its analysis and interpretation, must be carefully scrutinized. Accordingly, the subcommittee decided to begin the workshop with a presentation by Dr. Sommer and a review of the study design and methods used in Aceh. The subcommittee hopes that the review, summarized in this chapter, can contribute to the design of the planned series of studies which will investigate whether vitamin A also affects mortality in other settings. In this way, Sommer's experience can be used to design better studies.

The report of the Indonesian trial presented at the workshop and reviewed by the subcommittee described vitamin A supplementation in 450 villages in Aceh (Sommer et al., 1986). Through a process of random selection, 229 villages were assigned to a government vitamin A supplementation program and 221 were designated as control villages. In the 229 villages in the government program, children ranging from 12 to 71 months of age were given two doses of vitamin A 6 months apart. Each dose contained 200,000 IUs of vitamin A and 40 mg of vitamin E and was administered in capsule form by teams of local volunteers. One to 3 months after baseline examinations, the initial dose was given by snipping off the end of the capsule and expressing its contents into each child's mouth. All children with

signs of xerophthalmia were treated with vitamin A, referred to the local health unit, and excluded from the analysis. The effects on mortality were determined during the year following baseline studies by comparing mortality rates in treatment and control villages.

DESIGN

One limitation of the Indonesian study is that it was not a double-blind trial. That is, the study subjects in the treatment villages were aware that they were receiving vitamin A supplementation, and the investigators and field workers knew which communities received and which did not receive vitamin A capsules. A greater degree of contact with participants can be assumed to be necessary for the delivery of vitamin A, and it is conceivable that this may have increased public awareness of health issues, increased the demand for health services, and decreased mortality rates. It seems unlikely that these mechanisms were the cause of the substantial reductions in mortality observed in the Aceh study. Nonetheless, careful monitoring of the use of health services in control and treatment villages is recommended in future studies, if they are not to be double blind. Another concern to the subcommittee was the possibility of ascertainment bias. Because the field workers knew which communities were intervention sites, this knowledge could have influenced the ascertainment of deaths. A greater expectation of deaths in the control sites may have increased the intensity of the monitoring of mortality in these areas relative to the intervention communities.

For logistical reasons, randomization was at the village level and not at the household or individual level, and sample sizes were increased to compensate for the expected increase in variance of the estimated rates due to randomization by cluster. These factors should not be regarded as a limitation of the study. The analyses by Sommer et al. (1986) indicated that most background variables (e.g., socioeconomic status) were similar for the control and treatment groups. They did observe, however, that in the control groups, the prevalence of diarrhea ($p < .05$) was greater. The prevalence of xerophthalmia, stunting, and wasting was also greater, but not significantly, in comparison to

the treatment groups. These differences were greater in males than in females in a direction that would upwardly bias the estimate of vitamin A efficacy. In all these respects, control communities tended to have a poorer status at baseline than did the intervention communities. Thus, observed differences in mortality may have been due not to vitamin A but to differences in morbidity and nutritional status between the study groups (Costello, 1986). The interpretation of the results is made more difficult because the only measure of baseline vitamin A status included in the studies was prevalence of clinical signs of xerophthalmia.

MORTALITY ASSESSMENT

Examination of the demographic data suggests that there may have been underestimation of the population at risk and of the number of deaths. This is a serious matter, because the study was not double blind. If these estimates had been more accurate, there would not be as much reason for concern that there may have been ascertainment bias in the assessment of mortality. The age structure reported in the Indonesian study indicates that there was underenumeration of the population at risk at younger ages and that there were errors in the reporting of ages for the 60- to 71-month age groups.

For children 12 to 59 months of age, supplementation with vitamin A reportedly reduced annual mortality by 2.0 per 1,000, from 8.0 per 1,000 in control villages to 6.0 per 1,000 in treatment villages. Surprisingly, the effect of vitamin A was greater in the older children, the very group at smallest risk of death. For children 60 to 71 months of age, vitamin A supplementation reduced annual mortality by 3.8 per 1,000, from 5.3 per 1,000 in control villages to 1.5 per 1,000 in treatment villages.

When these results are considered on a sex-specific basis, another unexpected finding emerges: the reduction in mortality in the 12- to 71-month age group (from 7.4 per 1,000 in control villages to 4.9 per 1,000 in treatment villages) was principally due to the findings in males. For males 12 to 71 months of age, supplementation with vitamin A reportedly reduced annual mortality by 3.6 per 1,000, from 8.8 per 1,000 in control villages to 5.2 per 1,000 in treatment villages. The corresponding reduction in the death rate

for females was 1.1 per 1,000, from 5.5 per 1,000 in control villages to 4.4 in treatment villages.

These findings, while unexpected, could in fact be due to a peculiar distribution (by age and sex) of vitamin-A-preventable causes of death or a higher frequency of vitamin A deficiency in boys than in girls, a finding that has been observed in several studies (McLaren, 1986). These explanations, though consistent with the findings, must remain conjectural, since neither cause of death nor baseline vitamin A levels were recorded.

Both the child mortality estimates and the xerophthalmia rates in the study area were very low. The mortality rate in the 0- to 71-month group was approximately 6 deaths per 1,000 population per year in both groups, whereas national data estimates for Indonesia are about 13 to 18 deaths per 1,000 population per year, suggesting an underrecording of deaths in both intervention and control communities.

The 34% reduction for children 12 to 71 months of age at baseline recorded in Indonesia appears very large as percentages but not in comparative terms. The 2.5 per 1,000 reduction from initial levels of 7 deaths per 1,000 population is not large relative to the changes that occur as a country moves from high mortality to low mortality. For example, model life tables indicate that for a population with a life expectancy at birth of 40 years, the death rate in the 1- to 4-year age group is approximately 32 per 1,000. For a population with a life expectancy of 67.5 years, it is 3 per 1,000. To put the apparent program effect in Indonesia into perspective, the mortality reduction attributed to vitamin A represents less than 10% of the variation in the developing world between areas of low and high mortality (Coale and Demeny, 1966).

In future studies, better measures of mortality are needed. Although the Indonesian data enable investigators to compare mortality rates during the study period, they did not establish baseline mortality estimates. Such estimates are highly desirable in order to describe the study population, to examine changes in mortality within treatment and control groups, and to provide a reference point for generalizing the findings to other populations. Baseline comparability studies are not a requirement for an appropriately randomized trial; in their absence, however, skeptics can argue that baseline mortality differences account for the

findings. Continuous collection of data on morbidity and mortality data both before and after the intervention would be preferable in future studies.

MORBIDITY

In the Indonesian study, changes in morbidity were determined from limited data obtained from two 7-day recall surveys of morbidity: one conducted at the initiation of the study and one at the end. Any differences in incidence reported at the beginning and end of the study period in this manner will be difficult to interpret, because diarrheal and respiratory infections are subject to substantial seasonal variation and there is a substantial variance in morbidity that cannot be captured in a 7-day recall.

DURATION OF STUDIES

The Indonesian study suggests that differences in mortality data between treatment and control villages were increasing with time. As a consequence, an extended period of data collection would have been desirable to determine whether mortality reductions in the treatment group stabilized over time. The authors mentioned that the plan was for the government program to provide vitamin A to the control communities at the end of the study period (Sommer et al., 1986). This plan is worthy of consideration in future studies to determine whether mortality reductions occur in the control communities after treatment.

CONCLUSIONS

The availability of funding for replicating these studies presents a unique opportunity for enhancing the data.base in this critical area and for examining the extent to which the Indonesian findings can be reproduced in other populations.

Following is a recapitulation of the major findings of the subcommittee:

- Because the study was not a double-blind trial, the enumeration of morbidity and mortality is vulnerable to possible bias.

- The overall mortality rates reported in this study are low compared to national data, suggesting that ascertainment bias could have affected the results.

- There appears to have been underenumeration at younger ages and errors in age reporting in older children.

- The data indicate baseline differences between treatment and control groups that could lead to overestimates of the effect of vitamin A.

- The lack of adequate information on baseline levels of vitamin A in the groups studied limits the potential to generalize the findings to other populations.

- Mortality effects were found to be greater in older children who are at lowest risk of mortality.

- Mortality declines were observed only in males. Future studies are needed to confirm these findings.

- The Aceh study may provide only limited information about effects on diarrheal and respiratory infections, the presumed mechanisms for effects of vitamin A.

- Better measures of mortality and morbidity should be included in future studies both at baseline and during follow-up.

- Mortality differences between treatment and control groups seem to have increased over time, suggesting that future studies should be conducted for periods longer than 1 year to determine what the maximum effect might be.

Chapter 4

CRITIQUE OF PROPOSED RESEARCH

Proposals for research on the effects of vitamin A supplementation on mortality in the Philippines, Bangladesh, and the Sudan were submitted for review by the subcommittee before the workshop along with a proposal for a morbidity study in the Philippines. Two additional mortality studies summarized at the workshop (one in India and the other one in The Gambia) were not included in the subcommittee's deliberations and are discussed here only very briefly.

Table 4-1 provides a general overview of the six proposed studies. The study designs differ in many ways, including strategies for randomization, intervention in the control group, and measurement planned at baseline. However, the proposals of major interest to the subcommittee (i.e., those for studies in the Philippines, Bangladesh, and the Sudan) involve the use of similar treatment and outcome measures and sample sizes of 30,000 to 40,000 children.

In the following pages, each of the proposed studies is discussed, and recommendations are given for possible improvements in design and methods.

PROPOSED PHILIPPINE MORTALITY STUDY

The proposed research in the Philippines is a 3-year prospective study consisting of a half year each for start up and analysis and a 2-year period of observation. The study group would consist of approximately 20,000 to 30,000 preschool children divided into two study groups: one to be given a high dose of vitamin A (200,000 IUs with 40 IUs of vitamin E every 4 months), the other to be given a low dose of vitamin A (2,000 IUs

Table 4-1. Comparison of Vitamin A Supplementation Protocols

Study Design	Dose Administered	Outcome Measures	Baseline Studies
Philippines: Mortality Study Double-blind controlled community trial; randomization by village (stratified). Observation Period: 2 yrs.	Treatment 200,000 IU vitamin A with 40 IU vitamin E every 4 mos. Placebo 2,000 IU vitamin A with 0.4 IU vitamin E every 4 mos.	Prospective mortality survey. 7-day recall of fever, diarrhea, cough respiratory problems. 4-mo. recall of measles.[a]	Ophthalmological examination, conjunctival impression cytology
Philippines: Morbidity Study Double-blind clinical trial; randomization by individual (stratified). Observation Period: 2 yrs.	Treatment 200,000 IU vitamin A with 40 IU vitamin E every 4 mos. Placebo 2,500 IU vitamin A with 40 IU vitamin E every 4 mos.	Incidance and duration of respiratory, gastrointestinal, and skin and eye infections. Stool parasites.[a]	Ophthalmological examination, serum retinol levels, conjunctival impression cytology, anthropometry, morbidity physical examination
Bangladesh: Controlled community trial; randomization by village. Observation Period: 16 mos.	Treatment 200,000 IU vitamin A with 40 IU vitamin E every 4 mos. Placebo Alternative intervention to be determined.	Prospective mortality survey, morbidity.[a]	Ophthalmological examination, serum retinol level, and stool parasites (5% subsample), retrospective mortality, anthropometry
Sudan: Double-blind controlled community trial; randomization by household. Observation Period: 1 yr.	Treatment 200,000 IU vitamin A with 40 IU vitamin E every 6 mos. Placebo 40 IU vitamin E every 6 mos.	Mortality, morbidity, and nutritional status.[a]	Ophthalmological examination, serum retinol level, morbidity, anthropometry

[a]Outcomes are to be reassessed at the time of each vitamin A treatment.
[b]Up to 30% of the subsample may have been treated with vitamin A prior to the study.
[c]Estimated from survey of 3,461 children in the region.

Table 4-1. Continued

Assumptions Made to Calculate Sample Size	Estimated Sample Size (and Age)	Additional Information
Type I error = 0.05 Type II error = 0.10 25% reduction in mortality rate. 30% increase in sample size to account for clustering. 15% increase in sample size to account for losses.	40,000 (9 to 72 months)	Mortality rate = 11/1,000 for children 6 mos. to 5 yrs. old.
Type I error = 0.05 Type II error = 0.10 25% reduction in morbidity. 5% refusal rate. 10% noncompliance. 10% attrition.	666 children (6 to 71 months)	In Albay province 8.5% of 322 mothers reported nightblindness in children.
Type I error = 0.05 Type II error = 0.20 20% reduction in mortality. 15% reduction in effect estimate due to contamination.[b] 30% increase in sample size to account for clustering. 10% increase in sample size to account losses.	40,882 children (6 to 71 months)	Mortality rate = 25/1,000 for preschoolers. National vitamin A capsule distribution program serves 46%. Prevalence of nightblindness or Bitot's spots estimated to be 4.5%.
Type I error = 0.05 Type II error = 0.20 20% reduction in mortality.	21,000-30,000 (9 to 72 months)	Mortality rate = 20-25/1,000 in children less than 6 years Prevalence of Bitot's spots 9.5/1,000, 1.2/1000 for keratomalacia or corneal scars[c]

with 0.4 IUs of vitamin E every 4 months). The study objectives are to investigate the relative effectiveness of the high-dose treatment on cause-specific and overall mortality. Every 4 months, incidence of diarrhea, fever, and respiratory tract infections during the previous 7 days and prevalence of clinical signs and symptoms of xerophthalmia will be recorded. In a substudy of pregnant women and lactating mothers, investigators will examine the effects of a 10,000-IU daily dose versus a 100-IU daily dose of vitamin A in reducing neonatal mortality and early infant mortality and increasing birth weight.

Study Design

Randomization will be at the village level. The theoretical advantages to be gained by randomization at the household level, such as reduced sample size and control of potential confounding variables, are offset by the considerable logistical problems likely to be encountered in ensuring that the proper treatment is given to each child at follow-up. There was a consensus among the subcommittee members that village-level randomization is reasonable for such large-scale field surveys.

According to the proposal, child mortality rates in the Philippines are officially recorded as approximately 9 per 1,000 per year, compared to reported mortality rates in Indonesia of 13 to 18 per 1,000 per year. The estimation of sample size assumes a 25% reduction in mortality. The subcommittee was not certain whether this reduction should be expected, especially if mortality rates in the study population prove to be lower than the mortality rates encountered in the Indonesian study, which was the basis for the sample size projection.

The use of a controlled, experimental design contributes greatly to the strength of the study but raises ethical concerns. There was agreement among subcommittee members that the administration of low doses of vitamin A constitutes a control rather than an alternative treatment. There was also consensus among the subcommittee that the provision of very low vitamin A doses as the control is an acceptable procedure as long as adequate information is given to parents to obtain informed consent. Participants should be informed that they will receive either a high dose that would protect against ocular signs and symptoms or a very low and

unprotective dose of vitamin A. Parents must not be led to believe that all children participating in the study are ensured adequate vitamin A nutriture.

One strength of the study is its duration of 2 years, which enables investigators to examine seasonal effects. If the prevalence of vitamin A deficiency varies due to seasonal differences in availability of foods that are good sources of vitamin A, seasonal variation in the effects of supplementation on morbidity and mortality may be found. This would provide supplementary information on the relationship between the severity of vitamin A deficiency and the magnitude of treatment effects. For this reason, information on dietary intake of vitamin A is needed.

To document the baseline vitamin A status of the population, the investigators will measure initial and subsequent vitamin A status by conducting clinical examinations to determine the presence of xerophthalmia and by using a procedure that involves taking a filter paper impression from the conjunctiva, which is then examined histopathologically for the presence or absence of goblet cells (Hatchell and Sommer, 1984; Wittpenn et al., 1986).

At the workshop, there was considerable discussion concerning the usefulness of serum vitamin A measurements as an additional indicator of vitamin A status in the population. Clinical findings are likely to be rare, and the conjunctival impression cytology technique is unproven under field conditions. Although impression cytology assessment is a promising method and should be pursued, the subcommittee members agreed that biochemical assessment of serum vitamin A levels on a subsample of the preschool children in the population at baseline and at the end of the observation period would add significantly to the characterization of vitamin A status. This recommendation is made with full realization of the technical difficulties that exist in obtaining reliable vitamin A measures. These difficulties should not prove insurmountable.

A limitation of the study is that baseline mortality studies are not planned. It does, however, include baseline morbidity assessments, through a 7-day recall of fever, diarrhea, cough, or respiratory problems. Because reductions in the incidence or severity of gastrointestinal and respiratory diseases are the probable mechanisms for effects of vitamin A supplementation on

mortality, the inclusion of these data are highly relevant, as are data on morbidity from other infectious diseases, which should also be collected since infections reduce serum vitamin A levels and increase requirements. The reference period for the data is only 7 days; thus its usefulness is somewhat limited for characterizing the study population. The assessment of baseline nutritional status relies on arm circumference. Although this measure is practical under field conditions and is a useful indicator of nutritional status, the addition of height and weight measurements would strengthen the characterization of growth status of the children in the study population. To control for possible confounding effects of malnutrition, growth measurements should be made at baseline.

The subcommittee members concur that the lack of baseline measurements of mortality, serum vitamin A, and growth (beyond arm circumference, which is included) is a weakness of the design. There can be no question that this baseline information will be needed by policymakers to interpret and use the study results. It should therefore be included to meet this need.

Methods and Measurement Techniques

The importance of an accurate enumeration of deaths and the potential for underenumeration were recognized by the subcommittee. The use of key informants in the villages to report deaths has been a successful technique in past research in the Philippines. However, the present study is far more ambitious than those previous studies with regard to the number of villages under surveillance. Supervision of the key informants will need to be carefully controlled to minimize underenumeration of mortality and to achieve the ambitious goal of determining causes of child deaths within 2 weeks after death by interviewing surviving members of their families. Another possible approach would be to establish a more active mortality surveillance system in which informants would visit all study households on a regular schedule rather than waiting to be informed of deaths.

An active system of morbidity surveillance of a subsample would enhance the data collected in 7-day recall surveys every 4 months. Continuously collected data facilitate consideration of seasonal changes when ascertaining changes in morbidity, particularly when

there are data for a baseline period of at least 1 year. Continuous morbidity data also permit detailed investigations of changes in incidence and severity of infections. Seven-day recall surveys are more appropriate for collecting prevalence data and offer limited information about severity. For example, a desirable parameter of severity, duration of episodes, is best determined through continuous data collection. Another problem is that great care must be taken to time the 7-day recall surveys appropriately with respect to the seasons in order to facilitate the interpretation of changes in prevalence between any two survey periods. Although it yields superior data, an active morbidity surveillance system in a massive study of this kind presents enormous logistical problems unless it is done only for a subsample. From a design point of view, it is not necessary to monitor the entire sample, since infections are far more common than deaths.

An adequate assessment of baseline nutritional status in the study population and a determination of whether child growth is affected by vitamin A intervention will be useful in interpreting study results. If, as appears to be the case in the Indonesian study (A. Sommer, Johns Hopkins University, personal communication, 1987), the improvements in mortality are accompanied by improvements in weight gain among the supplemented children, the credibility of the mortality results will be enhanced. The decline in mortality is presumably associated with a decline in morbidity as suggested in earlier Indonesian studies (Sommer et al., 1984), and frequent morbidity has been clearly associated with impaired growth. Thus, beyond the substantial intrinsic scientific interest in the effects of vitamin A supplementation on growth, it would be advisable to include height and weight measurements to permit an evaluation of the congruency of mortality results with other measures of child health.

Although the substudy of effects of vitamin A on birth weight addresses some interesting issues, there are some methodological questions. For example, it may prove difficult to ensure compliance of the mothers in taking the daily dose of vitamin A. Ensuring the accurate measurement and recording of birth weight by traditional midwives will also be a challenge. Although this substudy is of interest, the subcommittee believes that resources devoted to it would be better used to strengthen the key aspects of the main mortality study,

principally by adding periodic home visits and measurements of serum vitamin A, height, and weight.

In the implementation of the studies, several potential problems were identified. The codes for treatment and control groups should not be broken to allow the investigators to perform substudies or for interim analyses. In addition, well-developed procedures for handling refusal to participate and for observation of capsule administration are needed.

Summary

In summary, the subcommittee drew the following conclusions:

- Village-level randomization is acceptable.
- The double-blind controlled design strengthens the study considerably.
- The low-dose control is acceptable if parents are informed that one of the two possible doses their children may receive is not protective against effects of vitamin A deficiency.
- A thorough evaluation of baseline child mortality in the study area is needed. This should include supervision of key informants or use of an active surveillance system.
- The 2-year study duration makes possible the analysis of seasonal variation in vitamin A deficiency.
- Conjunctival cytology assessment is a promising but as yet unproven method of determining vitamin A nutriture.
- Adding the assessment of serum vitamin A levels in a subsample of the population at least at baseline and at the end of the observation period would improve the study considerably.
- An active morbidity surveillance system in a subsample would enhance the data collected in 7-day recall surveys every 4 months.

- The morbidity survey at baseline should be expanded to include data for systemic infections in addition to respiratory and gastrointestinal infections.

- The study of the impact of vitamin A on pregnancy is unlikely to produce definitive results, and the resources needed for such a study could be used more effectively in expanded baseline studies.

- Dietary data are needed at baseline and during intervention. The latter can be obtained through interviews.

- Growth measurements, including height and weight, should be made both at baseline and during the course of the study to control for confounding due to differences in anthropometric status and to measure changes in nutritional status.

- Detailed procedures should be developed for resolving problems of coordination with other health programs in the study area and for management of refusals at various stages.

- Direct observation of capsule administration is very important and should be rigidly enforced.

- Codes of treatment and control groups should not be broken in order to allow the investigators to carry out interim analysis.

PROPOSED PHILIPPINE MORBIDITY STUDY

A second proposed study to be conducted in the Philippines is a double-blind placebo trial in children 6 to 71 months of age to compare the effect of administering 200,000 IUs of vitamin A with the effect of single 2,000-IU doses of vitamin A given every 4 months for 2 years. The outcomes to be studied include the incidence of respiratory and diarrheal disease, skin rashes, and conjunctivitis. Differences in the incidence of these outcomes between the treatment and control groups will be analyzed as a function of the time since administration, the dose, and the duration of

signs and symptoms. The anticipated sample size will be 333 children in each group (treatment and control). It is proposed that the study will be conducted in an area adjacent to the villages in which the larger study of vitamin A supplementation and mortality is to be conducted. Baseline and follow-up measurement of serum vitamin A status and nutritional status, as indicated by anthropometry, will be included. The study will be of substantial interest both for its contributions to knowledge of the relationship of vitamin A supplementation to morbidity and for its baseline descriptions of vitamin A and anthropometric status.

Study Design

Randomization will be at the individual child level with stratification by age and distance of the household from the local health clinic. Because of the relatively small scale of this project, such randomization is feasible.

Selection of sample size was based on incidence rates for diarrheal disease using data from previous studies in Bangladesh. The investigators assumed a 25% difference between the supplement and placebo groups in the annual incidence rate and mean number of days per episode of diarrhea due to specific organisms. Allowance was also made for a 5% refusal rate, a 10% noncompliance rate, and a 10% attrition rate, leading to the final sample size of 333 per group. These assumptions seem reasonable, although there appear to be no good data that would permit assessment of the comparability of diarrhea incidence in Bangladesh and in the Philippines.

Both the participant households and the study staff will be blinded to the identity of the assigned vitamin A group. The dosage and the schedule of administration as well as the nature of the placebo are the same as in the mortality study, thus permitting comparisons of the results of the two studies. The 2-year study will span several seasonal cycles and is likely to permit a detailed examination of seasonal trends in the treatment and control groups.

The baseline assessment of vitamin A status will include an ocular examination, measurement of serum vitamin A levels (at baseline and at 1 year), and conjunctival cytology (at baseline and at each treatment

with vitamin A). This thorough assessment of vitamin A status will be useful not only for this study but also as a reflection of the vitamin A status in the population of the study area. Nutritional status will also be assessed every 4 months by measuring weight, height, and arm and head circumference. Dietary data will also be collected at each vitamin A distribution.

Methods and Measurement Techniques

Intensive morbidity surveillance will be maintained by visits to the households every 2 days to determine if there are signs and symptoms in the study children. Children found to be ill will receive careful clinical follow-up to document the illness and to provide appropriate treatment. This level of follow-up should provide very accurate assessments of morbidity--more extensive than any of the other trials reviewed at the workshop.

Height and weight measurements will permit the assessment of nutritional status with generally accepted methods. The measurement of arm circumference will add significantly to the assessment of nutritional status, and the resulting data can be compared to the arm circumference measurements of children in the larger mortality field trial. Follow-up monitoring of growth will enable investigators to determine whether there is an association between nutritional status changes and morbidity differences in the treatment and control groups.

The thorough assessment and follow-up of vitamin A status using clinical, cytological, and serum vitamin A levels, as planned, will be of great value. These data, taken with the anthropometric data, can be used to obtain a more thorough baseline assessment and a clearer understanding of true effect.

Summary

This proposal describes a thorough and well-developed study plan that will provide information on the relationship of vitamin A supplementation to morbidity that is critical to the interpretation of the concurrent mortality study. The subcommittee's members concur that this morbidity study promises to provide

information that will not be gained in any of the other proposed investigations.

In particular, the subcommittee reached the following conclusions:

- The study is well designed, and the results should be of great interest to the scientific community.
- The planned follow-up strategy is likely to generate detailed and accurate morbidity information that is critical in assessing mechanisms of vitamin A effects, if found, on mortality.
- The inclusion of growth information will make possible analyses of the relationship between changes in morbidity and child growth.
- The population descriptors used should be identical to those in the mortality study so that the results can be extrapolated to the larger population.

PROPOSED BANGLADESH STUDY

In the proposed Bangladesh study, investigators will examine the effects on mortality resulting from the distribution of vitamin A capsules. For a comparison group, communities that will receive an alternative health intervention, such as another vitamin or immunization, will be used. The alternative treatment may also be given to the vitamin A-treated group. Approval by the government of Bangladesh is needed for this alternative treatment.

The study area will be in Jamalpur, chosen because a relatively low percentage (26%) of its children have participated in a government-sponsored vitamin A distribution program and because vitamin A deficiency is prevalent (no figures were given in the proposal).

In the first 12 months of the study, approximately 180 villages will be selected for research. The criteria for selection of the villages will be that the number of participants in the government vitamin A distribution program prior to the selection has been limited to less than 35% of eligible children. These

villages will be identified through administrative records and some form of household surveys. Complete household surveys will then be conducted in these villages. Information will be gathered on the prevalence of Bitot's spots, and mortality rates will be estimated retrospectively. This information will be used to stratify the 180 or so villages. Within strata, villages will be randomly assigned to one of two groups for future program activities: vitamin A treatment (A) or the alternative treatment (B). It is assumed that it will be possible to select villages where few children have already been treated. It is expected that the total population will include approximately 20,000 children between the ages of 6 months and 5 years. If the period of investigation is 16 months and the mortality rate in the age range under study is 0.025, the sample size should be large enough to detect a 20% reduction in mortality, given a Type I error of 0.05 and a Type II error of 0.20.

The first round of treatments with vitamin A and the alternative intervention will begin in the 12th month of the study. Three additional rounds will be made at 4-month intervals. Information on mortality and both respiratory and diarrheal morbidity will be collected through household surveys conducted during the visits in which the treatments are given. A final recording of mortality and morbidity will begin in the 28th month. Thus, the treatment phase of the intervention will last 16 months. The 4 months of the year in which data will be obtained in two successive years will coincide with seasonal peaks for xerophthalmia and child mortality. Extending the study period beyond a year is an attractive feature of the research design, since it allows repeated observations during the period that mortality is likely to be most sensitive to vitamin A intervention. This increases the statistical power of the study and permits examination of the cumulative effects of vitamin A supplementation.

A 5% sample of children in A and B villages will be selected for more intensive investigation of biochemistry, diet, and clinical symptoms. Data from the subsample will be used to study links between vitamin A treatment, vitamin A status, and morbidity. By analyzing the consistency between morbidity and mortality results, the substudy will serve as a test of the plausibility of the mortality results and will provide valuable supplementary information about morbidity.

Children who reach 6 months of age after the 12th month of the study may or may not be included in the study design. While overall mortality of these children is high, xerophthalmia is less common in this age group and thus there may be reduced risk of vitamin A deficiency over the ensuing 4 to 7 months of follow-up. Therefore, their deaths may be less likely to be affected by vitamin A supplementation, in which case their inclusion would not improve the study's power. This line of reasoning, however, has to be tempered by the findings reported from the Sumatra study by Sommer et al. (1986). Although that investigation was targeted to children 1 to 5 years of age, 82% of infants in the treatment villages received at least one capsule of vitamin A. For males 0 to 11 months of age, the death rate in the treatment villages was reported to be 17.8 per 1,000, compared to 29.9 per 1,000 among the controls. For females in the same age group, the death rates were 28.2 per thousand in the treatment group and 26.5 per 1,000 in the control group.

Attention should be given to the development of detailed procedures for managing refusals and loss to follow-up as well as for dealing with possible confusion due to the presence of multiple health programs in the treatment area. Direct observation of capsule administration should be enforced.

The subcommittee is concerned about the absence of a double-blind procedure for distributing and evaluating treatments. As a result, for any number of reasons, fewer deaths and illnesses may be recorded in the villages receiving the more efficacious treatment leading to overstatement of the program's effects.

The subcommittee does not believe that the lack of a double-blind design is reason to recommend against undertaking the study, although it does diminish its value. Although a double-blind design is highly desirable, several procedures can be used if necessary to compensate for the inability to use this design. Randomization at the household rather than the village level would be one possible method, although the gains from such a strategy are difficult to determine with certainty. Another is to give children in B villages a treatment that is similar in its overt characteristics to the vitamin A capsule treatment in the A villages. The absence of a double-blind design increases the attractiveness of using another vitamin distribution program in the B villages.

Methods and Measurement Techniques

To estimate baseline mortality, a 5-year birth history will be collected from all women at the beginning of the study. Subsequent rounds of interviews will be conducted to collect data on mortality since the previous round. Reports on deaths during the study period will be supplemented with data on deaths recorded by village monitors. This plan seems appropriate, but sizeable errors in the 5-year birth history can be anticipated. As was shown in the World Fertility Survey, dating of events in Bangladesh is among the least accurate in the world (Preston, 1985). By conducting baseline interviews of women to learn their cumulative lifetime number of births and deaths of the children, an additional estimate of mortality could be obtained for comparison. Total births and deaths are usually reported more accurately than subtotals within any particular time period. At the end of the assessment period, it would be desirable to repeat the retrospective questions.

Cause of death will be assigned by trained paramedic supervisors, about whom no further information is supplied in the proposal. Information about diarrheal disease and respiratory infections will be collected at each survey round for the full sample using a 7-day recall period and standard definitions. Dietary intake for each child will also be determined during each round of interviews, but the instrument used to make this determination has not been identified. Those exhibiting ocular signs of vitamin A deficiency will be given vitamin A capsules. For a 5% subsample, the investigators will collect additional data during each round. This information will include weight, height, body length, plasma vitamin A levels, and the presence of parasites in stools. Standard procedures will be used to collect these data.

Much of the first year of the project will be devoted to developing and pretesting the interview instruments, a plan that the subcommittee considers advisable.

Analytic Issues

The project presents attractive possibilities for integrating dietary, biochemical, and clinical data into

the assessment of the mortality effects of vitamin A distribution; however, the analytic plans have not been fully described in the proposal, nor is there any mention of the many hypotheses that could be investigated. The proposal assigns variables to several categories. For example, it lists serum vitamin A levels and preprogram distribution of vitamin A as "covariates, possibly confounding" of mortality. The subcommittee believes that serum vitamin A levels are more appropriately considered as either initial conditions or mediating variables through which the effects of vitamin A distribution can be expected to work. It may be convenient to introduce a hierarchy of mediating variables, placing serum vitamin A logically before respiratory and diarrheal disease. Preprogram levels of vitamin A distribution would seem to be more appropriately included along with program distributions as conditional variables.

Summary

The findings of the subcommittee can be summarized as follows:

- The subcommittee was concerned about the lack of a double-blind study design.
- An alternative, similar form of intervention in control villages would overcome some of the disadvantages of not having a double-blind design.
- The subcommittee believes that a preprogram surveillance system to obtain baseline mortality data would be preferable to the retrospective analysis now planned.
- Inclusion of dietary and biochemical data on a subsample of the study is a strength of the study.
- Some clarification of the relationships among possible confounding variables would be worthwhile.
- Analysis of vitamin A effects should account for the possible confounding influence of preprogram vitamin A distribution in the design.

- Inclusion of children who reach 6 months of age during the course of the study may strengthen the statistical power.

- Detailed procedures should be developed for resolving problems of coordination with other health programs in the study area and for handling refusals at various stages.

- Direct observation of capsule administration is very important and should be rigidly enforced.

PROPOSED SUDAN STUDY

Although the Sudan has been the site of recent and continuing political turmoil, Khartoum and its environs, where the study is to be conducted, has not been seriously affected. A strong feature of the study is the reported infrastructure of primary care services developed by the Department of Community Medicine of Khartoum University. This program covers the entire proposed study population. The population inhabits both banks of the Nile as it runs north from Khartoum for some 160 miles. Traditional midwives resident in the villages, which contain an average of 150 households, have been trained in certain primary care techniques and work under the supervision of a paramedical team. Each team is assigned to a district and works under the direction of the Department of Community Medicine at Khartoum University. These teams have conducted censuses and have made maps of the populations under their care. They have also attempted to develop a registration system of births and deaths. On site will be a Sudanese epidemiologist who completed his doctoral studies at Harvard University.

The availability of these basic population data and the presence of the health teams is an enormous advantage for any longitudinal study in the Third World. In the absence of a Sudanese representative to discuss this study at the workshop, it was difficult to assess the operational effectiveness of the program. It is important to know in greater detail the type and quality of the data on hand in order to assess the real advantages that this infrastructure will be able to confer on the study.

The proposal relies on an assumption that there is significant vitamin A deficiency in the area around Khartoum, but there is no supporting documentation. The knowledge that there is vitamin A deficiency in areas of the Sudan--that are much less stable and probably much less well off than the Khartoum environs--is not enough to support the assumption. To obtain meaningful results from this study, it is essential to know whether widespread vitamin A deficiency exists in the study area itself.

Study Design

The subcommittee questions the proposed household randomization procedure. In terms of inferring the effects of vitamin A in individuals, household randomization is preferable to the village randomization proposed in other studies; it would allow narrower limits of interpretation. On the other hand, effective household randomization--especially to keep track of all the individuals randomized by household in a situation as uncontrolled as most in the Third World--will not be easy and is bound to require an intense effort. The problems of randomization at this level are likely to be intensified by the methods used to reach the population. In the Sudan, the investigators plan to assemble all the households of one village at a given time in order to collect data, to assign treatment, to administer the treatment, and to examine individual children. This procedure is likely to lead to substantial systematic omissions, especially when used in conjunction with a household randomization scheme. It seems reasonable to rely on a resident health worker to serve as a central figure in sustaining the study at the local level and thus to know everyone in the village and where they reside. However, keeping track of 150 households under the proposed randomized scheme would undoubtedly stretch the capabilities of any such person and backup team and would require additional resources.

Treatment

The treatment, a 200,000 IU capsule of vitamin A with 40 IUs of vitamin E administered on two occasions at

6-month intervals, is precisely the same as that used by Sommer's team in the first Sumatra study. Since serum vitamin A levels appear to be sustained only for approximately 8 to 12 weeks after such a dose, a shorter interval of perhaps 16 weeks might enhance the magnitude of the effect. With the limited funds available, however, the additional cost of adding such a visit might be better used to extend observations over a longer period, such as 2 years, and to administer three treatments at 6-month intervals. The extension in duration is in any case advisable, since it would reduce sample size requirements, which, as discussed below, could be a problem in this study. The longer period would facilitate interpretation of seasonal effects of vitamin A, which are bound to be important in many areas.

One strength of the proposed study is administration of the capsule under direct observation. This will need to be maintained with rigid discipline.

Treatment of a control group with vitamin E capsules is a good solution to a masked alternative treatment, since most vitamin A capsules also contain vitamin E and vitamin E is not expected to influence mortality. Ethical considerations concerning the withholding of vitamin A treatment are minimized because all cases of active xerophthalmia will be treated. The remaining ethical issue is that of informed consent: the information to be conveyed to participants should be made more explicit in the final research protocol.

The suggested color coding of treatment and placebo capsules could cause field teams to guess their content and, if they form opinions, could lead to biased observation. With village-level randomization, the use of numbers to code treatments might be less difficult, although not simple.

Sample Size

Given the assumptions of 20% reduction in mortality, a Type I error of 0.05, and a Type II error of 0.20, the proposed sample size of 15,000 in each treatment group and the 1-year observation period will meet the needs of the study only in the abstract. The selection of sample size is based on the unrealistic assumption that there will be no attrition from refusals, dropping out, or migration. Allowances should also be made for the clustering of effects in households (or more especially in villages). A further narrowing of the margin for

detecting an effect may result from the assumed mortality level, which is based on the whole of the Sudan, but is probably substantially lower in the Khartoum area. Moreover, as discussed in Chapter 6, the assumption that mortality reduction resulting from vitamin A supplementation is proportionate to the mortality rate in the population is not necessarily sound. It is as plausible to expect an absolute reduction that is governed not by the existing level of mortality but by the extent of vitamin A deficiency in the population (which leads to different sample size assumptions and estimates). Since the population available for study is limited, it would be advisable, as noted above, to extend the observation period to increase the number of person-years in which outcomes could be observed. Because mortality is high in the youngest age group, a further increment in power would be gained if the sample were to include children from 6 to 72 months of age rather than 9 to 72 months, and if children reaching that age during the study were recruited.

For the intensive study of the effect of vitamin A on morbidity, a 1% subsample is to be drawn at random, but the proposal included no calculations of sample size. It may in fact be safe to assume that the frequency of respiratory diseases and diarrhea in the subsample is sufficient to allow for the detection of vitamin A effects of a magnitude to have public health significance. This may not be true for proposed further studies of the interactive effects of vitamin A with oral rehydration therapy, immunization, and birth intervals. The detection of these effects is not crucial to the aims of the study and need not command an increase in the sample size that is difficult to manage.

The same issues apply to a proposed case-control study of complications resulting from measles as a mediating variable in the possible impact of vitamin A on survival. Presumably, complicated and uncomplicated cases of measles will be compared with regard to their vitamin A treatment in the study. The code of treatment assignment should not and indeed need not be broken to accomplish this study.

Baseline Status Monitoring and Measurement Techniques

The existing infrastructure of primary health care services, if proved to be operating effectively, will

confer marked advantages on this study. For example, knowledge that already exists or can be quickly collected can be used in determining population structure, assembling the study population, making random treatment assignments, monitoring the study in progress, and ascertaining the outcome. The age of the participants and the preexisting death rates in the study villages should be well characterized to the extent possible. When randomization is at the individual or household level and a large number of units is to be randomized, baseline information is not an absolute necessity for study validity. On the other hand, information on baseline status could aid greatly in the interpretation of study results, since the initial status of the study population is likely to be a moderating condition for the effect of the treatment. Information of importance includes recent infectious illness, growth and nutritional status, biochemical and clinical indicators of nutritional status (weight, height, arm circumference, and if possible, skin-fold thicknesses), and dietary histories of foods containing vitamin A. With such baseline information, it is possible to detect conditional effects and estimate actual change in the treatment group, not merely a treatment difference. These data assume even more importance if they are collected at intervals during the study, since they may provide corroboration of treatment intake, illuminate some of mechanisms of treatment effects, and provide important collateral evidence of the occurrence and nature of the effects being sought. It cannot be taken for granted that these collateral effects will be as predicted from preexisting knowledge.

The investigators do intend to measure growth and to use this information to characterize nutritional status, but the subcommittee believes it would be wise for several reasons to add supplementary observations on the participants, both at the beginning and, to the extent possible, during the course of the study. For example, nutritional status may either interact with or confound the effects of vitamin A. In general, intensive observation of a randomly selected subsample should suffice.

The investigators have indicated that they intend to test the reliability of several measurements between observers, over time, and for the same observer at different times. As much as possible, the measurements should be standardized among all the studies discussed

at the workshop. The subcommittee believes that validity testing should be considered, especially for measurements of morbidity (in particular respiratory morbidity) and impression cytology, and for any other new measurement techiques that are used.

Summary

In addition to the problems of implementation already discussed, some others should also be considered. The provision of treatment under observation is an important strength of the study, but lack of coordination and errors such as the administration of treatment to controls are possibilities, especially in household randomization. If participating children are assembled at a central point for interviews and treatment, as proposed, the accompanying person may not be adult or may not be from the same household and thus may not be able to provide needed information. If information about the treatment is assigned to households with no address (only a location on a map), there is great potential for error.

It would be worthwhile to consider the possible effects resulting from the lack of coordination that might be produced by the proximity of three parallel ongoing projects--on oral rehydration, on immunization, and on family spacing--in the study community. Procedures should be developed to manage refusals at various stages, e.g., after randomization but before treatment. Procedures for following dropouts need to be developed, and the duration of follow-up should be defined.

- The health infrastructure described in the Sudan proposal is a strength of the study.
- The level of vitamin A deficiency in the areas near Khartoum should be assessed at baseline.
- The proposed household randomization has advantages over village randomization but may be very difficult to accomplish successfully.
- The proposed distribution of vitamin A capsules every 6 months may be less than optimal, since there is evidence that serum levels decline after 4 months (Flores et al., 1984).

- A longer study period (e.g., 2 years) would increase the probability of detecting differences and seasonal variations in mortality.

- A thorough evaluation of baseline child mortality in the study area is needed. This should include supervision of key informants or use of an active surveillance system.

- Although the sample size may not be sufficient to detect interactions among other treatments and vitamin A supplementation, these analyses are not crucial to the hypothesis.

- The sample size should be revised to allow for refusals, loss to follow-up, and estimated migration.

- Baseline information should include recent infections, anthropometric measurements, and to the extent possible, clinical and biochemical indicators.

- Growth measurements should be made both at baseline and during the course of the study to document and control for effects of nutritional status.

- Detailed procedures should be developed for facilitating coordination with other health programs in the study area and for handling refusals to participate at various stages.

- Direct observation of capsule administration is very important and should be rigidly enforced.

- Codes of treatment and control groups should not be broken to perform interim analyses.

- Additional resources may be needed, especially to lengthen the duration of the study and to modify the plan to visit the households rather than to use a community gathering place.

- Emphasis should be placed on a sound design for the main study rather than for substudies.

PROPOSED STUDIES IN INDIA AND THE GAMBIA

The subcommittee did not critique the proposed studies to be conducted in India and The Gambia, since they were not included in its task and proposals were not available for review. The designs were presented at the workshop, however, because these studies are likely to yield useful information on the relationship of vitamin A to child survival that should aid in the interpretation of results from the other four studies.

The proposed research in India will be conducted in the Nalgonda district of the state of Andhra Pradesh. It will include longitudinal follow-up of morbidity and mortality for 1 year and consideration of the relationship between xerophthalmia and mortality. At the end of the first year, the study group will be randomized to control groups or to groups treated with massive doses of vitamin A. At the end of 2 years, mortality in the two groups will be compared.

The research to be undertaken in The Gambia will include 6,000 infants under 2 months of age. Longitudinal observations will be made until each child reaches its third birthday and will include anthropometric, social, and demographic factors as well as mortality. Individual children will be randomized to treatment with 200,000 IUs of vitamin A or to a placebo given every 4 to 6 months. Serum vitamin A levels will be obtained annually for children and once for a 10% sample of mothers. Separate morbidity studies using a subsample may also be undertaken.

Chapter 5

METHODOLOGICAL CONSIDERATIONS FOR VITAMIN A FIELD STUDIES

Workshop participants considered methodological issues raised by the proposed studies. The following pages summarize the subcommittee's review of the methodologies for studies of this kind, including study design, comparability, randomization, selection of controls, unbiased assessment, determination of sample size, and ethical considerations.

STUDY DESIGN AND IMPLEMENTATION

Study Objectives

Nutritional studies are designed to provide answers to specific questions. Although the general question of interest in the workshop was whether increasing vitamin A intake in populations with evidence of deficiency would reduce childhood morbidity and mortality, the specific question for investigation was whether this can be done by periodic supplementation with capsules containing 200,000 IUs of vitamin A. These questions are comparative: to inquire about the effect of vitamin A supplementation implies that it is possible to compare rates of mortality and morbidity between supplemented and unsupplemented groups in which the only material difference is vitamin A intake. To be certain that such questions can be answered, therefore, the study design must ensure that treatment and control groups conform to the preceding requirement.

Selection of Study Population

Given the hypothesis of interest, evidence of vitamin A deficiency must be demonstrated in any population selected for study. This can be done clinically, e.g., by using prevalence rates of xerophthalmia as an index of endemic vitamin A deficiency, or by the use of biochemical markers, e.g., levels of serum retinol. Since populations will vary considerably in the severity and extent of vitamin A deficiency, which may in turn affect the success of supplementation, one needs to use methods of characterizing vitamin A status that can be compared across different populations. This will be important not only for comparing results of intervention trials in different countries but also for judging how the findings might apply to other areas. Ideally, generalizability will be enhanced if the studies are conducted in sites where deficiency ranges from moderate to mild. It is not clear at this time whether the choice of sites achieves this goal.

Further information on the relationship between vitamin A status and effects on mortality will be provided by studies of patterns of seasonal variation in both (Sinha and Bang, 1973). To maximize the likelihood of detecting effects of vitamin A on mortality, doses should be provided at intervals of no more than 4 months in order to maintain adequate serum retinol levels and the absence of clinical signs (Sinha and Bang, 1976). Effects of the timing of the dose on mortality should be studied to gain information that will be useful in the design of interventions.

Baseline Surveys

Although the selection of a study area may be based on past surveys of nutritional status, the selection should be validated through baseline studies before intervention is begun. These studies should include assessments of child mortality and morbidity due to diarrheal and respiratory disease--two pathways by which vitamin A deficiency might affect mortality (Sommer et al., 1984). Characterization of the baseline status of the study population with respect to vitamin A deficiency, child morbidity, and child mortality will also serve other useful goals:

- description of the extent of baseline comparability between treatment and control groups,
- adjustment of comparisons between groups for any slight differences at baseline,
- assessment of the effect of supplementation on the study population's vitamin A status, and
- interpretation of the findings of the four studies and generalization to other populations.

Intervention

To demonstrate whether an expected reduction in morbidity and mortality is due to vitamin A supplementation, one needs to ensure that treatment and control groups are comparable before intervention is begun, that follow-up is uniform for the two groups, and that no other factors that might affect the study end points or their assessment have intervened during follow-up and biased the results.

Randomization

Baseline comparability is ensured on average, i.e., in repeated samples, by random assignment of treatment and control interventions. This randomization can be done at several levels: individual, household, and community. The choice of the level of randomization is a matter of balancing considerations of study size with feasibility. Regardless of the level of randomization used, studies otherwise comparably conducted and designed to the same specifications of power and significance will have equal validity.

In general, studies with randomization at the individual level require fewer subjects to achieve power equivalent to those with household-level randomization, which in turn require fewer subjects than studies with randomization at the community level. The actual magnitude of the differences in total study size depends on the overall event rate in the study population, the variation in rates among clusters (households or communities), and the average cluster size (Cornfield, 1978). For example, data from the study in Aceh, Indonesia, suggest that randomization by village, as

opposed to randomization by individual, resulted in a 30% increase in the variance of estimated mortality rates (A. Sommer, Johns Hopkins University, personal communication, 1987). The increase in study size for community randomization must be weighed against the benefits of simplified field procedures.

With village randomization, one need only validate each village's assignment. This should not be a difficult task for the proposed studies, which involve 180 to 250 villages each. With individual or household randomization, current lists of treatment assignment must be maintained for all households and all children within each household. At follow-up, the previous treatment for each child must be validated by checking against such a list, and the correct treatment or control intervention must then be given. The mortality studies presented at the workshop will follow 20,000 to 40,000 children. Thus, it will be cumbersome to maintain lists of individuals for field use, even with the aid of microcomputers. Of even greater concern is the likelihood of error in administering vitamin A at follow-up, since each child's or household's previous treatment will have to be validated and the correct treatment readministered. Although randomization by households may not present an insurmountable problem, it will surely require great attention to detail and careful checks in the field.

Randomizing a large number of individuals or clusters ensures that any baseline differences between treatment and control groups are likely to be small but does not guarantee perfect agreement. Perfect agreement is not essential, however, since analyses can adjust for the small effects of any baseline differences that do emerge.

Stratified Randomization

The likelihood of strict baseline comparability can be improved by randomizing within strata on factors that bear a strong relationship to the outcome variables, such as serum vitamin A level at baseline, and that vary considerably among units to be randomized (villages, households, or individuals). As noted previously, strict comparability at baseline is not crucial to the validity of a study, because in moderate-size studies, adjustments can be made during data analysis either by stratification or by regression analysis. Thus, if data

have been collected on factors to be adjusted, unbiased comparisons can be made.

The rationale for stratified randomization rests not on considerations of validity (see Peto et al., 1976) but, rather, on efficiency (precision of estimates). In making decisions about stratified randomization, like those about cluster randomization, one must weigh the expectation of slight gains in precision against the additional complications involved in field work.

Control Group

Since the questions posed by the studies presented at the workshop are comparative, concurrent control groups are needed. The assignment of villages, households, or individuals to treatment or control groups should be made at random.

Children in the control group should derive some direct benefit from participating in the study. For example, one might consider immunizing the control children against a specific disease. To retain the integrity of the study design, the vitamin A treatment group should also receive the treatment provided to the controls.

Children with clinical signs of vitamin A deficiency should be treated with vitamin A, irrespective of their assignment to a treatment or control group. If serological evidence suggests severe deficiency, then supplementation should be given, even in the absence of clinical signs. Children in the household who are not in the preschool age group that is the focus for the study but who exhibit ocular signs or symptoms should also be treated. Although these maneuvers might cause slight complications in data analysis, the ethical imperative is preeminent.

Unbiased Assessment

Achieving baseline comparability between treatment and control groups is but one aspect of preventing biased comparisons. It is also necessary to ensure that follow-up has been uniform and that apart from treatment with vitamin A, no other factors affecting the study end points or their assessment have intervened to bias the results. The former objective is often secured in part

by double-blinding the treatment assignment--that is, not telling either the study subjects or the investigative team who is receiving vitamin A. If double-blinding is successful, then not only are claims of biased assessment more difficult to sustain, but the trial itself is likely to be conducted in a way that avoids a self-fulfilling prophesy.

Double-blinding of clinical trials is often accomplished by using treatment and control interventions that are identical in all apparent respects, occasionally including side-effects. However, this ideal is unattainable in many circumstances, for example, in a comparison of surgical versus medical treatment. When treatment assignment is known or is likely to be discovered, one must establish additional precautions to prevent biased assessment: standardization of all critical aspects of measurement, rotation of field staff through treatment and control areas, systematic audits of field procedures by persons other than field investigators, concealment of the major study hypotheses from persons involved in the field work, and withholding of the results of interim analyses from the investigative team.

The possibility of bias can be assessed through consistency analyses. For example, if overall mortality appears to be reduced by treatment with high doses of vitamin A, the apparent effect might be examined in relation to age, sex, village, time from initiation of treatment, duration of effect, and cause-specific mortality. Plausibility would be enhanced, for example, if most of the mortality decline in those treated with vitamin A is explained by the same constellation of causes across different studies.

Sample Size

There is no single answer to the question, "How large should a study sample be?" The sample size for a morbidity or mortality study depends on the expected baseline rate in controls, the magnitude of the treatment effect that, if present, one would like to detect, the levels of significance and power desired, the duration of study, the method of randomization (by individual or cluster), the expected rate of refusals to participate, and the expected loss to follow-up. Once these details are specified, calculation of study size is a routine matter (Pocock, 1983).

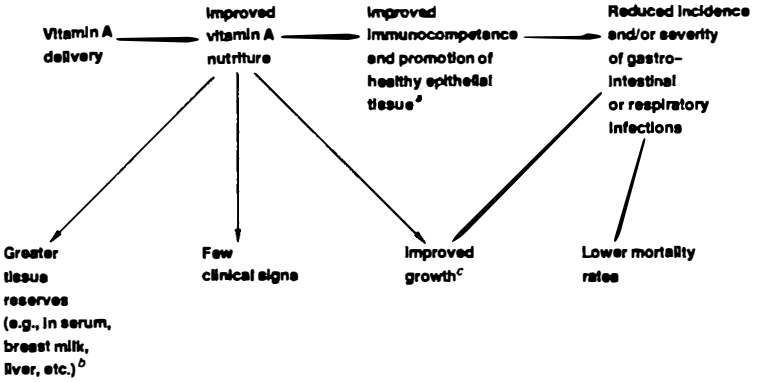
Any disagreement that occurs in planning the size of a study will involve the specifications outlined above. Since slight changes in specifications can produce major changes in projected sample size, there is ample opportunity for disputes. In the end, theoretical considerations must be balanced against pragmatic concerns such as resources and duration of the study.

One year is a minimally acceptable period of follow-up. Given the continued effect observed after 1 year in the Aceh study, 18 months or 2 years is preferable. In longer follow-up periods, potential latency in treatment effect can be revealed, seasonal effects can be detected, and more thorough analyses of cumulative effects and consistency of findings over time can be conducted. With longer follow-up, it becomes possible to determine whether an effect is sustained or merely transitory.

All the mortality studies are designed to detect, with high power, a 20% or greater reduction in mortality among children 6 months to 6 years of age, and they provide formal estimates of the necessary sample size. However, only the specialized study to be undertaken in the Philippines did this for morbidity, even though morbidity is an important component of these investigations. In the Indonesian study, rates of cough and diarrhea for children 12 to 71 months of age during the 7 days before interview were approximately 32% for control and treatment groups (Sommer et al., 1986). Approximately 22% of all children had a history of measles at any time in the past. Although these morbidity rates suggest that the studies under review are sufficiently large to detect any important effects of vitamin A on morbidity, confirmatory analyses of sample size and power would be useful.

Outcomes

A range of outcomes could be altered by an effective vitamin A supplementation program, but not all are suitable for inclusion in the proposed studies. Figure 5-1 proposes a conceptual framework for effects of vitamin A delivery on vitamin A nutriture, as ascertained by tissue samples or clinical signs. Included are effects on host resistance and the integrity of epithelial tissue. None of the proposed studies include these as outcome measures. A review of



[a] Not recommended for inclusion.
[b] Recommended, but assessment may vary by population (see Methods).
[c] Small effects should be expected.

FIGURE 5-1. Possible Outcome Variables (Recommended Unless Otherwise Specified)

current knowledge of the role of vitamin A in resistance to infection is included as an appendix to this report. Also, effects on the incidence and severity of infections, improvements in growth, and reductions in mortality are noted in Figure 5-1. Improved vitamin A nutriture is also shown to have a more direct effect on growth (i.e., independent of morbidity changes).

The need to include morbidity as well as mortality assessments has been emphasized in the section on the Philippine study designs. Both mortality and morbidity are of concern to public health authorities, and the demonstration of effects on morbidity would lend credence to any observed effects on mortality.

Determination of cause of death is also important, because this information could be used to assess the plausibility of results. If the proposed mechanisms through which vitamin A acts are the correct ones, and if mortality rates decline by as much as 25%, it should be possible to detect reductions in the proportion of deaths due to diarrheal and respiratory infections.

The prevalence of vitamin A deficiency would also be expected to change in a successful vitamin A intervention. Indeed, all the study proposals include the measurement of xerophthalmia. In addition to serving as an outcome measure, the inclusion of clinical assessments serves to identify those children who should be provided with vitamin A, irrespective of their intervention group. If all children with clinical signs are treated, declines in xerophthalmia are likely to occur even in control communities, but to a lesser extent than in intervention communities. Finally, however, it is important to ensure that the procedures used to assess vitamin A status are appropriate to the population (see section on vitamin A nutriture).

If morbidity rates are decreased, one would expect physical growth to improve. Although a reasonable outcome, effects on growth may be too small to detect easily in subsamples. Diarrheal diseases are known to affect growth, but the magnitude of these effects as measured in field studies is not large (Martorell and Yarbrough, 1983). Depending upon the level of reduction in disease prevalence, the anticipated effects on physical growth might be difficult to ascertain. Vitamin A supplementation may also have direct nutritional effects on growth, but evidence from field studies of the magnitude of these potential effects is lacking. Thus, the subcommittee believes that measurements of growth are less important than the measurement of other outcomes but that they should be included, at least in the baseline assessment, because they are useful as an indicator of nutritional status. Despite these problems with growth as an outcome, the subcommittee recommends that effects of vitamin A on child growth be analyzed if the data are available. Failure to detect effects on growth should not be construed as proof that the intervention is ineffective, however, or that the results are inconsistent. Finally, in assessing effects on growth, an attempt should be made to detect changes in indicators of wasting as well as stunting, since either may be plausibly affected by intervention with vitamin A.

Although host resistance to infection and integrity of epithelial tissues are potential outcome measures, these are not recommended for inclusion, because their measurement cannot be easily incorporated into field studies. The inclusion of assessments of vitamin A nutriture, xerophthalmia, morbidity, and mortality should, in the subcommittee's view, be sufficient for a

proper evaluation of response to vitamin A supplementation.

MEASUREMENT ISSUES

A fundamental aspect of study design and implementation in field trials of this kind is the accuracy of measurement for the major end points.

Mortality

There are two main types of methods for measuring infant and child mortality in developing countries: prospective and retrospective. Retrospective measurement involves some attempt to elicit statements from mothers about the number of live infants they have delivered and the number of their children who have died. It is important to ask specifically about live births, since mothers may otherwise include stillbirths in their response. Infants who died immediately after birth may be categorized along with stillbirths by the respondents and thus not reported.

Retrospective mortality data include two types of inquiries: those designed to provide counts of cumulative events and those requiring that events be placed accurately in time. There is substantial evidence showing that women are better able to supply information on cumulative events than they are to report the time of their occurrence (Preston, 1985). The inability to date events properly is vividly demonstrated in the erratic age distributions reported in most developing countries. Older women may also underreport some cumulative events. If it is deemed advisable to truncate the birth histories by limiting them to events in the recent past, it would also be useful to ask questions on the cumulative number of live births and surviving children. To do this, three sets of questions called the Brass series should be asked of the entire population of women or a representative sample thereof (United Nations, 1983):

- How many males (females) were ever born alive?
- How many males (females) are living with the mother?
- How many males (females) are living away from the mother?

In this way, one can avoid asking direct questions about the number of deaths that have occurred--questions that parents are unlikely to report in many cultural groups. If there is no cultural aversion to discussing child deaths, the list can include the question: "How many males (females) have died?"

In more than 40 countries, the World Fertility Survey made a considerable effort to collect fertility histories, which included dating deaths in time. This was done by obtaining complex maternity histories that occupied many questionnaire pages. Women were urged to reconcile any discrepancies in the histories. When they could not, a special computer program was used to reach a resolution. By and large, the World Fertility Survey was successful in doing this. Comparisons of fertility and mortality histories in several areas with reliable data from vital events registration systems (e.g., Sri Lanka and Chile) showed good correspondence between mortality levels and trends reported (Preston, 1985). In other countries, such as Bangladesh, the dating of events was much less reliable (Preston, 1985).

Indirect procedures for dating cumulative events reported in surveys have been developed by demographers. For example, by conducting surveys of women married less than 5 years, one can estimate the probability of their children dying before age 2 for the 1 or 2 years before the survey (United Nations, 1983).

Whether or not to include a maternity history in the baseline studies for research on vitamin A supplementation and mortality deserves some consideration. If a before-and-after study design were used, it would be very important to include such a component. In the proposed studies, however, the basic purpose of the baseline inquiry is to establish that preprogram mortality was equivalent in the treatment and control groups. Thus the Brass series, perhaps combined with a truncated history, seems to be a reasonable substitute (United Nations, 1983). Whether the Brass series or a truncated history is used, it is very important that the same questions be asked both at the beginning and the end of the surveillance so that mortality trends can be properly assessed.

In addition to retrospective methods, prospective techniques are also used although less frequently. Each of the studies discussed in the workshop includes prospective measurement of mortality in the form of follow-up household interviews at 4- to 6-month

intervals as well as surveillance systems in the villages to be studied. The quality of such data is enhanced if a roster of household residents during the previous visits is always available to determine whether any children have died since that time. Specific questions can be asked for each child on the list. If the study uses a continuous enrollment of children born into the households, the list must be updated at each survey, and it may be useful to record pregnancies as well. The use of a 4-month interval would be preferable, simply because the information requested would be more recent and thus more readily recalled. Loss to follow-up due to migration, which might be more likely in families with child deaths, would result in the loss of fewer person-months of data if more visits were made. Household visits made every 2 weeks provide very accurate information on mortality, whereas visits made only once a year lead to substantial omissions, especially of neonatal mortality.

Since two recording systems are to be used in the proposed studies (household visits and monitoring by key informants), it will be important to develop and use a carefully designed record-matching scheme to avoid duplicate reports of death.

The ideal study design would include both prospective and retrospective recording of mortality levels during a baseline period and during intervention. The best format for retrospective recording would be a complete maternity history, and the prospective studies should include frequent household visits. Obviously, the ideals must be pursued in the context of resource constraints. For the purposes of these studies, it should be sufficient to use prospective studies based on household visits made at 4-month intervals and the Brass questions for the retrospective inquiries.

Morbidity

Since decrements in the incidence or severity of gastrointestinal and respiratory illnesses are among the potential outcomes of vitamin A supplementation, morbidity data should be collected in all the proposed studies. The measurement of morbidity is complex. Seasonal variation in rates of infection is pronounced in many areas of the world, and comparison of baseline and intervention values requires caution. For example,

comparison of diarrhea rates collected at baseline during the summer with rates measured during the intervention phase in winter are not easily interpretable.

Continuous, prospective data collection is highly desirable but requires frequent home visits or examinations. Physical examinations provide data on morbidity events occurring at a single point in time. Since examiners are generally trained medical personnel, frequent visits become costly. Thus, investigators have relied on recall data from morbidity surveys conducted by carefully trained observers. The respondents in such studies are usually mothers.

Recall periods of days to months have been used by investigators. The longer the recall period, the greater the degree of underreporting due to forgetfulness. In Guatemala, even 2-week recall surveys of child morbidity were found to result in substantial underreporting. In each interview period, the number of days ill with diarrhea was underreported on average by 22% and for respiratory illnesses, by 12% (Martorell et al., 1976). The manner in which the questions are asked has important implications for reliability and validity. Open-ended questions (e.g., How has your child been in the last 2 weeks?) may not lead to the same answers as more direct questions (e.g., Did your child have diarrhea in the last 2 weeks?). The definition of morbidity events is another important element. In some studies, investigators have accepted the respondent's diagnosis of illnesses, whereas others have specified additional criteria. For example, some investigators record "diarrhea" whenever respondents so report; others also require a specified duration and stool frequency.

A few studies have asssessed the validity of recall morbidity surveys by comparing responses to physical examinations by qualified medical personnel. In one study, mothers' reports on the day of interview were compared with diagnoses made independently by a physician on the same day. Sensitivity values were found to be 66% and 92% for diarrhea and respiratory infections, respectively, and corresponding specificity values were 99% and 64% (Martorell et al., 1975).

Despite deficiencies in reliability and validity, data generated through recall morbidity surveys have been shown to be related, in the expected direction, to outcomes such as child growth (Martorell et al., 1975;

Mata et al., 1972; Rowland et al., 1977). This demonstration provides indirect confirmation of validity.

Since morbidity is far more common than mortality, its determination can be made from subsamples of the treatment and control groups from the mortality studies. Sample size requirements should be estimated by specifying expected effects and desired levels of power and statistical significance. Recall methods with reference periods not longer than 1 or 2 weeks should be used. By collecting information on respiratory and gastrointestinal signs and symptoms, researchers should be able to make adequate estimates of the incidence and severity of these events in treatment and control groups.

The proposed studies vary in the duration of their morbidity recalls. The Sudan proposal includes a 1-week recall of morbidity, and specific questions on diarrhea and respiratory diseases are narrowly defined. For the Bangladesh study, no particular methodology is described, although morbidity data collection is planned. For the proposed mortality study in the Philippines, the investigators plan to assess morbidity only at baseline. This is a deficiency of design that should be remedied. The separate morbidity study planned in the Philippines will use a sample from a nearby area and collect data during daily home visits; this should minimize recall bias. Because of the high degree of contact, however, mothers may be less willing to provide information. Planned pilot studies will provide information about this concern.

Assessment of Vitamin A Nutriture

There is no single, simple noninvasive method for assessing vitamin A nutriture under field conditions. In the proposed studies, the basic concern is to determine the presence and extent of systemic vitamin A deficiency in the study populations at baseline and to learn how the basal levels are changed by the intervention. Biochemical measurements should help to elucidate such changes, but by themselves they may not be conclusive. As a general rule, therefore, field studies in populations where vitamin A deficiency may be encountered should not rely only on measurements of serum vitamin A levels but instead should apply a

combination of dietary, biochemical, and clinical methods. The use of these methods in the proposed trials will strengthen the validity of the assessment of vitamin A status in the study populations. There is much that remains to be determined about the interrelationships of the various measures of vitamin A nutriture.

Dietary Intake Assessment

Dietary assessments that accurately reflect usual intakes are difficult to conduct. Yet, for a valid assessment of vitamin A status among communities and by individuals within communities, an indication of the level of dietary intake is needed. The objective of the dietary assessment in the proposed studies is not to quantify the vitamin A intake of individual children or subjects but, rather, to obtain a representative picture of the customary pattern of intake of vitamin A food sources by the population of interest (e.g., preschool children and pregnant women). Fortunately, the formidable methodological problems inherent in the field application of a method for dietary assessment are lessened when the purpose is restricted to determining intake of a particular nutrient that is contained in substantial amounts in only a limited number of foods. Among the populations to be studied in the proposed field trials, carotenoids rather than preformed vitamin A are likely to constitute two-thirds or more of the dietary intake. Vitamin A-active carotenoids are widely distributed in dark-green leafy vegetables, yellow vegetables, yellow cereals and tubers, yellow citrus and other yellow fruits, and red palm oil. Unfortunately, where nutrition-related blindness is prevalent, only very limited amounts of these carotenoid-containing foods are found in the diets of children during the vulnerable period of weaning and after weaning during the preschool years.

In the proposed studies, the choice of a dietary survey method will depend on various factors, such as resources available, accuracy expected, and type of population, and should be made by a professional with expertise in dietary field studies. This person should also be responsible for the validation and supervision of the procedures chosen. Recently, simplified methods for assessing vitamin A intake among literate adult

populations have been developed for use in epidemiological studies (Block et al., 1986; Hankin et al., 1984; Willett et al., 1983). The basis for simplification is to limit the survey questions to the frequency of consumption of specific portion sizes of a limited number of vitamin A-containing foods that constitute about 90% of total intake. An adaptation of this approach to make it relevant to local food supplies, consumption patterns, and socioeconomic conditions has been proposed by a task force and is being tested for reliability when administered by trained community health workers (IVACG, 1986). Application of such an approach to a community by collecting the data for a subsample of subjects in both experimental and control populations would provide the information needed to evaluate possible relationships among seasonal variations in patterns of mortality or morbidity, food availability or consumption, and, indirectly, vitamin A status. It would also serve to document that there were no significant quantitative differences in the intake of vitamin A in food during the study period and among and between experimental and control populations.

The committee favors including in each study at least a baseline qualitative assessment of the availability of vitamin A-containing food sources within communities and their presence in the diets of preschool-age children. If possible this assessment should include some quantitative components such as frequency of consumption of these sources in a random subsample. The assessment should be repeated periodically to cover seasonal variations during the study period. Selection of an appropriate, simplified, rapid method from those already developed is strongly recommended.

Biochemical Assessment

In the Aceh area, the population was presumed to suffer from marginal or partially depleted body reserves of vitamin A--a condition that may or may not have included clinical evidence of deficiency, e.g., xerophthalmia. It is generally accepted by the scientific community that liver reserves <20μg/g constitute a state of depletion or marginal vitamin A status; however, the amount of time that liver reserves <20μg/g can be maintained without signs, symptoms, or alterations in

serum levels is not known but seems to be directly related to the severity of depletion. Olson (1982) calculated that liver reserves <20 μg/g would afford protection against deficiency for 4 months in adults with no dietary intake of vitamin A. For children with liver reserves <20 μg/g, the time required for progression to symptoms would still be substantial but shorter than for adults with no dietary intake and the same liver retinol concentration.

An understanding of the correlation between concentrations of vitamin A in liver tissue and reductions in serum levels and the appearance of mild, moderate, and severe ophthalmological symptoms and signs is needed as a basis for an evaluation of health risks at the population level. Unfortunately, reliable data are limited and only tentative inferences can be made. Amédée-Manesme et al. (1984) reported abnormal relative dose-response tests and conjunctival impression cytology in children with vitamin A levels <20 μg/g of liver. In addition, they showed that serum levels bear little relationship to total liver vitamin A levels when these are >10 μg/g (Amédée-Manesme et al., 1984). Nightblindness, a mild, reversible clinical sign, has been demonstrated to occur occasionally at plasma levels >30 μg/g in diseased adults (Carney and Russell, 1980) and in children with plasma levels >20 μg/dl (Sommer et al., 1980). However, plasma levels <10 μg/dl are usually associated with clinical eye symptoms and signs and are invariably associated with liver levels <10 μg/g. On the basis of these studies, it can be inferred that in humans, serum levels <10 μg/dl reflect severely depleted vitamin A reserves. Animal studies have shown that clinical eye signs are manifested only when liver reserves are critically depleted.

In populations with marginal vitamin A status, the prevalence of serum levels <10 and <20 μg/dl may be very low. There is likely to be a higher prevalence of levels ranging from 20 to 30 μg/dl (Pilch, 1985). The interpretation of values in this range is very difficult, since nondietary factors play a part. Such borderline situations are unlikely for the study populations in the proposed trials, except possibly in the Sudan, where no preliminary biochemical data are available, and in The Gambia, where clinical deficiency seems to be less common. When the prevalence of marginal deficiency is high, an intervention that alters some nondietary factor, such as immunizations, programs that

introduce potable water or sanitation, or a home garden program that improves vitamin A intake, could bring about changes in the distribution of serum levels. The nondietary programs may relieve stress factors, such as the prevalence of chronic and acute infections, that usually depress serum levels independently; in these cases, a change in the serum distribution curve may not reflect a true change in vitamin A nutriture per se, i.e., body stores. On the other hand, a change in the serum distribution curves resulting from a program that does or could influence intake, e.g., a large periodically administered dose or increased intake from fortified foods or home-grown vegetables, would reflect a true improvement in vitamin A status.

For the type of epidemiological studies proposed, knowledge of the distribution of vitamin A nutriture in the study populations is particularly useful where vitamin A deficiency is known to occur. Separate curves should be developed and interpreted for preadolescent children, for adolescents, and for adults. When the prevalence of serum levels <10 μg/dl in young children is greater than 5% or greater than 15% for serum levels <20 μg/dl, WHO and IVACG consider that there is a deficiency problem at the public health level (Arroyave et al., 1982; WHO, 1982). Hence, distribution curves by age group and by sex can be used to describe grossly the baseline vitamin A status of a population. A shift in the distribution toward a decline in the prevalence of low serum levels in the population indicates that an intervention program has been successful in improving the blood supply of retinol to the body tissues (Arroyave et al., 1981; Solon et al., 1979).

Because of their promising, but as yet unproven, value as diagnostic tools, conjunctival impression cytology (Wittpenn et al., 1986) and the relative dose-response test (RDR) (Flores et al., 1984) may be used for a subsample of the study population if circumstances permit. Recent research described on page 17 (O. Amédée-Manesme, INSERM, Paris, personal communication; Amédée-Manesme et al., in press) suggests that the results of these tests would reinforce the diagnosis of marginal vitamin A status and that subsequent changes in serum levels are due to changes in vitamin A status, that is, before xerophthalmia symptoms are evident.

Clinical Assessment

The methodology and criteria for the clinical assessment of vitamin A deficiency have been standardized by WHO (1982) and Sommer (1982). The proposed studies plan to use these clinical criteria as end points. Corneal xerophthalmia (X2, X3) and Bitot's spots (X1B) are sufficiently objective that they can be diagnosed by trained nonspecialists under supervision of an experienced ophthalmologist. Nightblindness (XN) is more difficult to measure, since its occurrence can be identified only through histories of the study children obtained from mothers or guardians by interviewers from the study location. Because this approach may be highly subjective, it should be validated in the different populations. The use of distribution curves for serum levels could be helpful. Interviews to determine nightblindness should be conducted by well-trained field personnel who are regularly supervised and who adhere to well-developed quality control procedures.

Measurement of Anthropometric Status and Growth

The effects of vitamin A supplementation on growth may be too small to detect in subsamples of the study population. However, baseline anthropometric status is worth assessing to characterize the nutritional status of the cohort.

Measurements of weight and length or height are widely accepted as the fundamental parameters in anthropometric assessment of children and can be used to characterize the degree of wasting (low weight for height), stunting (low height for age), and low weight for age. These indicators can provide a general guide to the nutritional status of the population, and since they are commonly a component of anthropometric studies in the United States and abroad, it is possible to compare results with those for many other populations. If age is difficult to determine, weight-for-height measurements can be used as an indicator of acute malnutrition. Weight-for-height data alone are seriously limited as a nutritional indicator, however, since they provide no indication of more chronic malnutrition and they vary by season and, to some extent, among different racial groups.

Arm circumference may be a useful measurement to include, preferably in addition to weight and height.

Small values of arm circumference appear to be about as well correlated as other nutritional indicators with health outcomes such as morbidity and mortality, although data in this area are limited (Chen et al., 1980). In preschoolers, arm circumference without adjustment for age may be used as a rough measure of nutritional status, since arm circumference changes relatively little from age 1 to 4 years. Measurements of arm circumference can vary widely when field staff are not carefully trained and supervised to ensure that there is consistency in the way these measurements are taken. Moreover, arm circumference data are not as readily interpretable as indicators of nutritional status, since there are relatively few reference data on other populations for comparison. Thus, this measurement alone is probably better than having no anthropometric assessment at all but is much more useful as a measure of nutritional status when assessed along with weight and height.

ETHICAL ISSUES

During the course of the workshop and in subsequent deliberations of the subcommittee, the methods for controlled studies of the effect of vitamin A supplementation were the main focus of the subcommittee's efforts. Also discussed in considerable detail were the ethical implications of controlled studies including the extent of scientific uncertainty about the value of various study designs and their results, the possible risks and benefits to participants, the procedures for informed consent, and the soundness of the hypothesis to be tested.

Although the importance of vitamin A for health has already been demonstrated for people with severe vitamin A deficiency, as manifested by ophthalmological signs or symptoms (see section on biochemical assessment), similarly convincing evidence is lacking for the effects of vitamin A on mortality among people with marginal vitamin A status (i.e., with no clinical signs or symptoms). The Indonesian study (Sommer et al., 1986) did not provide definitive information on this matter because of shortcomings noted by the subcommittee (e.g., lack of a double-blind design, baseline differences between treatment and control villages, lack of data on serum retinol levels at baseline and on preprogram mortality, and the pattern of mortality differences in older boys).

Although laboratory studies in animals and clinical data on humans suggest a relationship between vitamin A and resistance to infection, these data, reviewed in Appendix C, are not conclusive. All but one member of the subcommittee believe that Sommer's findings, together with data from animal and laboratory research, do not provide adequate proof that treatment of marginal vitamin A status has a positive effect on morbidity and mortality. For this reason, they concluded that the recent field studies in Indonesia should be replicated. Included in Appendix D at the end of this report is a statement by Frank Chytil who questions the need for these studies.

Implications of the Results of the Controlled Trials for Program Design

The subcommittee recognizes that xerophthalmia is a preventable outcome of vitamin A deficiency and is accompanied by other systemic manifestations. It also recognizes that the risk of blindness due to severe vitamin A deficiency provides strong justification for interventions to control the deficiency. The existence of persuasive reasons for establishing vitamin A supplementation programs does not lessen the importance of demonstrating convincingly whether or not such programs will affect child health and survival in a significant way.

Because limited resources are available to public health and nutrition planners in developing countries, not all needed programs can be implemented. Difficult choices are often based on anticipated effects, and the prospect of reducing child mortality by periodic dosing with vitamin A could result in the decision to defer or abandon other public health interventions. For this reason, it is important to undertake adequately controlled trials to determine and quantify the efficacy of the proposed intervention and also to identify any possible adverse effects that could result from the intervention. The majority of the subcommittee believes that the true ethical imperative is to ensure that these program decisions are guided by a sound evaluation of the probable outcomes. For this reason, all but two subcommittee members concluded that more controlled studies of the kind reviewed at the workshop are needed as a basis for decisions involving competition for limited program resources.

Choice of Study Design

The subcommittee recognizes the complex ethical issues inherent in the conduct of controlled clinical trials, particularly when randomization and use of placebos are involved. Responsible scientists writing on this subject often disagree over the application of scientific principles to specific research protocols (Shaw and Chalmers, 1970). An example is the Medical Research Council's clinical trials of folic acid supplements as a preventive measure against neural tube defects (Beardsley, 1983).

The subcommittee recognizes the value of nonexperimental studies and believes that they should be conducted when experiments are not feasible. However, a given controlled study is likely to yield information of greater scientific validity than a nonexperimental study. Since nonexperimental designs are likely to produce results that can have several plausible interpretations, a consensus within the scientific community is likely to be reached more rapidly if the most rigorous applicable study designs are used. The view of the majority was that randomized controlled trials are preferable to nonexperimental studies for rapidly and unequivocally gaining the information needed to determine the level of deficiency at which supplementation with vitamin A would affect child survival. The majority of the subcommittee concurred with USAID's decision to sponsor the controlled trials described in this report.

A second minority report, by Lincoln Chen (Appendix E), agrees with the majority of the subcommittee that mortality consequences of marginal vitamin A deficiency remain uncertain and unproven. However, it takes the position that individuals with marginal vitamin A nutriture are at increased risk of developing severe deficiency and hence are at increased risk if assigned to the control group in a placebo study design. It advocates consideration of alternative, less rigorous study designs and urges that final designs be worked out in conformity with local and international ethical standards.

In the proposed studies involving time-staggered introduction of vitamin A capsules, or intensive vitamin A distribution in selected populations already receiving vitamin A through regular government services, some children would receive vitamin A supplementation and

others would not. The populations of some geographical areas can be used as a control for populations with intensive vitamin A distribution only if regular government services are less than fully effective in delivery of vitamin A. Nonetheless, there would still be a treated group and an untreated or ineffectively treated control group. Without the benefit of randomization in a controlled trial, vitamin A treatment could be confounded by other factors affecting the study outcome. Another suggested design, retrospective case-control studies, would be less satisfactory than other designs, since it would be difficult to ascertain prior vitamin A intake and there would again be a strong possibility of confounding, which may not be controlled adequately in the analysis.

Risks and Benefits

To minimize the risk of severe vitamin A deficiency in any study design, participants should be screened before the studies begin to detect and treat those with any clinical signs of xerophthalmia (e.g., nightblindness, Bitot's spots, or corneal xerophthalmia). Similarly, clinical signs and symptoms should be monitored at 4-month intervals to detect and treat those who develop symptoms of xerophthalmia during the course of the study.

The risk of excessive intake arising from the provision of vitamin A capsules containing 200,000 IUs to children every 4 months is judged to be very small (Bauernfeind, 1980). The study protocols stipulate oral administration of 200,000 IUs of retinyl palmitate in oil (with 40 IUs of vitamin E) to preschool children, either every 6 months or every 3 months. In considering the safety of this approach, two possible effects deserve mention. The first is chronic or long-lasting toxicity resulting from the frequent ingestion of large amounts of vitamin A for prolonged periods, often weeks or years. Chronic toxicity leads to the accumulation of excessive amounts of vitamin A in the body. The second is transient intolerance, which is observed immediately after ingestion of a large dose. This is due to a temporary rise in the circulation of retinol or retinyl esters that are not bound to retinol-binding protein.

These concerns have been seriously considered by the group of experts in the International Vitamin A Consultative Group (IVACG). Their report, entitled The

Safe Use of Vitamin A (Bauernfeind, 1980), presents a critical review of the world literature on the subject, discusses the physiological disposition of vitamin A (e.g., absorption, retention) by the human body at normal and excessive levels of intake, and, most importantly, analyzes the substantial body of experience in various countries with programs to deliver high doses of vitamin A. At 200,000 IUs in oil (in most cases with 40 IUs of vitamin E), most of the studies report no manifestations of intolerance; a few describe transient post-administration malaise or vomiting, which disappear within a few hours. Abnormalities suggesting prolonged cellular effects (as described in chronic toxicity) have never been reported, even in studies where the 200,000 IU dose was administered every 4 months. Therefore, IVACG recommends that treatment consist of one capsule (200,000 IUs) every 4 to 6 months to children over 1 year of age. It also recommends that "infants less than 1 year and very small or low weight children should be given one-half of the capsule dose (100,000 IUs)."

The level of vitamin A supplementation for pregnant women deserves special consideration. Studies in experimental animals have shown that excessive intakes of vitamin A during gestation may produce congenital abnormalities (Rosa et al., 1986). A few observations in humans, reported in the same paper, suggest that the same may occur in pregnant women with daily intakes of 25,000 to 150,000 IUs during the critical period of organogenesis, and in one case, abnormalities were associated with a single excessive accidental dose.

In fact, major malformations have been reported only in infants born to women who regularly took vitamin A supplements containing 25,000 IUs or more daily during early pregnancy (Underwood, 1986). There are no confirmed reports of teratogenicity in humans at lower doses and none from women consuming regular dietary sources of either preformed vitamin A or carotene. The proposed study in the Philippines will provide pregnant women with 10,000 IUs daily as a supplement to their regular diet. WHO and IVACG warn that massive doses should not be given during pregnancy but that daily supplements of 10,000 IUs can be given safely at any time during the gestational period "in geographic areas or under conditions where the intake is known to be inadequate and there is little opportunity for dietary improvement of vitamin A status" (Underwood, 1986).

The majority of the subcommittee concluded that all children, including those in control communities, derive some benefits from participating in the studies as described above. For most of these children, a salient benefit is that both treatment and control groups will be screened and treated for severe vitamin A deficiency. It is unlikely that xerophthalmia would be detected or treated if the studies were not implemented.

The subcommittee also urges that study subjects receive additional direct benefit from participating in the study. Among the options discussed were improvements in primary health care, including immunizations against infections such as tetanus and measles. To ensure the validity of the study results, these benefits should be extended equally to control and intervention communities.

Ethical Safeguards

In its consideration of the proposed protocols, the subcommittee took into account USAID's procedures for soliciting proposals. In compliance with USAID requirements, each of the proposals reviewed by the subcommittee had been examined by a human subjects review board in each of the U.S.-based universities planning to conduct the study or was required to undergo such a review before beginning the study. USAID's procedures for the protection of human subjects involve adherence to the U.S. Codes used by the Department of Health and Human Services (CFR, 1983). For studies taking place in other countries, similar codes, such as those used by WHO, may also be used. Studies of the kind reviewed by the subcommittee are also subject to review in the countries where the fieldwork will be done by the government bodies responsible for coordinating and approving research carried out with foreign assistance. Furthermore, studies carried out in conjunction with a local research or educational institution will be governed by applicable review procedures in those institutions. To ensure that every possible ethical safeguard is taken, the subcommittee also recommends that a data and safety monitoring board be established for each of the trials, that the board should have access to interim results of the studies, and that it should be independent of the principal investigators.

The need to secure informed consent was recognized by the subcommittee, but a review of the specific procedures

for this was considered to be beyond the scope of this report. The subcommittee emphasizes, however, that the advisability of obtaining consent before or after randomization should be considered in the development of study procedures.

Conclusions

- Because it is not known whether marginal vitamin A deficiency affects mortality, at what level of deficiency the effect on mortality may occur, or whether the magnitude of the effect may vary in different populations, it is ethical to randomize children without clinical signs to either treatment or control groups.

- Double-blind designs offer the best potential for reaching unequivocal conclusions regarding the value of vitamin A supplementation in lowering mortality risk in children with mild or moderate deficiency.

- A convincing assessment of the mortality effects of vitamin A supplementation in populations with avitaminosis A will have major implications for resource allocation and design of child health programs.

- The risks associated with xerophthalmia should be minimized by screening and treating children with any clinical signs. Without the screening and treatment program provided by these studies, all the participants would be at greater risk.

- Control groups should receive some additional direct benefit from participating in the study. For example, they could be immunized against tetanus or measles. To ensure comparability of results, the treatment groups should also receive the interventions administered to controls.

- Each study must undergo thorough review of ethical considerations by institutional review boards and governments.

- **At the end of the study period, administration of vitamin A to the control group should be considered in light of the available findings. If the benefit: risk ratio is favorable, supplementing the control areas with vitamin A would permit a validation of benefits in the randomized controlled trial by a comparison of the controls before and after supplementation.**

Chapter 6

SUMMARY OF SUBCOMMITTEE'S FINDINGS

Two undisputed goals of public health efforts in developing countries are to lower child morbidity and mortality rates and to improve nutritional status. Although these two goals are closely related, the response to specific interventions need not be similar for the two types of outcomes. For example, oral rehydration therapy is generally regarded as an approach that will lower case-fatality rates for diarrhea but will affect neither morbidity rates nor nutritional status.

In recent years, international and national agencies have placed increased emphasis on ways to improve child survival and have striven to identify measures, such as oral rehydration therapy and vaccinations, that are simple, relatively inexpensive, and effective in lowering mortality rates. The most recent intervention to attract the attention of the international community is based on the hypothesis that community distribution of vitamin A supplements, when given to populations with marginal vitamin A status, will lower child mortality rates. If proven effective, there is no doubt that vitamin A programs will surface as a cost-effective measure for lowering mortality in areas where the vitamin A status of the population is compromised.

EXPECTED OUTCOMES

The main hypothesis of the study protocols reviewed at the workshop is that the provision of periodic massive doses of vitamin A will reduce child mortality rates. The work of Sommer et al. (1986) in Aceh, Indonesia, was used as a basis for estimating sample size. The investigators who developed the proposed study designs

assumed that mortality rates in the 1- to 5-year group will be reduced by 20% to 25%. The reported decrease in Aceh was 35% to 40% and may have been even greater, because untreated individuals in villages randomized to treatment with vitamin A were included in the analysis and because children with clinical signs of xerophthalmia, and thus probably at greatest risk of death from vitamin A deficiency, were treated with vitamin A and excluded from the analysis. All the planned studies will be similar to the Aceh study in these respects.

An anticipated 20% to 25% decline in mortality rates for children 1 to 5 years of age, which was used to calculate the sample sizes, may be unrealistically high for areas of the world where vitamin A deficiency is not as pronounced as in Indonesia and where rates of diarrheal and respiratory infections are relatively low. Although the true impact on mortality in those areas may be considerably lower than the 34% reported in the Aceh study, the effects may still be important from a public health point of view, but the proposed studies may not have sufficient statistical power to detect lower but still important mortality declines. A conservative approach would be to design studies capable of detecting some minimum effect on mortality that can be expected to have important implications for public health, regardless of the setting. Although the subcommittee did not consider what this level would be, it believes that the figure would be substantially lower than 25%. The subcommittee also concluded that an increase in the samples to a size capable of detecting, for example, a 10% reduction in mortality would be so costly as to be prohibitive. For this reason, the adoption of lower estimates of the expected impact on mortality is not recommended. The investigators should also make careful allowances for refusals to participate, loss to follow-up, and other factors that may increase the required sample size. Where doubt exists about the effect of these factors, generous margins of error should be included.

If comparable research instruments are used, it may be possible to pool the data from the three studies to permit special analyses and overcome deficiencies due to loss and other factors. However, although combining the data sets might produce sample sizes adequate to demonstrate statistical significance for small mortality changes, it would be difficult to determine the public

health significance of combined results from such diverse studies. Moreover, consistency among the results of separate studies, even if they are not statistically significant, may be a more persuasive basis for policy than pooled results of several studies.

As has been discussed, adequate vitamin A nutriture is essential to the healthy function of epithelial tissues, thereby contributing to the ability of the individual to resist infections, especially in the respiratory and gastrointestinal tracts. Thus, the presumed mechanism by which vitamin A supplementation effects mortality is through reductions in morbidity, such as respiratory and gastrointestinal infections.

The subcommittee concluded that ascertainment of effects on morbidity should be given a high priority for several reasons. First, demonstration of plausible mechanisms would add to the persuasiveness of the mortality findings. Second, since episodes of infection are far more common than deaths, the sample sizes required for the detection of morbidity effects are much smaller than those required for mortality ascertainment. Thus, only a subsample of the study population is required to demonstrate changes in morbidity. Third, in the event that the true mortality effect is lower than that predicted in the proposals, the studies could show whether there is at least an effect on morbidity, thus providing information required to assess whether a negative finding may have been due to insufficient statistical power. Morbidity effects may also have direct relevance to the design of child survival programs. For example, demonstrations of reductions in the incidence or severity of diarrhea or measles brought about by vitamin A supplementation would be indicative of a corresponding potential for reducing mortality. Inclusion of a more detailed morbidity substudy in a subsample would benefit the Bangladesh, Sudan, and Philippine studies and should therefore be considered.

The subcommittee strongly recommends that baseline anthropometric measures be included, even though there is a lower possibility that vitamin A will influence child growth. If feasible, child growth measurements might be extended beyond the baseline; however, effects on growth may be too small to be detected.

The impact of vitamin A interventions may vary in relation to the prevalence and severity of vitamin A deficiency. Thus, to interpret the results as accurately as possible, the study population should contain the full

spectrum of vitamin A deficiency. In Bangladesh and the Philippines, vitamin A deficiency is known to be endemic, as it is in India. No data are available on vitamin A nutriture in the Sudan, according to the investigators, although they suspect that the prevalence of vitamin A deficiency may be high. Thus, the results of the proposed studies may not be informative about effects in communities where the prevalence and severity of vitamin A deficiency are low, such as in Latin American populations where clinical signs of deficiency are rare and biochemical measures are required to detect deficiency status. The proposed study in The Gambia may be more informative, since clinical signs are rare.

Ethical issues were carefully considered by the subcommittee, but unanimity could not be reached. The majority of the subcommittee concluded that there is a sound basis for controlled studies of the effect of vitamin A supplementation on child mortality and that double-blind designs are the preferred approach where possible. It concluded that in view of the importance of the results for resource allocation and program design, the benefits outweigh the risks to participants. Two subcommittee members did not agree with the majority view and raised questions regarding the ethical soundness of the studies, each for different reasons, and each drew different conclusions, which are given in Appendixes D and E.

STUDY DESIGN

The workshop included considerable discussion of overall study design issues, which are discussed in some detail in Chapter 4. Because of the Indonesian study results showing increasing divergence in mortality levels over time, the subcommittee recommends that the duration of the studies should be at least a year, preferably longer. The subcommittee also considered carefully the merits of individual, household, or village level randomization and concluded that although village level randomization would require some increase in sample size to correct for clustering effects, it would not detract from the validity of the studies and would solve logistical problems.

The subcommittee also recognized the importance of accurate, precise measurement of mortality--the principle outcome variable of the studies. It therefore recommends

specific procedures for avoiding biases in ascertainment of outcomes (see Mortality Measurement section in Chapter 4) and endorses the use of double-blind study designs. For baseline mortality data, it recommends the use of the Brass question series (United Nations, 1983) in addition to prospective measurement and suggests that the same series be repeated at the end of the study. The subcommittee also suggests that household rosters be used for mortality surveillance and emphasizes the importance of careful record matching to avoid duplication of death reports.

All studies should include an adequate assessment of vitamin A nutriture, child mortality, and morbidity. The use of comparable methods to determine vitamin A status will be of great value in interpreting the studies, as would standardization of procedures for morbidity recall data. The subcommittee also recommends that measurements of serum vitamin A levels should be supplemented either by analysis of conjunctival histopathology, by the relative dose-response test, or by both for a subsample of the population. This would permit the interpretation of borderline serum vitamin A levels (20-30 mg/dl). Inclusion of measures of immunocompetence is not recommended for large field studies of this kind. The proposed studies may provide an opportunity to assess the reliability and validity of new measurements that have not previously been accepted for field use such as conjunctival cytology.

The use of a stratification scheme prior to randomization is not recommended unless the stratification variables, such as the presence of health facilities in the village, can be easily ascertained. For other factors that may require adjustments for baseline inequalities, such as mortality, stratification can be performed when the data are analyzed, provided the pertinent data have been collected.

Uniformity and completeness in analyses will also add to the interpretation of the studies. In this workshop, the subcommittee did not consider which analyses are essential, but it did recognize the need for timely and complete reporting of central findings.

Descriptive data about the study areas should be readily available, and information concerning the effects on morbidity, growth, and vitamin A nutriture, not just mortality, should be reported. This information will be required to assess the consistency of results both within and between studies and thereby to determine the

prevalence of deficiency and permit investigators to estimate the potential effect of vitamin A supplementation in preventing child deaths among those with mild or moderate deficiency.

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APPENDIX A
WORKSHOP ON METHODOLOGIES FOR FIELD TRIALS OF VITAMIN A SUPPLEMENTATION

Subcommittee on Vitamin A Deficiency Prevention and Control
Committee on International Nutrition Programs
Food and Nutrition Board
National Research Council, Washington, D.C.
Board Room

Monday, August 18, 1986

8:30 a.m Continental breakfast

9:00 a.m. Introductions
...Dr. Reynaldo Martorell

Welcome on behalf of the National Research Council
..Dr. Sushma Palmer

9:15 a.m. Purpose of the workshop
..Dr. Reynaldo Martorell

9:30 a.m. Summary of Aceh study in Indonesia
..Dr. Alfred Sommer

9:50 a.m. Discussant
..Dr. Reynaldo Martorell

10:00 a.m. Presentation of study protocol for the Philippines
..Drs. Carmencita Reodica and Antonio Perlas

10:30 a.m. Discussant
..Dr. Frederick Trowbridge

10:45 a.m. BREAK

11:00 a.m. DISCUSSION: Philippines Study Protocol

12:15 p.m. LUNCH

1:15 p.m. Presentation of study protocol for the Sudan
..Dr. M. G. Herrera

1:45 p.m. Discussant
..Prof. Mervyn Susser

2:00 p.m. DISCUSSION: Sudan Study Protocol

3:15 p.m. BREAK

3:30 p.m. Presentation of planned study in India
..Drs. Vinodini Reddy and
K. Vijayaraghavan

4:00 p.m. DISCUSSION: Planned study in India

4:30 p.m. Presentation of Philippines
morbidity substudy
..Dr. Michele Forman

5:00 p.m. DISCUSSION: Morbidity Protocol

5:30 p.m. Reception (The Rotunda)

6:30 p.m. Dinner (Members' Room)

Tuesday, August 19, 1986

8:30 a.m. Continental breakfast

9:00 a.m. Review of study protocols and discussions
..Dr. Reynaldo Martorell

9:30 a.m. Presentation of Bangladesh Study Protocol
..Drs. Sloan, Habicht, and Chowdhury

10:00 a.m. Discussant
..Dr. Samuel Preston

10:15 a.m. BREAK

10:30 a.m. DISCUSSION: Bangladesh Study Protocol

11:45 a.m. DISCUSSION: study design requirements,
uniformity, and interrelationships of
studies

Discussion of research methods including:

- criteria and methods for identifying vitamin A status
- confounding variables
- sampling issues
- outcome measurement and ascertainment: morbidity and mortality

12:30 p.m. LUNCH (in Board Room)

1:30 p.m. Continuation of discussion of research methods

3:00 p.m. Closing remarks
..Dr. Reynaldo Martorell

3:30 p.m. WORKSHOP ADJOURNMENT

APPENDIX B

AUGUST 1986 WORKSHOP PARTICIPANTS, OBSERVERS, AND STAFF

WORKSHOP ON METHODOLOGIES FOR FIELD TRIALS OF VITAMIN A SUPPLEMENTATION

Subcommittee on Vitamin A Deficiency
Prevention and Control
Committee on International Nutrition Programs
Food and Nutrition Board
2101 Constitution Avenue, NW, Board Room
Washington, DC 20418
August 18-19, 1986

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APPENDIX C

VITAMIN A DEFICIENCY AND INFECTION

Early studies suggested a relationship between vitamin A deficiency and susceptibility to infections (Green and Mellanby, 1928). Studies in deficient animals (Wolbach and Howe, 1925) as well as postmortem studies of deficient children (Blackfan and Wolbach, 1933) demonstrated that there had been histological changes in epithelial tissue that had presumably rendered the subjects more vulnerable to infectious agents. Animal studies also demonstrated that keratinized epithelium responded to vitamin A (Wolbach and Howe, 1933). Nevertheless, the exact role of vitamin A in host defense mechanisms is still far from clear, especially in humans. Indeed, a direct association has been questioned repeatedly because nearly all the early animal and clinical studies (Blackfan and Wolbach, 1933; Bloch, 1921; McLaren et al., 1965) were confounded by multiple concurrent deficiency states, particularly protein energy malnutrition, that result from the inanition characteristic of vitamin A-deficient diets (Beisel et al., 1981; Krishnan et al., 1974).

In a report of a workshop, Beisel et al. (1981) suggested, "In generalized malnutrition, it is virtually impossible to define causal relationships between individual nutrients and abnormalities in immune responses." However, Darip et al. (1979), using an animal model that circumvented the confounding effects of protein energy malnutrition, demonstrated that rats severely deficient in vitamin A were more susceptible to infection when held under conventional conditions and died because of infections. In studies by Rogers et al. (1970), rats maintained under germfree conditions developed the symptoms and signs of vitamin A deficiency but survived for several months beyond conventionally

reared rats with vitamin A deficiency. However, using a mouse model, Hof and Wirsing (1979) found that when dietary vitamin A was reduced but not absent, that is, the animals were marginally deficient, the defense mechanisms of the host were not appreciably altered by their deficiency. In another study in mice, Hof and Wirsing (1979) demonstrated that a high dose of vitamin A may influence certain resistance mechanisms, but they did not recommend that vitamin A be used to increase resistance to infections for the following reasons:

"the beneficial effect seems to depend on the pharmacological form and route of application;
"the beneficial effects are achieved only by high doses approaching hypervitaminotic amounts;
"the consequences of high intake of vitamin A are detrimental for some resistance mechanisms."

They concluded that "the term anti-infective vitamin does not hold absolutely true for vitamin A, although certain anti-infective properties cannot be denied."

Morbidity from naturally occurring infections was not reported in other studies in rats that did, however, demonstrate that mild vitamin A deficiency altered the immune response (Nauss et al., 1979, 1985a), increased the severity of experimental corneal herpes simplex virus infections, and resulted in a high incidence of corneal epithelial ulceration and necrosis (Nauss et al., 1985b).

In a recent review of the literature concerning the potential role of retinoids in infectious diseases, Dennert (1984) concluded that data from animal studies are not sufficient to warrant conclusions and that epidemiological studies in humans are needed. In most epidemiological studies, however, investigators have been unable to successfully control the factors that may confound the association of vitamin A deficiency with increased incidence or severity of infections. Reports such as those by Oomen et al. (1964) based on admissions to a children's ward in Indonesia suggest that there may be an association between vitamin A deficiency and protein energy malnutrition as well as other specific infectious diseases, including gastroenteritis and bronchopneumonia. A soon-to-be published report on a study in India (Milton et al., in press) states that an increased risk of respiratory but not diarrheal infections was found among children with mild

xerophthalmia--a finding that differs from those reported by Sommer et al. (1984). These discrepancies and others noted previously in this report suggest that observed associations may reflect the depletion of vitamin A reserves by infections rather than an increase in the incidence of infection from vitamin A deficiency. A recent study in Brazil also suggests this type of relationship (Campos et al., in press).

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APPENDIX D

STATEMENT CONCERNING NEED FOR PLACEBO GROUP

Frank Chytil

Listed below are my specific objections to the use of placebo groups in the experiments proposed to document further that vitamin A deficiency is accompanied by higher morbidity and mortality in children. I sincerely believe that the designs discussed by the committee are based on insufficient or incorrect knowledge or interpretation of the well documented time-dependent deleterious effects of vitamin A deficiency in humans.

The essential nature of vitamin A (retinol) deficiency is clearly established but not widely recognized. Prior to designing experiments in an attempt to explain biologic or pathologic phenomena it is our duty to be familiar with the progress achieved in the field in question. We should realize that vitamin A deficiency manifests itself primarily as a systemic disease resulting from the alterations of cellular differentiation in many if not all epithelia of various organs, including the bronchopulmonary tree, digestive and the genito-urinary tract (Wolbach and Howe, 1925). Although these effects were first reported sixty years ago and have been amply confirmed since then, the description of the primary function of retinol in most textbooks is still limited to the involvement of this

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micro-nutrient in vision. It is, therefore, not surprising that the involvement of vitamin A in normal cellular differentiation and maintenance of the proper differentiated state which is essential for the existence and survival of an animal is not generally known or appreciated by ophthalmologists. Unfortunately, even professionals involved in the epidemiology of vitamin A deficiency are unsure on this essential point. For example, the following statement was published in 1986 in an authoritative publication "One theory is that vitamin A is necessary to maintain the body's mucus membranes, the surfaces of the respiratory, urinary and intestinal tracts which protect against entry of bacteria and viruses" (McLaren, 1986). I should like to stress that the occurrence of metaplastic changes in the vitamin A deficient epithelia and their reversibility with administration of vitamin A is not a theory but a well documented fact (Wolbach and Howe, 1925, 1933). Consequently there is no need simply once again to experimentally corroborate this "theory."

Poor appreciation of the systemic effects of vitamin A deficiency is probably the reason that the studies in Indonesia led to the "discovery" that vitamin A deficiency is accompanied by a higher incidence of morbidity and mortality which could be in part lowered by vitamin A intervention (Sommer et al., 1986). Higher mortality in children with vitamin A deficiency had been previously reported (McLaren et al., 1965). Need we give a "placebo" to children living in an area of demonstrated vitamin A deficiency to corroborate that the systemic effects of vitamin A deficiency are deadly?

Another clearly established fact sometimes casually cited is that ophthalmologic effects of vitamin A are the last symptoms of deficiency (Select Committee on Hunger, U.S. House of Representatives). Xerophthalmia which can be rather easily diagnosed is preceded by pathologic alterations in organs other than the eye and must be viewed as only a part of the vitamin A deficiency syndrome. This fact was established even before vitamin A was chemically characterized (Bloch, 1921, 1924). A serious error in interpretation of the effects of vitamin A deficiency occurs when xerophthalmia is delineated as a starting point for the necessity of vitamin A intervention. What about those children who will eventually become xerophthalmic but are already systemically deficient? Beyond any doubt some of them will die needlessly.

The most frequent cause of death is bronchopneumonia as first described by Bloch (1921, 1924) in Denmark and confirmed in the U.S. (Blackfan and Wolbach, 1933; Wolbach, 1937). These and other subsequent publications suggest that vitamin A supplementation to children living in an area of vitamin A deficiency may lower the incidence of pneumonia and diarrhea (differentiation of intestinal epithelium is also influenced by vitamin A) and prevent fatal effects of all kinds of infectious agents.

Admittedly more is known about the systemic lesions in vitamin A deficiency from experiments with animals than in humans. On the other hand, the lesions and their consequencies strikingly resemble those observed in humans (Blackfan and Wolbach, 1933; Bloch, 1921). Should we use the children in the placebo group as experimental animals to corroborate results that are already well documented?

In conclusion, the lack of availability of vitamin A leads to systemic effects which precede but are not as easily diagnosed as xerophthalmia. Xerophthalmia occurs late in the vitamin A deficiency syndrome. Thus, in the area in which a high frequency of xerophthalmia has been observed, many children are expected to be vitamin A deficient but have no evidence of xerophthalmia. In other words, systemic effects of vitamin A deficiency may be already existing in these children. By designing a placebo group as proposed by the investigators and sanctioned by the other members of this committee and by treating only those children already suffering from xerophthalmia, the other children in the placebo group will be placed in needless jeopardy. These children, who may already be suffering systemic effects from vitamin A deficiency, will be subjected to further possible consequences of vitamin A deficiency, namely death. The essential nature of vitamin A is well known and documented and all children living in an area of vitamin A deficiency should be given vitamin A supplementation as would be the case with immunization against an infective agent. Vitamin A is not an experimental drug and should not be treated as such.

Therefore, I urge the members of the committee to consider whether or not designing a protocol leaving several thousand children as a "placebo" group in an area with high morbidity in addition to xerophthalmia, would serve the purpose of helping underdeveloped countries through AID funding.

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APPENDIX E

STATEMENT CONCERNING ETHICAL REVIEW[1]

Lincoln C. Chen

The report provides an excellent review of the scientific merits of field research methodologies associated with proposed studies on this subject in the Philippines, Bangladesh, the Sudan, India, and the Gambia. It concludes that because the mortality impact of Vitamin A supplementation to children is scientifically unproven, it is ethically feasible to conduct random, double-blind field trials of Vitamin A capsular distribution in comparable treatment and control population groups. Such a definitive ethical conclusion is unwarranted, in my judgment, for the following reasons.

My basic premises are: (1) Uncertain and unproven are the morbidity and mortality consequences of mild-moderate deficiency in populations; (2) Severe Vitamin A deficiency is associated with clinically significant disease; (3) The definition and measurement of mild-moderate deficiency are unclear; However, (4) among mild-moderate deficient individuals and populations, the risk of progression to severe deficiency and/or clinically significant disease is higher than in normally nourished individuals.

In my judgment, the research designs recommended in the report involving placebos or control populations, blind or not-blind, wherein Vitamin A is withheld from mild-moderate deficient individuals in the control groups are ethically questionable. Although the morbidity and mortality consequences of withholding in

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the control/placebo populations are scientifically uncertain, the risk of severe deficiency and/or clinically significant disease must be absorbed by the mild-moderate deficient individuals in the control/placebo populations.

In discussion and correspondence with the subcommittee, it has been pointed out to me that all the proposed research designs call for identification and treatment of severely deficient individuals, benefits of the knowledge generated would outweigh risks, and alternative quasi-experimental designs would be less rigorous scientifically. While I agree with these comments, none of the proposed research designs address the issue of risk to the mild-moderate deficient study subjects in the control/placebo groups. Also, the benefits gained are universal while the risk is absorbed by these study subjects. Finally, a broader array of complementary, ethically unassailable, lower cost approaches, while less rigorous scientifically, could add weightage to elucidation of the study hypothesis--as has been the case in most scientific issues that involve studies in human populations.

Another approach to the ethical issue would have been to view vitamin A as an essential nutrient like calories or water, not an experimental health technology such as a new vaccine. In the latter case, as efficacy is uncertain and provided safety is assured, double-blind controlled studies are often recommended. In the former case of an essential nutrient, this same approach could be inappropriate. An example would be controlled double-blind studies to examine the mortality impact of caloric supplementation in known calorie deficient populations. The calorie example parallels the Vitamin A issue to the extent that the hypothesis is reasonable, the consequences of severe deficiency are scientifically accepted, the definition/measurement and mortality consequences of mild-moderate deficiency are scientifically uncertain.

A more balanced report would have examined alternative methodologies--such as (1) retrospective case control studies of mortality associated with acceptance or refusal of vitamin A supplements in population groups receiving capsular distribution; (2) time staggered introduction of vitamin A capsules along with other service components into ongoing or planned primary health care programs; and (3) intensive vitamin A distribution in selected populations in comparison to

populations receiving regular governmental services, including vitamin A supplements.

The report should have highlighted better the ethical issues at stake, the scientific uncertainties, the need for local ethical review and judgment, and the diverse views of subcommittee members. Its recommendations should encourage involved US and host country investigators and institutions to work out final designs in conformity with local and international ethical standards. The current report's conclusion providing blanket ethical endorsement of all proposed control/placebo studies is unwarranted.