Attachment 1. A-000035 Library Date 6/5/97

(FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. M. Mujibur Rahman Trainee Investigator (if any) _____

Application No. 97-008 Supporting Agency (if Non-ICDDR,B) _____

Title of Study Effect of simultaneous zinc and vitamin A supplements on the bioavailability of vitamin A in children

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects — Yes (No)
   (b) Non-ill subjects — (Yes) No
   (c) Minors or persons under guardianship — (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects — Yes (No)
   (b) Social Risks — Yes (No)
   (c) Psychological risks to subjects — Yes (No)
   (d) Discomfort to subjects — Yes (No)
   (e) Invasion of privacy — Yes (No)
   (f) Disclosure of information damaging to subject or others — Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) — Yes (No)
   (b) Use of fetal tissue or abortus — Yes (No)
   (c) Use of organs or body fluids — Yes No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study — (Yes) No
   (b) Procedures to be followed including alternatives used — (Yes) No
   (c) Physical risks — (Yes) No
   (d) Sensitive questions — (Yes) No
   (e) Benefits to be derived — (Yes) No
   (f) Right to refuse to participate or to withdraw from study — (Yes) No
   (g) Confidential handling of data — (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No
5. Will signed consent form be required:
   (a) From subjects — Yes (No)
   (b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   NA Questionnaire or interview schedule *

   * If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
   2. Examples of the type of specific questions to be asked in the sensitive areas.
   3. An indication as to when the questionnaire will be presented to the Cttee. for review.

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

M Rahman

Principal Investigator

Trainee

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: Date: 4 May, 1997

RRCApproval: Yes/ No Date:

ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuations) Effect of simultaneous zinc and vitamin A supplements on the bioavailability of vitamin A in children

2a. Name of the Principal Investigator(s) (Last, Middle, First).	2b. Position / Title	2c. Qualifications
Rahman Mujibur Mohammad	Associate Scientist	MBBS, MPH

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out.
Clinical Sciences Division

Contact Address of the Principal Investigator
4a. Office Location: CRSC, CSD

4b. Fax No:880-2-883116
4c E-mail:mujib@cholera.bangla.net
4d. Phone / Ext: 871751-60/2314

5. Use of Human Subjects	5a. Use of Live Animal	5b. If Yes, Specify Animal Species
Yes ☐	☐	Yes
No ☐	☐	No

6. Dates of Proposed Period of Support (Day,Month,Year - DD/MM/YY) 01.07.97

7. Cost Required for the Budget Period
7a. *Ist Year($):76,612 2nd Year($): 25,166 3rd Year:*
7b. Direct Cost($) 95,120 Total Cost ($) 101,778

8. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers.
The protocol has been revised according to the reviewer's comments and is approved.

George J. Fuchs, MD — Name of the Division Director

Signature

6 May 1997 — Date of Approval:

9. Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

10. Signature of PI

Date: 6/5/97

Table of Contents

✓ Check here if appendix is included

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(TYPE TEXT WITHIN THE SPACE PROVIDED).**

Principal Investigator M. Mujibur Rahman

Project Name: Effect of simultaneous zinc and vitamin A supplements on the bioavailability of vitamin A in children

Total Budget
Beginning Date : July 1, 1997 Ending Date: July 30, 1998

~~Project summary~~

To examine whether combined zinc and vitamin A supplementation leads to better health and vitamin A nutrition status as compared with vitamin A supplementation alone, 460 children aged 1 to 3 yr will be studied in a randomized double blind controlled trial. Children will be randomly assigned to receive either i) vitamin A (200,000 IU, single dose on day 14), or ii) zinc acetate (20 mg, daily for 14 days), or iii) vitamin A (200,000 IU, single dose on day 14) plus zinc acetate (20 mg, daily for 14 days), or iv) placebo. Venous blood will be drawn on admission, day 21, and 3 mo after supplementation for MRDR test, serum retinol, retinol binding protein (RBP), and zinc assay. A urine sample will be collected from a subset of children for 3 days after vitamin A supplementation to measure the urinary loss of retinol, if any. Cell mediated immunity (CMI) will be measured on enrolment and on day 21 using multi-test CMI skin test. Weight and height measurement will be taken on admission, 1 month and 3 month. The children will be followed up at home weekly for diarrhea and respiratory tract infection morbidity (RTI) for a period of 3 mo. The major outcome variables will be pre- and post-supplementation liver vitamin A stores, serum retinol and RBP, plasma zinc levels, cell mediated immunity (CMI), morbidity (diarrhea and RTI), and growth (weight and height gain). Improvement of vitamin A and RBP levels, CMI response, growth, and reduction of morbidity will be compared among the above 4 groups and also between the vitamin A and vitamin A+zinc group (to examine the zinc-vitamin A interaction). This will be a community study carried out in Bangladesh over a period of 2 years. The estimated budget is $101,778.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/Specialty	Role in the Project
1. M. M. Rahman,	Associate Scientist/Public Health Nutrition	P.I.
2. M. A. Wahed,	Associate Scientist/Head Nutr.,Biochemistry,ICDDR,B	Co-Investigator
3. A.H. Baqui,	Scientist/Director MCH-FP Ext Project	Co-Investigator
4. George J Fuchs,	Director, Clinical Sciences Division	Consultant
5. J.O. Alvarez,	Prof & Chairman, Dept. of International Health, UAB	Consultant

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Compared to zinc or vitamin A supplementation alone simultaneous zinc and vitamin A supplementation -

1. will increase the synthesis of retinol binding protein (RBP), improve liver vitamin A stores and increase serum retinol levels.
2. will improve growth.
3. will enhance immunity.
4. will reduce morbidity.
5. Zinc supplementation alone will increase the synthesis of RBP

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

1. To evaluate the efficacy of combined vitamin A and zinc supplements in improving the serum RBP and retinol levels, and liver vitamin A store (measured by the modified relative dose response (MRDR) test).
2. To measure the effect of vitamin A plus zinc supplementation on the growth of children.
3. To examine the effect of vitamin A plus zinc supplementation on cell mediated immunity (CMI).
4. To evaluate the interaction of vitamin A and zinc on respiratory infection .
5. To examine whether zinc supplementation alone increases the serum retinol concentrations compared with placebo.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).

Vitamin A deficiency : Public health problem

Vitamin A deficiency is an important public health problem in developing countries (WHO 1982, Sommer et al. 1982, WHO 1995). It is one of the most important causes of blindness in children (Cohen et al. 1985). World-wide, an estimated ten million children under the age of six years develop some degrees of xerophthalmia and about a million go blind (Habte, 1989). Other than blinding malnutrition, vitamin A deficiency is a major cause of morbidity and mortality in children (Sommer et al. 1983, Sommer et al. 1984,). Even mild vitamin A deficiency has been associated with an increased rate of infection (Sommer et al. 1984, Milton et al. 1987).

Vitamin A supplementation

Vitamin A supplementation either as single large dose or weekly low doses have substantially reduced mortality in children (Sommer et al. 1986, Rahmatullah et al. 1990, Hussey and Kellen, 1990, West et al., 1991). A recent meta-analysis (Fawzi et al. 1993) showed that vitamin A supplementation resulted in a 30% reduction (Odds ratio :0.70, 95% CI:0.56-0.87) in overall mortality and a 61% reduction in measles related mortality (odd ratio: 0.39, 95% CI: 0.22-0.66). Vitamin A supplementation has consistently shown a reduction of measles relatèd complications (i.e. pneumonia) and mortality (Elison JB, 1932, Barclay et al. 1987, Hussey et al. 1990, Hussey et al. 1993, Madhulika et al. 1994).

Risk of diarrhea and respiratory disease were reduced and physical growth improved in children after large dose of vitamin A supplementation (Lie et al. 1993). In contrast, some other studies failed to show a demonstrable effect of vitamin A supplementation in reducing disease morbidity (Rahmatullah et al., Abdeljaber et al. 1991, Ramakrishnan et al. 1995, Dibley et al. 1996) except in measles (Hussey and Klein 1990). A recent study has shown that vitamin A

supplementation early in infancy reduced the duration of respiratory infection although incidence of diarrhea and respiratory infections remained same (Rahman et al. 1996). However, increased CMI response was observed in children whose vitamin A status improved after supplementation (Rahman et al. 1997).

Considering the public health importance of vitamin A deficiency and the beneficial effect of vitamin A supplementation on child survival many developing countries, including Bangladesh have initiated large dose vitamin A supplementation programs as recommended by WHO (WHO, 1982). However, recent studies in Bangladesh have shown that despite 3 large doses (50,000 I.U each) at monthly intervals in infants under six months about 60% remain vitamin A deficient (Rahman et al. 1996, Mahalanabis et al. 1996). Acute respiratory infection and fever were strongly associated with a deficient vitamin A status in these post supplemented infants (Rahman et al. 1996). Likely cause of sustained vitamin A deficiency after supplementation are increased catabolism of vitamin A or the high retinol excretion in urine that accompanies fever (Alvarez et al. 1995, Stephensen et al 1994). Also very recent data from India and Indonesia showed that vitamin A supplementation fails to reduce the morbidity in children (Ramakrishnan et al. 1995, Dibley et al. 1996).

Lack of improvement of vitamin A after supplementation may also be due to the simultaneous deficiency of other micronutrients (such as zinc) which limit the bioavailablity of vitamin A (Solomons et al., 1980, Smith et al. 1980). In developing countries where protein energy malnutrition is highly prevalent multiple micronutrient deficiency usually co-exists. Hence, vitamin A supplementation alone fail to improve the vitamin A levels.

Zinc deficiency

Zinc deficiency is also a public health problem worldwide (Sandsted et al. 1991). Clinical zinc deficiency among infants, children, adolescents, and pregnant women has been described in both developing countries and industrialized countries, including the United States (Sandsted, 1991). Experiments in humans and animal models have established the essential role of zinc in many physiologic functions, including immunity, taste acuity, dark adaptation, wound healing, lipid metabolism, protein synthesis, sexual function and cognition (Sandsted, 1981, Hambidge, 1986).

Zinc deficiency is associated with infections such a diarrhea in children (Tomkins et al. 1993, Kay et al. 1975, Moynahan EJ, 1974). Zinc deficiency is a cause of childhood growth failure, particularly stunting (Allen, 1994). On the other hand, diarrheal illnesses cause excessive stool zinc losses leading to deficiency (Castillo-Duran et al. 1979). Hence, there is a vicious cycle.

Zinc supplementation

Several supplementation studies have shown that zinc supplementation reduces the severity and duration of diarrhea (Sazawal S. et al. 1995), improves immunity and enhances growth (Castillo-Duran et al. 1987, Ninh et al. 1996). The beneficial effects of zinc supplementation has also been observed

on reduction of incidence of persistent diarrhea and on growth (Sazawal S. et al., 1996, Roy SK, personal communication).

Zinc supplementation has been shown to increase growth in children in a number of studies (Walravens et al. 1983, Simmer et al.1988,Schlesinger et al. 1992, Khanum et al. 1988). However, some other studies failed to demonstrate a beneficial effect of zinc on growth (Hemalatha et al 1993, Mahalanabis D, personal communication).

Impaired immune function has been documented in zinc deficiency (Schlesinger et al. 1974, Suskind 1977, Beisel et al. 1981). Zinc supplementation has been shown to reverse the immune functions such as leukocyte function and cell mediated immunity (Weston et al. 1977, Allen et al. 1981, Schlesinger et al. 1992).

Existing Data on Zinc- vitamin A interaction

An understanding of the interaction between zinc and vitamin A has been only recently elucidated (Smith JC, 1980, Solomons NW 1980). The role of other micronutrients such as zinc on vitamin A metabolism was first suspected in 1939 when Patek et al. observed that certain cirrhotic patients with impaired dark adaptation did not improve after vitamin A therapy. It is probable that these patients had zinc deficiency (Halsted JA et a. 1968). After several years, Valle et al. suggested a possible metabolic relationship between zinc and vitamin A (Vallee et al. 1956, Valle et al. 1957). The basis for this relationship were : 1). Zinc is an integral part of the metalloenzyme alcohol dehydrogenase in yeast and horse liver (Valley et 1957). 2). The eye (a target site of vitamin A metabolism) is one of the highest zinc containing organs in the body (Underwood et al. 1977). 3). Zinc-deficient swine showed low serum vitamin A, which was not reversed by vitamin A therapy (Stevenson & Earle 1956).

Animal studies

Zinc deficiency has been accompanied by a depression of circulating retinol in a number of mammalian species including swine (Stevenson et al. 1956), lambs (Smith JC et al. 1977), and rats (Smith JC et al. 1973, Smith JE et al. 1974, Brown ED et al., 1976, Smith JC Jr et al. 1976, Carney SM et al., 1976, and Duncan et al. 1978). Vitamin A supplementation alone failed to revert this vitamin A deficiency. However, after giving either zinc supplementation or zinc containing diets, the vitamin A levels in those animals improved, suggesting that the low vitamin A levels were related to the zinc deficiency (Stevenson et al. 1957, Smith et al. 1973, Ette et al.1979). Ellen et al. (1975) demonstrated that in zinc depleted rats , plasma vitamin A concentration returned to normal after 6 days of repletion with a zinc sufficient diet.

The essentialness of zinc for mobilization of vitamin A was extensively studied by Smith et al. (1973). Using both germ-free and conventional rats, Smith et al. demonstrated that animals fed a zinc-deficient vitamin A diet had plasma vitamin A concentrations nearly 50% lower than rats fed a zinc-adequate

diet. Vitamin A supplementation alone did not revert the plasma vitamin A levels to normal. Vitamin A, but without zinc supplementation, only resulted an accumulation of vitamin A in the liver. However, zinc therapy alone for 14 days resulted in an increase of plasma retinol levels to within normal range with a concomitant decrease in liver vitamin A concentration. These findings suggest the important role of zinc in mobilization of vitamin A from liver to the plasma, as well as in the maintenance of normal serum retinol concentrations.

The postulation that zinc deficiency interferes with vitamin A metabolism was further supported by the simultaneous reduction of retinol binding protein (RBP), a specific transport protein essential for vitamin A transport. RBP is consistently low in both plasma and liver in zinc deficient animals (Smith GE et al. 1974, Smith et al. 1980, Solomons et al. 1980) which suggests that the low plasma vitamin A levels in the zinc deficiency might be due to an impaired ability of the deficient rat to mobilize hepatic vitamin A in the form of retinol-RBP complex (Smith JE 1974). A recent study (Mobarhan et al. 1992) has demonstrated that zinc deficiency reduces hepatic cellular retinol binding protein (cRBP) in rat, again indicating that zinc is an essential element for intra-cellular transport of vitamin A, in addition to its well-established role in the intercellular transport of vitamin A.

Human studies

Unlike animal studies, data on zinc-vitamin A interaction in human is limited. Most studies of zinc-vitamin A interactions in humans have been in adult patients with liver or pancreatic diseases.

In 1939, Patek and Haig first reported several cirrhotic patients with abnormal dark adaptation who were resistant to vitamin A therapy. Valle et al. (1957) suggested that the poor response to vitamin A therapy in certain cirrhotic patients was due to altered zinc metabolism characteristic of that condition; both serum and liver zinc levels are known to be low in patients with cirrhosis (Valle et al. 1956, Halsted et al. 1968).

Morrison et al. (1978) studied five alcoholic cirrhotic who had low serum zinc concentration and abnormal dark adaptation. Two patients were first treated with oral vitamin A alone for two to four weeks with an unsatisfactory response. However, after the addition of oral zinc sulfate (220 mg/d) for one to two weeks, their final dark adaptation thresholds returned to normal. There were also three patients who were treated with zinc sulfate without prior vitamin A therapy, and they also responded with normal dark adaptation tests. In another study by Russel et al. (1978), two of the 12 cirrhotic patients with abnormal dark adaptation treated with vitamin A did not improve. Serum zinc concentrations of these two patients were low (< 65 ug/dl). When zinc (220 mg per day) was given, the dark adaptation became normal after one week.

Smith et al. (1977) suggested that cystic fibrosis patients exhibited several anomalies of the vitamin A and zinc metabolism as seen in deficient animals, including low blood levels despite vitamin A supplementation and adequate liver vitamin A stores . This anomaly suggested a defect in the transport of the vitamin A from hepatic stores to the periphery (Smith et al. 1972). A reduced circulatory retinol and RBP, and significant correlation

between the two, have subsequently been demonstrated in cystic fibrosis patient (Smith et 1972, Jacob et al. 1978).

Palin et al. (1979) supplemented cystic fibrosis patients with zinc sulfate which resulted in no effect on taste acuity, serum zinc, or vitamin A concentration. However, this was not totally unexpected as no preexisting evidence of zinc deficiency was identified in any of the patients.

The situation with respect to protein energy malnutrition is much more complex than in hepatic or pancreatic diseases and the data less easily interpreted. There seems to be an synergistic relationship between PEM and vitamin A deficiency leading to more severe ocular manifestations (Oomen et al. 1976). In PEM children, there is defective synthesis or release of RBP (Smith et al. 1973, Smith et al. 1975) which might prevent the improvement of serum retinol levels despite supplementation. Zinc is essential for protein synthesis (Terhune et al. 1972) which suggests that RBP is one of the hepatic proteins most sensitive to zinc deficiency. Several studies have found low plasma zinc in PEM (Smith et al. 1964, Sandstead 1965, Hansen et al.1969, Kumar & Rao 1973,Kutumbale et al. 1976). The earlier report by Smith et al. (1973) showed a significant correlation between zinc and vitamin A in the plasma of children. A later study in 74 preschool children (Denver Head Start Program) found no correlation between plasma zinc and serum retinol level (Hambidge et al. 1976). However, plasma zinc was low in 34% of the children and a near equal percentage (35%) had either low or deficient levels of serum retinol. Henkin and Smith (1972)found that serum zinc, vitamin A, RBP, and pre-albumin were depressed in viral hepatitis patients. However, no significant correlation between zinc and vitamin A was found. In contrast, serum zinc and RBP were significantly correlated although the correlation coefficient was low (r=0.286).

Shingwekar et al. (1979) studied the effect of zinc supplementation on plasma RBP and vitamin A levels in Indian children with vitamin A deficiency and PEM. Forty mg of zinc was given to PEM children for 5 days and 10 days to vitamin A deficient children. Initial mean plasma zinc concentrations was 57 ug/dl in PEM children and 71 ug/dl in vitamin A deficient children. In the vitamin A deficient group, the plasma retinol and RBP increased slightly, but not significantly. However, improvement of both the parameters were significant in the PEM group. In contrast, a recent study in Thailand failed to demonstrate an interaction between zinc and vitamin A on the improvement of RBP and retinol (Udomkesmalee et al. 1992). A major difference was that the malnourished Indian children were more severely deficient in zinc and vitamin A compared with the Thai children.

Animal studies have consistently showed a reduction of plasma vitamin A concentrations in zinc deficient animals. RBP which is essential for vitamin A transport was low both in liver and plasma suggesting a defect in vitamin A transport in zinc deficient animals. Supplementation studies clearly demonstrated that zinc supplementation improve the mobilization of vitamin A from liver to the circulation. Human studies suggested that zinc supplementation is beneficial to vitamin A metabolism in conditions where zinc deficiency is prevalent. Most of those studies were done in adults with liver, pancreas and other diseases. In children, the data are limited and definitive clinical studies in children are needed in order to precisely ascertain the beneficial effects of a combined therapy using zinc and vitamin A.

Significance and Rationale: In developing countries where protein energy malnutrition is highly prevalent, children usually suffer from mulitiple micronutrient deficiencies, particularly zinc deficiency (Sandstead et al. 1965; Hansen et al. 1969; Kumar et al. 1973, Kutumbale et al. 1976) and vitamin A deficiency (Cohen et al. 1985). In Bangladesh more than 90% of children suffer from varying degree of PEM; 6.5% being severely malnourished (weight/age <60%) (Bangladesh Bureau of Statistics, 1992). These children are therefore likely to have vitamin A deficiency as well as zinc deficiency. Recent report from Bangladesh have shown that more than 60% of children remain vitamin A deficient despite large doses of vitamin A supplementation (Rahman et al. 1995; Rahman et al. 1996, Mahalanabis et al. 1997). Silmultaneous zinc deficiency may be the cause for the failure to improve vitamin A status in children. Although the interaction between vitamin A zinc has been shown in several animal and human studies (Smith et al. 1980; Solomons et al. 1980) the role of zinc on vitamin A metabolism has not been precisely examined in children. The present study will examine the role of simultaneous administration of zinc with vitamin A on improvement vitamin A status, immunity, growth and on RTI reduction and also the effect of only zinc supplementation on vitamin A metabolism. If the results of this study show that simultaneous zinc and vitamin A supplementation significantly improve the vitamin A status, immunity, growth and reduce morbidity in children as compared to conventional vitamin A supplementation, it will lead to a new strategy for combined zinc-vitamin A supplementation instead of vitamin A supplementation alone. Such a finding would have important clinical and public health implications.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. (DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).

Study design: Double blind randomized controlled trial.

Children will be randomized to receive either of the followings:

a). Vitamin A
b). Zinc
c). Zinc+vitamin A
d). Placebo

Protocol layout

	Admission (d0)	day 21	1 mo	3 mo
Supplementation	Zinc (20 mg/d for 14 days/or vitamin A (200,000 IU single dose on day 14)			
Modified relative dose response (MRDR) test (retinol, RBP, C-reactive protein, and zinc assay)	X	X		X
Anthropometry (Weight, height)	X	X	X	X
CMI test	X	X		
Morbidity	Weekly home visits for 3 months			

In a subset of children (20 receiving vitamin A and 20 receiving vitamin A plus zinc) urinary retinol and zinc will be measured for 8 hour daily for 3 days after supplementation. They will be randomized separately.

Subject selection:

Eligibility criteria:

Age: 12 mo to 36 mo
Sex: Either

Exclusion criteria:

a). Presence of systemic infection such as diarrhea/dysentery, respiratory tract infection with or without fever, measles and other diseases requiring hospitalization at the time of enrolment (or during the preceeding 1 week).
b). Presence of clinical signs/symptom of vitamin A deficiency (e.g. night blindness, conjunctival xerosis, corneal ulcer, and xerophthalmia)
c). Presence of clinical signs of zinc deficiency (skin ulcer and excoriation).
d). Children with wt/age (% NCHS median) less 60% or with edema.
e). Received vitamin A capsule within last 6 months.

Outcome variables:

Major

a). Pre and post supplementation serum retinol binding protein (RBP).
b). Pre and post supplementation MRDR values and serum retinol levels.
c). Pre and post supplementation serum zinc levels.
d). Respiratory infections morbidity during post supplementation follow up period. *
e) Cell mediated immunity.
f). Weight and height gain. *

Minor:

a). Urinary loss of retinol and zinc.
b). Diarrhea morbidity.

Project area

The study will be implemented in Bangladesh where vitamin A deficiency is a major public health problem (Cohen et al. 1985, WHO 1995). More than 90% of children in Bangladesh suffer from varying degree of protein energy malnutrition (PEM); 6.5% being severely malnourished (wt/age < 60%, NCHS median) (Bangladesh Bureau of Statistics, 1992). Although the prevalence data on zinc deficiency in Bangladesh is not available, from the high prevalence of PEM it is conceivable that zinc deficiency will be highly prevalent. Children

living in the urban slums of Dhaka, the capital city of Bangladesh will be studied.

Vitamin A deficiency in Bangladesh

A large-scale national blindness survey (1982-83) provided the most up-todate estimate of vitamin A deficiency (VAD) in the country. Rural areas had the following rates of clinical xerophthalmia: XN=3.6%, X1B=0.9%, corneal involvement=0.1%. Urban slum areas:XN=2.8%, X1B=1.6%, corneal involvement=0.2%. The total xerophthalmia rate was 4.6%. The sample design was proportional to the population distribution, found primarily in rural areas, and thus was similar to the overall rates which were: XN=3.5%, X1B=1.0%, corneal involvement=0.1%. This translates to 11 cases per 10,000 or 11 times greater than what WHO considers to be a severe public health problem. Biochemical data from Matlab (1990) found 20% of preschool children to have serum retinol levels < 0.35 umol/l.

Zinc deficiency in Bangladesh

There is no national data on the prevalence of zinc deficiency in Bangladseh. However, clinic based data have shown that 45% children attending ICDDR,B hospital (coming from urban and peri-urban community for treatment of diarrhea) are zinc deficient when serum zinc was taken as an indicator (SK Roy, personal communication). In Bangladesh more than 90% of children suffer from varying degree of PEM; 6.5% being severely malnourished (weight/age < 60%) (Bangladesh Bureau of Statistics, 1992). From the high prevalence of PEM it is conceivable that the prevalence of zinc deficiency in Bangladesh would be high.

Sample size

Total sample size will be 460. Sample size was calculated based on all the outcome variables and the maximum number was considered as the total sample (Annex 2).

Consent

Informed consent will be obtained from the parents of all eligible children. The study procedures (supplementation, blood drawing, risk involved and their rights and responsibilities) will be explained in details to the parents and they will read the consent form. The children whose parents will give written consent will be enrolled for the study.

Treatment allocation (Randomization)

All eligible children will be randomized to receive either i). vitamin A (200,000 IU single dose on day 14), or ii). zinc acetate, 20 mg elemental zinc (2XRDA) daily for 14 days, or iii) zinc acetate (20 mg elemental zn daily for 14 days) plus vitamin A (200,000 IU single dose on day 14), or iv) placebo. Block randomization will be used to assign approximately an equal number of children into each groups. All enrolled children will be given serially

arranged identity numbers. The supplementation preparation in test and placebo groups will be identical in appearance and will be packaged in identical small dark containers so that these are protected from light. The containers will be arranged in sequence of drugs or placebo that correspond to randomization numbers. These numbered containers which correspond to I.D. (identity number) of the prospective children under study will then be assigned to children according to the container number. The master randomization list will be sealed and kept safely with a responsible person at ICDDR,B who is not related to the study in any way.

Training of the Personnel

After recruitment all the research health assistants and physician will be given practical training regarding subject enrolment, data collection, case detection, management and referral. The training will be provided by the ICDDR,B. The training will be evaluated by pre- and post-test. Periodic evaluation and refresher training will be given every 6 months.

The physician will conduct baseline physical examination, and subsequent management of patients. He/she will draw blood for MRDR test and supervise the health assistants at the field level.

The health assistants will recruit children in the study, take the history and fill the data forms, carry out the MRDR and CMI test and measure anthropometry and do the morbidity follow up.

Development and field testing of instruments

The data collection instruments (forms) will be designed before beginning of the study. They will be field tested, revalidated and revised according to the pretest. Same forms, and instruments (weight and height machine) will be used throughout the study.

Supplementation procedure

Supplementation will be given daily for 14 days. A study health worker will administer the medication.

Blood collection

Modified Relative Dose Response (MRDR) test will be done on enrolment, day 21 and 3 months. Each time 2 ml venous blood will be obtained. Serum retinol, RBP, and plasma zinc will be measured from the same samples. The sample for retinol assay will be collected in a zinc free container container (to avoid contamination) wrapped with black carbon paper to avoid light. Blood samples will be transported to ICDDR,B Laboratory within an hour where it will be centrifuged and serums will be seperated for serum retinol (A1 and A2), RBP, C-reactive protein, and zinc (in a zinc free container). The samples will be preserved at -70° C until analysis.

Modified Relative Dose Response

Relative dose response (RDR) and modified relative dose response (MRDR) test are used to indirectly measure the liver vitamin A stores and are indicators of marginal vitamin A deficiency (Flores et al. 1984, Tanuminhardjo et al. 1990). Serum retinol concentrations, although homeostatically controlled over a broad range of liver reserves, can be significantly affected by infections and other nutrient deficiencies. Therefore, RDR and MRDR test were developed to ascertain whether liver reserves are adequate. MRDR test offers several technical advantage over other assessment procedures such as, it requires only one blood sample, it is highly responsive to therapeutic doses of vitamin A and can be used for evaluating the effects of intervention programs in population (Tanuminhadjo et al. 1996, Tanuminhardjo et al. 1996 b). In comparison with the measurement of serum retinol concentrations only, the MRDR test is better able to discriminate between vitamin A adequacy and inadequacy (Tanuminhadjo et al. 1996, Tanuminhardjo et al. 1996 b). Indeed MRDR test provides both a response ratio as well as an initial serum retinol concentration. In Bangladesh, however, the MRDR test proved less reliable, possibly because of low dose of 3,4-didehydroreinyl acetate (DRA) used for the MRDR test (Wahed et al. 1995). However, Tonuminhardjo et al. (1996) refined and revalidated MRDR test using different DRA doses and suggested a simplified MRDR test with standard doses for different age groups which is higher than the earlier dose (Tanuminhardjo et al. 1996 b). A dose of 5.3 µmol DRA has been recommended for children younger than 6 years.

In a vitamin A-depleted state, apo-retinol binding protein (RBP) accumulates in the liver because of a lack of free retinol in liver cells with which to combine. After a small oral dose of DRA is administered, DRA is hydrolyzed in the gut to 3,4-didehydro retinol (DR), absorbed, and then reesterified, mainly with palmitic acid, in intestinal mucosal cells. When DR esters reach the liver on chylomicron remnants, they are hydrolyzed to the alcohol, which in turn is bound to accumulated apo-RBP in liver cells and secreted into the plasma as the holo-DR-RBP complex. A single blood sample taken an appropriate time after dosing can be analyzed, and a DR to retinol (R) can be calculated (MRDR ratio). In developing countries, a molar DR:R of 0.06 is provisionally used as a cutoff to indicate a marginal vitamin A status. This cutoff ratio is based on the responses of subjects to large doses of vitamin A.

Oral dosing material and MRDR test

DRA was synthesized from retinoic acid (Barua et al. 1972, Tanuminhardjo et al. 1989). The compound was carefully purified on an 8%-water-deactivated alumina column and dissolved directly into 100% corn oil by sonication. An appropriate working dilution was made with corn oil to dose the children with a volume of 200 µL oil.

The MRDR test procedure has been described elsewhere (Tanuminhardjo, 1996). Briefly, the MRDR test will be performed by giving a single oral dose of 5.3 µmol DRA dissolved in corn oil (Tanuminhardjo et al. 1996 b). High-fat, low vitamin A-containing snacks (i.e. fried potato or ice cream) will be provided after dosing to ensure absorption of the DRA. 5 hours after the DRA dose blood samples will be drawn from an antecubital vein.

Laboratory analysis

Samples will be analysed in the ICDDR,B Nutrition & Biochemistry laboratory.
Retinol and DR will be measured by High Performance Liquid Chromatography (HPLC) (Wahed et al. 1995).
Retinol-binding protein (RBP) will be measured by `radial immunodiffusion using a commercially available kit from Behring Diagnostics (La Jolla, CA).
Zinc will be determined by atomic absorption spectrophotometric method (AOAC).

C-reactive protein will be measured by Turbidimetry method.

Morbidity data

Diarrhea and respiratory infection morbidity will be collected by weekly home visits for a total of 3 months.

Definitions

Diarrhea

Diarrhea will be defined as 3 or more liquid stools in 24 hours (for ≥ 1 day) or passage of bloody stool of any frequency. The exact number of days that the children experienced watery, mucoid or bloody stools will be recorded. If only watery type of stools occur throughout the episode, it will be recorded as watery diarrhea, while the presence of blood and mucus in any stool during the episode will be considered as dysentery.

Respiratory infection

Fever, cough, fast breathing and chest indrawing will be recorded separately. Lower respiratory tract infection will be defined as cough (difficult breathing), and fast breathing (≥ 50 breaths per minute in a child aged < 1 year; ≥40 breaths per minute in a child >12 months) with or without chest indrawing (WHO, 1993).

Episode

Three or more symptom-free days will be considered to differentiate a new episode from the previous one.

Case management

Once diagnosed, all children will be treated for diarrhea and respiratory tract infections. Diarrhea will be treated with oral rehydration solution (ORS) at home. Children with dysentery will be given appropriate antibiotics. Those who needs intravenous fluid will be referred to the ICDDR,B hospital.

Respiratory tract infections will be treated with oral antibiotics. Those with severe illness (e.g. respiratory distress, inability to eat, lethargy, convulsions etc.) will be referred to ICDDR,B or Children's Hospital.

Research health assistants will detect the cases and provide ORS. Children requiring antibiotics and further treatment will be seen by the physician.

Anthropometry data

Weight and height will be measured on admission and at the end of 3-month follow up. The weight will be measured with a portable electronic balance with a precision of 10 g (Secca, Germany). Length will be measured using a locally maid pediatric length board with a precision of 1 mm. Supine length will be taken in children less than or equal to 2 years of age and for older (> 2 yrs) children standing height will be measured using a vertical height board of same precision.

The multi-test CMI

The method for measuring cell mediated immunity is based on the delayed type hypersensitivity test and has been described elsewhere (Corriel et al. 1985, Black et al. 1989). Briefly, the Multi-test CMI (Institute Merieux, Lyon, France) is a plastic applicator consisting of eight sterile test heads preloaded with seven glycerinated tests antigens and a 70% glycerin-saline negative control. The test antigens are tetanus toxoid, diphtheria toxoid, streptococcus, tuberculin (PPD), candida, trichophyton, and proteus antigens. The multi-test CMI will be applied through tightened skin on the volar surface of each forearm. Test heads 1 to 4 (tetanus, diphtheria, streptococcus and tuberculin) will be applied to the left arm and test 5-8 (control, candida, trichophyton and proteus) will be applied to the right arm. The average of maximum horizontal and vertical induration will be taken as the induration for a particular test. The CMI test will be performed on enrolment and 21 days.

Urine collection

40 children will be separately randomized (20 in vitamin A and 20 in vitamin A plus zinc group). A health worker will observe the child at home during 8 h at day time (9 am to 5 pm) and will collect urine using pediatric urine collection (PUC) bags for 3 days after supplementation. Only male children will be recruited to ensure proper and complete urine collection. The samples will be preserved at -70° C until analysis.

Data management

Base-line data, morbidity, growth data and data of serum and urine retinol, RBP and plasma zinc will be recorded in pretested forms. Forms will be checked by health assistants in the field for completeness and for obvious mistakes, and errors will be corrected on the spot. In 10% of cases, data collection will be repeated by one of the investigators and if any error

detected will be corrected. After completion, each form will be visually checked by one of the investigators for completeness and will then be sent to the data entry technicians. For the sake of standardization any intra-observer and inter observer variations will be monitored on a monthly basis. Monthly meetings will review the progress of work and any problems arising in the field work. The same forms, equipments, definitions and methods will be used throughout the study period. Data will be entered twice (using interactive checks), each time by a different person and will be compared. Then the data will be checked by the data manager by logical and range checks.

Study Schedule

0-3 months:	Recruitment and training of study personnel; development of study materials and field testing of study materials; procurement of supplies.
4-18 months:	Conduction of supplementation, data collection and data checking; monitoring and quality control.
19-24 months:	Finish laboratory assay, data analysis and preparation of manuscripts and reports.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. (TYPE WITHIN THE PROVIDED SPACE).

FACILITIES AVAILABLE

The study will be carried by the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B). The existing facilities of the urban MCH-FP Extension project of ICDDR,B that conducts operational research and provide assistance to agencies of the Government of Bangladesh and to NGOs through a partnership effort as the "URBAN MCH-FP Initiative" will be utilized. The urban MCH-FP extension project has surveillance system and we will use those to identify our subjects.
The study site will be Lalbagh and we will study the children in slums belonging to the Urban MCH-FP surveillance. Lalbagh is in the older part of the Dhaka metropolitan city. The means of communication from that area is car, auto rickshaw and rickshaw. It takes less than half hour to reach the center from Lalbagh area by baby taxi or car.
The laboratory analysis of blood samples will be done in the Nutrion and Biochemistry laboratory of ICDDR,B. This laboratory has facilities for analysing many macro and micronutrients including retinol, zinc and iron. ICDDR,B Laboratory has been measuring zinc and retinol since 1977 and has been participating in the quality control program of NIST, HPLC, atomic absorption spectrophotometry, and Radial immunodiffusion kits (for measuring retinol, zinc, and RBP respectively) are available at ICDDR,B.

Physician to examine and treat the study children, and health worker for collecting and recording data will be hired.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (TYPE WITHIN THE PROVIDED SPACE).

DATA ANALYSIS

Initial statistical analysis will include descriptive methods such as means, median, standard deviations, frequency distributions, scatter plots and cross tabulations. After evaluating base-line data for comparability, plasma zinc, serum RBP and retinol levels, proportion of children with abnormal MRDR (vitamin A deficiency), will be compared among various groups particularly between vitamin A vs. vitamin A plus zinc group to examine the zinc vitamin A interaction. Proportion of children with positive CMI tests will be compared among various groups. Similarly morbidity (episodes/child-year and cumulative days with LRI) will be compared. Length increment per 100 cm of initial length and weight increment per kg of the initial weight will be compared between different groups. Height/Length for age, weight for age, and weight for height/length will be calculated using the NCHS standard. The mean Z-scores will be compared between the vitamin A and vitamin A+Zinc groups as well as with placebo. The statistical difference will be compared using Student's t-test for normally distributed data and Mann-Whitney test for skewed data. For categorical data, Chi-squared test will be done. Stratified analysis will be done by Mantel-Haenszel Chi-square test. Multiple linear regression analysis will be done to see the effect of confounders (baseline nutritional status, breastfeeding, and serum retinol, zinc and C-reactive protein) on serum retinol and RBP. Finally a multiple logistic regression analysis will be done to examine the effect of various factors on diarrhea and RTI risk.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

12. ETHICAL ASSURANCE FOR PROTECTING HUMAN RIGHTS

Vitamin A and zinc supplementation with the proposed dose are safe in children. There is no physical, psychological or social risk involved either from zinc/vitamin A supplementation or from blood drawing, CMI testing, weight/height measurement and collection of information on morbidity.

On the other hand, if the hypothesis proves correct, the potential impact on child survival programs is immense. The addition of zinc to vitamin A supplementation programs currently in place not only in Bangladesh but worldwide will result in a significant increase in cost/benefit ratio and will greatly reduce morbidity and mortality among infants and children.

The information about the identity will be kept confidential with the principal investigator. Data will be used for publication for scientific purposes. However, the information about the individual's identity will not be disclosed. The results will be published as a group without mentioning the name and address of the subject.

The children can be withdrawn from the study any time by the the parents/guardians if they want so.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not applicable

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

Dissemination and Use of Findings

The findnigs of the study will be published as original publications in peer reviewed journals. The results will also be presented at ICDDR,B scientific meetings and also at national and international meetings. This research findings will also be used for the disseration leading to a DrPH degree of the PI Dr. M. Mujibur Rahman.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. (DO NOT EXCEED ONE PAGE)

Collaborativ Arrangements

This research will be done under the Clinical Sciences Division in colloboration with Community Health Division (MCH-FP extension project).

This will be a collaborative study between the ICDDR,B and the University of Alabama at Birmingham (UAB), USA. ICDDR,B and UAB have a 7 year history of successful collaboration in research and training. This project will be conducted primarily at the ICDDR,B under the direct supervision of Dr. M.M. Rahman, a physician and associate scientist at the Center who has been trained at UAB under Dr. Jose Alvarez. ICDDR,B and UAB have signed a general collaborative agreement which has provided the administrative and academic frame to various research projects carried out to in the past few years. Dr. Alvarez will visit Dhaka several times during this 2 year project, once supported by this grant and supported by UAB/Sparkman Center funds on subsequent visits.

A copy of the collaborative agreement between ICDDR,B and UAB is attached with the proposal.

Biography of the Investigators

Give the following information for key professional personnel, beginning with the principal investigator/program director. Photocopy this page for each person.

Name	Birthdate
Mohammad M. Rahman - Principal Investigator	02/27/62

Title

Associate Scientist

Education Begin with baccalaureate or other initial professional education and include postdoctoral training.

Institution and location	Degree	Year conferred	Field of study
University of Alabama at Birmingham, Alabama, U.S.A.	M.P.H.	1995	Public Health Nutrition
Dhaka Medical College, University of Dhaka, Bangladesh	M.D.	1986	Medicine

Research and/or Professional Experience

Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any National Institute of Health study section and/or Federal Government Public Advisory Committee. List in chronological order the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. *Do not exceed a total of two (2) pages per biographical sketch.*

Position Held (1986-present)

1986-87 Assistant Surgeon, Dhaka Medical College Hospital, Dhaka, Bangladesh

1987-91 Medical Officer, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh..

1992-93 Assistant Scientist, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh

1994-present Associate Scientist, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) Dhaka, Bangladesh

Member

1. Delta Omega Honorary Society for public health (Upsilon Chapter), USA
2. Bangladesh Medical Association, Bangladesh
3. Bangladesh Nutrition Society, Bangladesh
4. Bangladesh Society of Public Health, Bangladesh

Honors

Delta Omega honory society award for academic excellence (in MPH program) and contribution to pulic health.

List of publications during past 3 years

Rahman MM, Islam MA, Mahalanabis D, Chowdhury S. Biswas E. The Impact of Health Education on Feeding Leafy Vegetable at Home to Children of Urban Poor Mothers of Bangladesh. Public Health 1994;108:211-8.

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Rahman MM, Mahalanabis D, Alvarez JO, Wahed MA, Islam MA, Habte D, Khaled M A . Acute respiratory infection prevent improvement of vitamin A status in young infants supplemented with vitamin A. J Nutr, 1996;126:628-633.

Rahman MM, Mahalanabis D, Alvarez JO, Wahed MA, MA Islam, D Habte. Effect of early vitamin A supplementation on cell mediated immunity in infants younger than 6 mo. Am J Clin Nutr 1997;65:144-8.

Rahman MM, Mitra AK, Mahalanabis D, Wahed MA, Khatun M, Majid N. Absorption of nutrients from an energy dense diet liquefied with amylase from germinated wheat in infants with acute diarrhea. J Pediatr Gastroenterol Nutr, 1997 [In press].

Rahman MM, Mahalanabis D, Ali M, Mazumder RN, Khatun M, Majid N, Wahed MA. Absorption of macronutrients from an amylase-treated energy dense liquid diet in children with acute dysentery. Acta Paediatr, 1997 [In press].

MA Islam, Rahman MM, Mahalanabis D, Rahman AKMS. Death in a diarrhoeal cohort of infants and young children soon after discharge from hospital: risk factors and causes by verbal autopsy. J Trop Pediatr 1997 [in press]

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Majumder RN, Kabir I, Rahman MM, Mahalanabis D. Absorption of macronutrients fron a calorie dense diet in malnourished children during acute shigellosis. J Pediatr Gastroenterol Nutr, 1997 [in press].

ME	POSITION TITLE
sé O. Alvarez	Professor and Chair

UCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
iversidad Peruana Cayetano Heredia, Lima Peru	B.Sc.	1968	Human Biology
hns Hopkins University, Baltimore, Maryland	Ph.D.	1973	Biochemistry

EARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key onnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public ory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative er publications pertinent to this application. DO NOT EXCEED TWO PAGES.

SITIONS HELD (1973 - PRESENT):

3 - 1976 Assistant Professor of Biochemistry, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru.
6 - 1980 Associate Professor of Biochemistry, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru.
6 - 1980 Regional consultant for Post-graduate Training in Biological and Health Sciences, UNESCO/UNDP.
1 - 1983 Professor of Biochemistry and Nutrition, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru.
1 - 1982 Visiting Professor, Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama (while on a Fogarty International Fellowship, NIH).
2 - 1984 Vice-President for Research and Development, AB Chimica Laboratories, Inc., pharmaceuticals and diagnostic reagents; based in Lima, Peru.
4 - 1988 Associate Professor, Department of International Public Health Sciences, School of Public Health, University of Alabama at Birmingham, (UAB), Birmingham, Alabama.
5 - 1988 Associate Professor, Department of Nutrition Sciences, UAB.
8 - Present Professor, Department of Public Health Sciences, UAB.
8 - Present Director, Division of International Health and Public Health Nutrition, Department of Public Health Sciences, UAB.
9 - Present Director, Division of Public Health Nutrition, Department of Nutrition Sciences, UAB.
8 - Present Professor, Department of Nutrition Sciences, UAB
- Present Chairman, Department of International Health (formerly Dept. Of Public Health Sciences), UAB.
- Present Interim Director, John J. Sparkman Center for International Public Health Education, UAB.

lications: (Selected from 61 publications)

rez, José and Biltonen, R. Nucleic Acid-Solvent Interaction. Biopolymers 12:1815-1828, 1973.

rro, J.; Romero, G., Cazorla, A. and Alvarez, José. Ambient Temperature and Hypoxia: A differential Study of Liver Catalase d Cytochrome Oxidase in Guinea Pig. The Johns Hopkins Medical Journal 139:82-86, 1976.

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rez, José; Alegria, A., Merino, F. and Niemmeyer, H. Desarrollo de las Ciencias Biologicas en los Paises Andinos. Plan egional para el Postgrado. PNUD/UNESCO RAL 76/006, Ed. Oficina Regional de Ciencia y Tecnologia para America Latina y aribe (UNESCO), Montevideo, Uruguay, 1979.

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ra, Kervan; Aguilar, Rosario and Alvarez, José. The Oxygen Consuming System of the Liver of Mice Exposed to Simulated igh Altitude. Int J Biometeorology 25(4):293-297, 1981.

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Beggers, Y.H.; Cutter, G.R., Acton, R., Alvarez, J.O., Bonner, J.L., Goldenberg, R.L., Go, R.C.P. and Roseman, J.H. A Positive Association Between Maternal Serum Zinc and Birth Weight. Am J. Clin. Nutrition, 51:678-84, 1990.
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Higgins, N.D., Khaled, M.A., Cornwell, P.E. and Alvarez, J.O. Nutritional status and immune function in cocaine and heroin abusers and in methadone treated subjects. Res. Comm. Subst. Abuse 12:209-215,1991.
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Incharuniti, N., Lewis, C.A., Sauberlich, H.E., Perkins, L.L., Go, R.C.P., Alvarez, J.O., Macaluso, M., Acton, R.T., Copeland, R.B., Cousins, A.L., Gore, T.B., Cornwell, P.E., Roseman, J.M. Plasma homocyst(e)ine, folate and vitamin B_{12} levels and risk for early onset coronary artery disease. Am. J. Clin. Nutr. 59: 940-948, 1994.
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Alvarez, J.O., Salazar-Lindo, E., Kohatsu, J., Miranda, P., and Stephensen, C.B. Urinary Excretion of Retinol in children with Acute Diarrhea. Am. J. Clin. Nutr. 61:1273-6, 1995.
Rahman, M.M., Mahalanabis, D., Wahed, M.A., Islam, M., Habte, D., Khaled, M.A., and Alvarez, J.O. Conjuntival impression cytology fails to detect subclinical vitamin A deficiency in young children J.Nutr. 125:1869-1874, 1995.
Wahed, M.A., Alvarez, J.O., Khaled, M.A., Mahalanabis, D., Rahman, M.M., and Habte, D. Comparison of the MRDR and RDR in the assessment of vitamin A status in Malnourished Children. Am. J. Clin. Nutr. 61:1253-6, 1995.
Kelly, P.E., Yang, Y.L., Alvarez, J.O., and Smoot, T.M. Vitamin A depletion in HIV infection and AIDS. AIDS 10:14, 1996.
Rahman, M.M. Mahalanabis, D., Wahed, M.A., Islam, M., Habte, D., Khaled, M.A., and Alvarez, J.O. Acute Respiratory Infections Prevent Improvement in Nutritionally Compromised Peruvian Children. J. Nutr. 126:628-633, 1996.
Rahman M.M., Mahalanabis, D., Alvarez, J.O. Wahed, M.A., Islam, M.A., Habte, D. Effect of early vitamin A supplementation on cell mediated immunity in infants under 6 months of age. Am. J. Clin. Nutr 65:144-8, 1997.

APPENDIX 1

International Centre for Diarrhoeal Disease Research, Bangladesh

Voluntary Consent Form

Title of the Research Project:

Principal Investigator:

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

INFORMED CONSENT- PROTOCOL FOR EVALUATION OF THE EFFECT OF SIMULTANEOUS ZINC AND VITAMIN A SUPPLEMENTATION ON THE BIOAVAILABILITY OF VITAMIN A

Explanation of Procedures

You are being asked to allow your child to participate in a research study designed to evaluate the efficacy of combined zinc and vitamin A supplementation in improving health and your child's ability to fight diseases. Vitamin A and Zinc are currently being safely used alone in children to improve health. The present study will examine the combined effect of vitamin A and zinc administered simultaneously.

If you decide to let your child participate in the study he/she will be randomly assigned by random computer assignment to receive either vitamin A or zinc or both or none. The medication will be given daily in the morning for 14 days. We will collect blood 3 times from your child:on enrolment and again after 21 days and 3 months: Each time 2 ml venous blood will be drawn to measure the vitamin A and zinc levels. The immunity will be measured on enrolment and again after 21 days. The immunity will be measured using a kit known as multi-test CMI test kit. The multi-test CMI kit contains 8 needle heads those contain eight antigens and will be administered on the child's arm by pricking. The test result will be obtained after 72 hours by measuring the skin induration. We will visit your home once a week and ask whether your child had diarrhea and respiratory infections and will record it. The weight and height of your child will be measured on recruitment and after 1 and 3 months. The study is sponsored by the Thrasher Research Fund, a non-profit organization based in the United States, that funds research that will improve the health of children worldwide.

Risks and Discomfort: There is no apparent side effects from vitamin A or zinc supplementation with the proposed dose. The blood drawing and immunity testing

also do not involve any risk. Only slight discomfort (mild pain) from needle prick or bruising due to extravasation of blood may occur.

Benefits

The benefits your child will receive from participation include free medication, examination, and medical treatment for 3 months. Your child's participation may also provide valuable information about the benefit of combined zinc and vitamin A supplementation.

Alternative Treatment

There are alternatives to vitamin A and zinc supplementation such as increasing intakes of vitamin A and zinc containing diets. However, these are long term measures for prevention of the deficiency of these micronutrients. For acute deficiency high dose supplementation in medicinal form is desirable.

Confidentiality

The information gathered during this trial will be kept confidential. The results of supplementation including laboratory tests may be published for scientific purposes, however, identity of your child will not be revealed. In addition, the Food and Drug Administration may monitor the trial records and the individual conducting the review may see the child's name in the file folder.

Withdrawal without Prejudice

You are free to withdraw your consent and to discontinue participation of your child in this project at any time without prejudice against future medical care your child may receive at this institution. Any significant new findings that develop during the course of the study which may affect your willingness continue to in the research will be provided to you by the study physician.

Costs to Subject from participation in Research

There will be no cost to your child from participating in research. All medications, examinations, and medical treatment will be free of cost during the 3 months study period.

Payment for Participation in the Research

You will be paid no compensation to participate your child in the study.

Payment for Research-Related Injuries

UAB has made no provision for monetary compensation in the event of physical injury resulting from the research and in the event of such injury,

medical treatment is provided, but is not provided free of charge. The ICDDR,B, the implementor of the study will provide treatment required as a result of research-related injuries at ICDDR,B hospital, but will not provide other compensation in the event of physical injury resulting from the research.

Questions

If you have any question about the research, Dr. Mohammad Rahman will be glad to answer them. Dr. Rahman' number is 871751?Ext 2319. If you have any questions about compensation, medical treatment for research related injuries or rights as a research subject, Dr Rahman will also answer them.

Legal Rights and Signature

You are making a decision whether or not to have your child participate in this study. Your signature indicates that you have decided to allow your child to participate, that you have read (or been read) the information provided above and that you have received a copy of this consent form.

Signature of Investigator/ or agents **Signature of Subject/ Guardian** _____

Date: **Date:**

সম্মতি পত্র ঃ ভাইটামিন ও দস্তার কার্যকারিতার উপর গবেষণা।

গবেষণাটা কি?

আমরা শিশুদের স্বাস্থ্যের উন্নতি এবং রোগ প্রতিরোধক ক্ষমতা বৃদ্ধিতে দস্তা ও ভাইটামিন 'এ' এর কার্যকারিতার উপর একটা গবেষণা করতে যাচ্ছি। বর্তমানে ভাইটামিন 'এ ' এবং দস্তা উভয়ই শিশুদেরকে দেয়া হচ্ছে এবং শিশুদের উপর এদের কোন খারাপ প্রতিক্রিয়া নেই। প্রস্তাবিত গবেষণায় আমরা দস্তা ও ভাইটামিন 'এ' এর সম্মিলিত কার্যকারিতা পরীক্ষা করব। আপনার শিশুকে এই গবেষণায় অংশ গ্রহণ করানোর ব্যাপারে আপনার সম্মতি চাচ্ছি।

আপনি যদি আপনার শিশুকে এ গবেষণায় অংশ গ্রহণ করাতে রাজি থাকেন তবে আপনার শিশুকে ভাইটামিন 'এ' অথবা দস্তা, অথবা দস্তা ও ভাইটামিন 'এ' দুই এর মিশ্রণ এর সিরাপ খাওয়ানো হবে। উক্ত সিরাপটি প্রত্যহ একবার করে মোট ১৪ দিন খাওয়ানো হবে। আমরা আপনার শিশুর শরীর থেকে চা চামচের এক চামচেরও একটু কম পরিমাণ রক্ত মোট তিনবার নিয়ে পরীক্ষা করব: প্রথমবার শুরুতে, দ্বিতীয়বার ২১ দিনের দিন এবং তৃতীয়বার তিনমাস পরে। এই রক্ত থেকে আমরা শরীরের ভাইটামিন 'এ' ও দস্তার পরিমাণ নির্ণয় করব। এ ছাড়াও আপনার শিশুর শরীরে রোগ প্রতিরোধক ক্ষমতা গবেষণার শুরুতে ও ২১ দিন পরে পরীক্ষা করব। এ পরীক্ষাটি করতে শিশুর হাতের চামড়ার উপর একটি যন্ত্র দিয়ে সুইয়ের খোঁচা দিয়ে ওষুধ ঢুকিয়ে দেয়া হবে এবং ৭২ ঘন্টা পর চামড়ার উপর কতটুকু ফুলে উঠে তা মেপে দেখা হবে। আমরা আপনার বাড়িতে প্রতি সপ্তাহে একবার আপনার শিশুকে দেখতে যাব এবং ডায়রিয়া বা নিউমোনিয়া হল কিনা তা জিজ্ঞেস করব। আপনার শিশুর ওজন ও উচ্চতাও শুরুতে এবং প্রতিমাসে একবার মেপে দেখব। থ্রেসার রিসার্চ ফান্ড নামে আমেরিকার একটি অলাভজনক প্রতিষ্ঠান এ গবেষণার খরচ বহন করবে।

গবেষণাজনিত ঝুঁকি ও অসুবিধা

ভাইটামিন 'এ' ও দস্তা খাওয়ানোর জন্য কোন রকম ঝুঁকি নেই। এ ছাড়াও রক্ত নেওয়া ও রোগ প্রতিরোধক ক্ষমতা পরীক্ষায় কোন রকম ঝুঁকি নেই। কেবলমাত্র সুঁচ ফোটানোর জন্য সামান্য ব্যাথা অনুভূত হবে এবং রক্ত নেওয়ার যায়গা সামান্য কালচে দাগ হতে পারে।

যে সব সুবিধা পাবে।

গবেষণায় অংশ গ্রহণ করার জন্য আপনার শিশু ৩ মাস বিনামূল্যে স্বাস্থ্য পরীক্ষা ও চিকিৎসা পাবে। আপনার শিশুর অংশ গ্রহণ থেকে দস্তা ও ভাইটমিন 'এ' এর উপকারিতা সম্পর্কে মূল্যবান তথ্য পাওয়া যাবে।

অভিভাবকের অনুস্বাক্ষর

বিকল্প চিকিৎসা

উন্নত খাবার থেকে ভাইটামিন `এ' ও দস্তা পাওয়া যায়। কিন্তু এগুলো হচ্ছে দীর্ঘ মেয়াদী ব্যবস্থা। জরুরী ক্ষেত্রে বেশী মাত্রায় ভাইটামিন `এ' ও দস্তা ওষুধ হিসাবে অতি প্রয়োজনীয়।

গোপনীয়তা

এ গবেষণার সকল তথ্য গোপনীয় রাখা হবে। কেবলমাত্র গবেষণার ফলাফল প্রকাশিত করা হবে। কিন্তু কোন অবস্থাতেই আপনার শিশুর নাম ঠিকানা প্রকাশ করা হবে না। এছাড়াও ফুড এবং ড্রাগ এডমিনিষ্ট্রেশন গবেষণার নথি পত্র পরীক্ষা করে দেখতে পারে।

গবেষণা থেকে নাম প্রত্যাহার:

আপনি যে কোন সময়ে বিনা শর্তে গবেষণা থেকে আপনার শিশুর নাম প্রত্যাহার করতে পারবেন। এতে আপনার শিশুর স্বাভাবিক চিকিৎসার কোন ব্যাঘাত ঘটবে না। গবেষণা থেকে নতুন কোন ফলাফল পাওয়া গেলে তাও আপনাকে জানানো হবে।

গবেষণায় অংশগ্রহণের খরচ

গবেষণায় অংশ নেওয়ার জন্য আপনাকে কোন অর্থ ব্যয় করতে হবে না। সকল প্রকার পরীক্ষা ও ওষুধ বিনামূল্যে দেয়া হবে।

পারিশ্রমিক

এ গবেষণায় অংশ গ্রহনের জন্য আপনাকে বা বাচ্চাকে কোন প্রকার অর্থ প্রদান করা হবে না।

ক্ষতিপূরণ

গবেষণাজনিত কোন ক্ষতি হলে UAB কোন রকম আর্থিক ক্ষতিপূরণ দেয়না। এ ধরণের কোন অসুবিধা হলে চিকিৎসা প্রদান করার ব্যবস্থা রয়েছে তবে তা বিনামূল্যে নয়। আইসিডিডিআর,বি হাসপাতালে গবেষণাজনিত অসুবিধার জন্য চিকিৎসা প্রদান করা হবে , কিন্তু কোন প্রকার আর্থিক সাহায্য দেয়া হবে না।

অভিভাবকের অনুস্বাক্ষর

জিজ্ঞাসা

আপনার যদি গবেষণা বিষয়ক কোন প্রশ্ন থাকে তবে ডা: মোহাম্মদ রহমান এর সঙ্গে যোগাযোগ করবেন। ডাঃ রহমানের ফোন নং ৮৭১৭৫১-৬০, এক্সটেনশন ২৩১৯। গবেষণার একজন অংশগ্রহণকারী হিসাবে আপনার অধিকার বা চিকিৎসা বা ক্ষতিপূরণ ইত্যাদি বিষয়েও ডাঃ রহমানকে ৮৭১৭৫১-৬০, এক্সটেনশন ২৩১৯ নম্বরে ফোন করতে পারেন।

আইনগত অধিকার এবং দস্তখত

আপনি আপনার শিশুকে এ গবেষণায় অংশ গ্রহণ করানো বা না করানোর ব্যাপারে সিদ্ধান্ত নিতে যাচ্ছেন। নিম্নে আপনার স্বাক্ষর নিশ্চিত করছে যেন আপনি উপরে লিখিত তথ্য পড়ছেন/ বা আপনাকে পড়ে শোনান হয়েছে এবং তার পরই আপনি আপনার শিশুকে এ গবেষণায় অংশগ্রহন করার অনুমতি দিচ্ছেন।

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অভিভাবকের স্বাক্ষর তারিখ

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চিকিৎসক/গবেষকের স্বাক্ষর তারিখ

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সাক্ষীর স্বাক্ষর তারিখ

Annex 2. Sample size calculation

1. For serum vitamin A levels.

There has been no community study in Bangladesh showing the effect of simultaneous zinc and vitamin A on serum vitamin A level. However, a recent study in Bangladesh (Rahman et al. 1996) has shown that mean+SD vitamin A levels after large dose vitamin A supplements was 0.74±0.23. In a study in Thailand Udomkeshmalee et al. (1990) has shown that the mean+SD concentration of vitamin A in normal children was 1.06±0.31. We can assume that zinc plus vitamin A supplementation will increase the retinol levels to normal levels as shown in Thai children.

Based on the above assumptions:

Mean serum retinol concentrations:
in the vitamin A supplemented children u_1=0.74 µmol/L
in vitamin A plus zinc supplemented children= u_2=1.06 µmol/L
SD for mean serum retinol:
in the vitamin A supplemented children δ_1=0.23 µmol/L
in vitamin A plus zinc supplemented children= δ_2=0.31 µmol/L
Expected difference due to zinc and vitamin A supplementation=D=u_2-u_1
If we want to detect the above difference with an assumed:
Type I error=0.05 and Type II error=0.1
Then children in each category

$$N=(Z_{1-\alpha/2}+Z_\beta)^2(\delta_1^2+\delta_2^2)/(u_2-u_1)^2$$
$$=(1.96+1.28)^2(0.23^2+0.31^2)/(1.06-0.74)^2, \quad \text{where, } Z_{1-\alpha/2}=1.96,\ Z_\beta=1.28.$$
$$=16$$

Serum retinol concentrations in placebo children of the above study was 0.61±0.28. If we assume that only zinc supplementation will increase the serum retinol concentration at least by 25%, then the sample size in each group (to test the hypothesis 5) as calculated above will be 74.

2. Proportion of vitamin A deficient children

Wahed MA et. al. (1995) in a study in Bangladeshi children found that 45% children remained vitamin A deficient after vitamin A supplementation (Wahed MA, personal communication). We expect that after zinc+vitamin A supplementation only 10% will be vitamin A deficient (DR:R: >0.06).

Based on this assumption:

p_1=0.45, q_1=0.55, p_2=0.1, and q_2=0.9

assuming type I error=0.05 and Type II error=0.1 the number of children in each category will $N=(p_1q_1+p_2q_2)/(p_1-p_2)^2*10.5$

$n=(0.45x0.55+0.1x0.9)/0.35^2*10.5$

$= 29$

3. For serum RBP levels.

There has been no community study in Bangladesh showing the effect of simultaneous zinc and vitamin A on serum vitamin A level. The mean RBP (mg/dl) levels in the urban community of Bangladesh is 2.0±0.74 (Wahed MA, personal communication). If we expect a 25% rise of RBP with vitamin A and zinc supplementation then the samples in each group as calculated in (1) will be 47.

4. Diarrhea morbidity

A recent study in Bangladesh has shown that mean±SD episodes of diarrhea (per child-year) 3.68±2.92 (Mitra et al., personal communication). Several studies have shown that vitamin A supplementation does not have any effect on diarrhea morbidity (Rahmatullah et al. 1991). So we can assume that diarrhea episodes in vitamin A supplemented children will be the same. However, we expect that zinc+vitamin A supplementation will reduce the diarrheal episodes at least by 25%. Based on this assumption:

Mean diarrheal episodes

in the vitamin A supplemented children $u_1=3.68$

in vitamin A plus zinc supplemented children= $u_2=2.76$

SD for mean diarrheal episodes

in the vitamin A supplemented children $\delta_1=2.92$

in vitamin A plus zinc supplemented children= $\delta_2=2.92$

Expected difference due to zinc and vitamin A supplementation=$D=u_1-u_2$

If we want to detect the above difference with an assumed:

Type I error=0.05 and Type II error=0.1

Using the formula as in (1), the number of children in each category will be

N=210

5. Lower respiratory tract infection (LRTI)

A recent study in Bangladesh has shown that mean±SD episodes of LRTI (per child-year) 5.62±3.2 (Mitra et al., personal communication). Several studies have shown that vitamin A supplementation does not have any effect on diarrhea morbidity (Rahmatullah et al. 1991). So we can assume that LRI episodes in vitamin A supplemented children will be same. However, we expect that zinc+vitamin A supplementation will reduce the LRI episodes at least by 25%. Based on this assumption:

Mean LRI episodes

in the vitamin A supplemented children $u_1=5.68$

in vitamin A plus zinc supplemented children= $u_2=4.26$

SD for mean LRI episodes

in the vitamin A supplemented children $\delta_1=3.2$

in vitamin A plus zinc supplemented children= $\delta_2=3.2$

Expected difference due to zinc and vitamin A supplementation=$D=u_1-u_2$

If we want to detect the above difference with an assumed:
Type I error=0.05 and Type II error=0.2
Using the formula as in (1), the number of children in each category will be N=80

6. Weight gain

A recent study in Bangladesh showed that children aged 1-2 years gain 97±45 g/kg initial weight over 3 months (Rahman MM et al, unpublished data). If we assume that children receiving only vitamin A will gain similar weight and those receiving vitamin A+zinc will gain 25% more than this (58±37 g/kg). To detect the above difference at 5% significance level and 80% power, the sample size in each group as calculated in (1), will be 95.

7. Height gain

The study as mentioned in (6) the height increment in children aged 1-2 years was 2.62±1.02 cm over 3 months (Rahman MM et al, unpublished data). If we assume that children receiving only vitamin A will gain similar height and those receiving vitamin A+zinc will gain 25% more than this (3.27±1.02 cm). To detect the above difference at 5% significance level and 90% power, the sample size in each group as calculated in (1), will be 34.

If we take the largest number (diarrhea morbidity), the sample size in 4 groups will be 210x4=840. Adding 20% for expected loss from follow up due to migration or death, the total number of children will be 1008. However, for time and budget constraints we will not be able to recruit this large number of children.

Detailed Budget for New proposal

Project Title: Effect of simultaneous zinc and vitamin A supplements on the bioavailability of vitamin A in children

Name of PI: Dr. M. Mujibur Rahman

Protocol Number: Name of Division: CSD

Funding Source: Thrasher Amount Funded (direct): 95,120
Total: 101,778 Overhead (%) 7%

Starting Date: July 1, 1997 Closing Date:. June 30, 1999

Strategic Plan Priority Code(s):

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	**Position**	**Effort%**	**Salary**	**1st Yr**	**2nd Yr**	**3rd Yr**
	Dr. M.M. Rahman	Associate Scientist	50		5400	5400	
	To be recruited	Research Physician	100	4800	4800	2400	
	To be recruited	Health Asstt	100		2400	1200	
	To be recruited	Health Asstt	100		2400	1200	
	To be recruited	Health Asstt	100		2400	1200	
	Sub Total				17400	11400	
	Consultants						
	Local Travel				16,800	5,280	
	International Travel				5,000		
	Sub Total				21,800	5,280	
	Supplies and Materials (Description of Items)						
	Office supplies				500	300	
	Supplement (Vitamin A/zinc)				1,000		
	Didehydroretinol (for MRDR test)				2,000		
	CMI Kits				8,000		
	Medicines				800	300	
	Personal computer				3,500		
	Sub Totals				15,800	600	

	Other Contractual Services			
	Repair and Maintenance			
	Rent, Communications, Utilities			
	Training Workshop, Seminars			
	Printing and Publication		200	
	Staff Development			
	Sub Total		200	

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges			
	Pathological Tests			
	Microbiological tests	300	200	
	Biochemistry Tests	300		
	X-Rays	100		
	Patients Study			
	Research Animals			
	Biochemistry and Nutrition	16,000	5,240	
	Transport	200	100	
	Xerox, Mimeographs etc. (illustration)	700	500	
	Sub Totals	16,600	6,040	
	Other Operating Costs			
	Capital Expenditure			
	Total Direct Cost	71,600	23,520	
	Indirect cost (7%)	5,012	1,646	

TOTAL PROJECT COST **76,612** **25,166**

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

Specific functions of personnel

Principal Jnvestigator: will supervise the over all activities of the project. He will play the leading role in implementing the research at the field, planning for data entry, cleaning, analysis, and report writing.

Research Physician: will perform the physical examination, case diagnosis, management, referral and supervision of the health assistants at the field levels.

Health Assistants will recruit study subjects, collect data, arrange for MRDR tests, CMI test, measure anthropometry and do morbidity follow up. They will maintain records of the study subjects.

Travel Costs

Domestic: The domestic travel cost is mainly for follow up at home of study subjects. Each subject will be followed up for 24 times. Each follow up costs is $2.00. Therefore, for 460 subjects the total cost would be 460 x 24 x 2 = $22,080.

Personal Computer: For data entry, analysis and report keeping the P.I will need a personal computer which will cost approximately $ 3,500

Salary : Except the PI, all personnel (1 physician and 3 health assistants) will be recruited for this research project only. So, their full salary will be provided from the project fund.

The Principal Investigator will be supported from ICDDR,B for 50% of his salary for 50% of his time in ICDDR,B Hospital. The remaining 50% will be supported from the project fund.

Sample analysis : Sample analysis for serum retinol, RBP, and plasma zinc and urine retinol and zinc for 400 subjects will cost 21,240.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included ☑
2. Approval of the Division Director on Face Sheet ☑
3. Certification and Signature of PI on Face Sheet, #9 and #10 ☑
4. Table of Contents ✓
5. Project Summary ✓
6. Literature Cited ✓
7. Biography of Investigators ✓
8. Ethical Assurance ✓
9. Consent Forms ✓
10. Detailed Budget ✓

RESEARCH PROPOSAL TO THRASHER RESEARCH FUND

Project Title: **Effect of simultaneous zinc and vitamin A supplementation.....**
PI: JO Alvarez

Overall the objectives and design of the proposal appear to be good and well throughout. However, details of some aspects of the design are missing. In particular it is unclear how and where the biochemical analyses will be performed. Since the proposed biochemical analyses require specialized equipment and expertise in running the assays, this whole area needs to be addressed. This is especially true for the zinc assays.

Furthermore, the specific plans for processing the blood samples are missing. Collecting and processing urine and blood samples in the field is difficult. A detailed, well thought-out plan is needed. It is often not clear who will be recording the data--a field worker and or the parent/guardian.

In the Supportive Preliminary Data section it is stated that "zinc deficiencies are highly prevalent" —(in Bangladesh)— with no reference or supporting data. Please clarify.

Specific comments:

Study design: (pg 5)
Rather extensive: without a placebo group the data may be less definitive. In at least two places in the proposal a "placebo" is indicated, please clarify. This reviewer suggests a semi-placebo group be included which would be a vitamin/mineral supplement with zinc and vitamin A omitted. This would markedly strengthen the design and should allay ethical concerns.

The two-week supplementation period is rather--perhaps too-- short, especially to expect an effect on growth and maybe other indices.
The doses for zinc and vitamin A are high, especially for 1 yr infants.
I realize these levels have been given in a few recent studies but the long-term consequences have yet to be assessed, especially for the 40 mg of zinc per day.

The exclusion criteria are unclear re whether hospitalization at enrollment(a) is required or is a criteria for exclusion? Is (d) wt/age referring to WAZ score?

Data collection and analysis:
Urinary retinol (pg5):

The 8 hour collection will not give meaningful information for zinc metabolism. There is general agreement that a 24 hour collection is necessary for accurate zinc assessment. Relating urinary zinc to creatinine has not been successful. Will it be adequate to assess vitamin A excretion? The subset of males will be 12-36 months old. Details of how the collections are planned are necessary. I serious doubt if non-contaminated collections (for zinc) can be done under these field conditions. Even in a metabolic ward it is difficult. How would the authors collect in these situations? What vessels etc would be used and how would they be checked for zinc contamination?

Reviews due by

RBP =

RESEARCH PROPOSAL TO THRASHER RESEARCH FUND
Project Title: Effect of simultaneous zinc and vitamin A supplementation.....
PI: JO Alvarez

Score: 4.3

I would score the proposal high for design and merit but the overall score is lowered because of reservations about lack of details regarding methodology. The authors should be given an opportunity to expand on their plans for sample collection and analysis. They may have adequate plans for the processing and analysis but just did not outline them in the proposal. They have extensive experience with vitamin A but apparently little in zinc metabolism. Indeed, the purported high prevalence of zinc deficiency in Bangladesh was not documented with any preliminary data. I am also concerned that the apparent high prevalence of protein deficiency may confound the results. That is, other essential nutrient(s) may be more limiting that zinc. On the other hand I think the hypothesis should be tested. Lastly, less emphasis should be placed on bioavailibility --this is difficult to quantitate without using an isotope-- and more on functional outcome (effect on diarrhea and respiratory infections).

Sample collection =

analysis = 3

(NIST)

Blood collection:

The authors state that 2 ml of blood will be collected yet they propose to collect both serum, for retinol and RBP and plasma, for zinc. Do they propose to draw multiple tubes to get both serum and plasma? Would each one be 1 ml? Would the samples yield adequate amounts of serum and plasma to perform the proposed assays with extra for reruns, etc?

How would the samples be processed? Would they be put on ice after the draw? How long between the time of drawing and centrifugation? Where would the samples be centrifuged? The zinc samples must be stored in zinc-free containers. Would the children be fasted? Fasting is desirable for serum zinc? Does it make a difference for zinc or retinol determinations?

Most serious re the zinc analysis is the planned method for zinc analysis--"emission spectrochemical—after low temperature ashing". The method described nearly 25 years ago has long been abandoned and replaced by atomic absorption spectrophotometric techniques described throughout the literature. In addition, no mention was made of how the analysis will be monitored for accuracy. Certified standard reference standards are recommended.

Retinol binding protein

Apparently these kits are no longer available. Do the investigators have a source and if not can they develop an ELISA?

Modified Relative Dose Response Test

As the investigators are aware, this test is highly variable and its ability to accurately assess vitamin A status has not been firmly established. As indicated the previously published paper using the technique in Bangladesh(Wahed et al.1995), concluded that it lacked reliability. Although a higher dose may help, the "provisionally used" cutoff value remains a problem. To the investigators credit, they are aware of potential problems of interpretation.

Cell Mediated Immunology (CMI):

These kits are rather expensive and the limited results are variable in the literature, with the subjects often being extremely deficient before an effect may be noted. On the last page of the proposal (before the literature review #8 CMI) a recent study by Rahman et al 1996 is cited as employing CMI with little difference in the effect of vitamin A on immune response. However, it appears that these data were not included in the paper, at least this reviewer could not find it and it is not described in the methods section. Was the difference in response to vitamin A significant? If this test were omitted, it appears that the number of subjects could be markedly lowered from 340 children so it may be worth it. Although the budget lists $8,000 for the cost of the kits a conservative estimate if the kits are purchased would be $17,000 ($25x340x2=$17,000). Is this a typo?

Sample size calculations:

Some of the assumptions used for sample size calculations are questionable. For most of the parameters the authors assume there will be a 25% change between the vitamin A only and the vitamin A plus zinc treated groups. Are these reasonable estimates? Can serum RBP increase 25%? Can diarrhea decrease by 25%?

The last paragraph of the proposal is confusing since it states that the sample size chosen (340) will not be adequate to assess "diarrhea morbidity". However diarrhea morbidity is one of the specific aims of the project. Please clarify.

Impact on child health-

The project clearly addresses a major child health problem. Vitamin A deficiency in Bangladesh is a severe problem. This proposal attempts to improve on the traditional therapy of giving vitamin A alone for a vitamin A deficiency. The proposed treatment with vitamin A and zinc would be a significant finding if this treatment could improve the biochemical and functional vitamin A status of these children.

Practical Application-

There would be an immediate and clear application if the proposed new therapy is an improvement.

Funding Considerations-

The budget appears reasonable, indeed rather conservative compared to costs in the US or most other countries. The cost of the analysis appears under estimated, especially for the CMI kits. Details of the costs for analysis of specific analyses is not given eg zinc, RBP, vitamin A, but lumped together at $18,140. The price of computers is coming down and a good one can be purchased for < the $3500 listed. Also the cost under Travel should be justified and explained. (in one case it is $2.00/follow-up visit and another it is $20.00). Who receives these monies? Are the subjects paid?

Purpose and Objectives-

The project aims are strong although they may over ambitious, especially in regard to growth due to the short period of supplementation. One of the main aims of the study (to assess the interaction of vitamin A and zinc on childhood diarrhea morbidity) may not be reached because the projected sample size is too small according to PI's assessment (see last paragraph of the proposal). This is unfortunate since the functional parameters (diarrhea and respiratory infections morbidity) are the most definitive indices to determine the "bioavailability of vitamin A" as indicated in the title. Because of this reviewer's concerns of the MRDR to measure bioavailability as well as the other proposed indices, I suggest that the emphasis be changed to assessing the efficacy of a combination of zinc and vitamin A (compared to either alone) for combating childhood morbidity of diarrhea and respiratory infections

Evaluation-

The parameters proposed to evaluate the treatments are adequate. The question is if the biochemical parameters can be successfully analyzed with precision and accuracy, especially for zinc.

Project Site

The site seems very appropriate. The authors have demonstrated that this area has a high incidence of vitamin A deficiency in the age group they will work with. There is a high probability that zinc deficiency is prevalent although this has not been documented.

Replicability-

Treatment with vitamin A and zinc together could easily be transferred and adapted to other areas.

Effect of simultaneous zinc and vitamin A supplementation on the bioavailability of Vitamin A.

Impact on Child Health. This project addresses a child health problem of global dimensions. Programs for vitamin A prevention are already established in many countries. Further research is still necessary to determine optimal intervention strategies for the prevention and treatment of zinc deficiency and to determine in effectiveness of these programs. Thrasher's involvement in this general area over the next decade is likely to have a major impact on progress towards definitive prevention/treatment programs.

Practical application. The potential for immediate benefit to child health is very strong and interventions based on the outcome of this study should be culturally acceptable feasible economically and present no insuperable, technological barriers.

Funding Considerations. The budget appears reasonable.

Purposes and Objectives. The strength of the project aim is that it specifically addresses a potentially important interaction between zinc and vitamin A. Whilst there are several indications that zinc deficiency impairs vitamin A metabolism, this project is expected to make an important contribution to our understanding of the importance of this interaction.

Project Design. My major concern about the project design, if I understand the protocol correctly, is the wide variation in clinical status of young children who it is proposed to admit to this study. Specifically, it appears that they have, at the time of enrollment, to have a systemic infection such as diarrhea/dysentery, respiratory tract infection with or without fever, measles, and other diseases requiring hospitalization. This is a very heterogenous population, which may yield a wide mix of results. For example, two weeks of zinc therapy in a young child with dysentery is unlikely to impact zinc status to the same extent as it would a young child without diarrhea.

Evaluation. Evaluation component is adequate.

Project Site. Project site is suitable.

Replicability. This project would be transferable and adaptable.

While this proposal has substantial merit, my concerns about the heterogencus population are reflected in my scoring. There is no doubt that large-scale studies of the effectiveness of different intervention studies to prevent/treat zinc deficiency in young children in the developing world are a nigh priority. It seems to me unlikely that a 2-week course of zinc therapy is likely to prove to be among the most effective approaches. There may be some merit in linking the kind of studies proposed here to an intervention that is perhaps more attractive from a public health perspective.

Overall Project Score: 3.8

need for well-controlled, double-blind supplementation studies to definitively establish if there is an interaction of zinc and vitamin A in humans (Udomkesmalee et al. 1990). Involvement of the Thrasher Research Fund is likely to be a significant factor to ensure that this important question is addressed.

Practical Application

There is a potential significant and immediate benefit to child health if zinc is determined to enhance vitamin A utilization in the high percentage of children who do not respond adequately to vitamin A supplementation. The potential benefit is a substantial and consistent impact of vitamin A supplementation on morbidity as documented for childhood mortality. Large-scale zinc supplementation presents unique technical problems. There are no stores of zinc in the body, so that zinc supplements may need to be given often and perhaps daily to ensure adequate utilization of vitamin A. Alternatively, supplemental zinc might be provided through food fortification or targeted administration to high-risk groups. There is already a compelling rationale to develop systems for zinc supplementation in developing countries as a result of a potential role in prevention and management of diarrheal diseases in young children (Sazawal et al. 1995, Sazawal et al. 1996).

Funding Considerations

The budget is modest in terms of the scope of the proposed research. The potential for funding from other organizations or agencies is uncertain given the current fiscal climate.

Purpose and Objectives

The objectives of this study will be to determine whether biochemical and functional indices of vitamin A status can be improved in a population of high-risk Bangladeshi children by supplementation with a combination of vitamin A and zinc as compared with supplementation with vitamin A alone using a double-blind intervention design. There is a clear need for well-controlled, double-blind supplementation studies to address this problem. A particularly new and unique aspect of the proposed research is investigation of potential interactive effects of vitamin A and zinc supplementation on cell-mediated immunity.

Project Design

Strengths:

Study population: The proposed study population is well-suited to detect a biochemical interaction of vitamin A and zinc. There is a high prevalence of overt vitamin A deficiency in Bangladesh and a probable high prevalence of zinc deficiency as indicated by widespread protein energy malnutrition. In a recent study in Thailand, children with marginal vitamin A and zinc status who were supplemented with both nutrients did not have detectable changes in plasma retinol or RBP due to zinc treatment (Udomkesmalee et al. 1992), although there were functional improvements in terms of night blindness and normalization of conjunctival epithelium. In an earlier study in India, children with severe protein energy malnutrition showed significant increases in plasma vitamin A and RBP after only 5-10 days of oral zinc supplementation (Shingwekar et al. 1979). Thus overt rather than marginal

II 3

Project Peer Review - Thrasher Research Fund

Project Title: Effect of Simultaneous Zinc and Vitamin A Supplementation on the Bioavailability of Vitamin A

Principal Investigator: José O. Alvarez, Department of International Health, University of Alabama at Birmingham, United States

Coprincipal Investigator: Mohammad Rahman, International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh

Impact on Child Health

Vitamin A deficiency is a major nutritional problem among preschool-age children in developing countries with approximately 14 million suffering related eye damage and 190 million at risk worldwide (United Nations, 1987). Vitamin A supplementation substantially reduces childhood mortality (Sommer et al., 1986; Beaton et al., 1993) by mechanisms which are less clear but are thought to involve reduction in infectious disease incidence and/or severity (Dibley, 1996). Prospective trials do not provide consistent support for a primary preventive effect of vitamin A supplementation on the incidence of childhood illnesses (Abdeljaber et al. 1991, Rahmathullah et al. 1991, Stansfield et al. 1993, Vijayaraghavan et al. 1990). However, the VAST intervention study in Ghana reported reduced clinic visits and hospital admissions for vitamin A-supplemented children (Ghana VAST Study Team 1993); a trial in Brazil reported reduced episodes of severe diarrhea (Barreto et al. 1994); a trial in India reported reduced incidence of measles in children less than 23 months of age (Bhandari et al. 1994).

The inconsistent findings with regard to morbidity from childhood infectious illnesses may reflect the inconsistent efficacy of vitamin A supplementation. The investigators' recent studies at the International Centre for Diarrhoeal Disease Research in Bangladesh document a high prevalence of vitamin A deficiency that is refractory to vitamin A supplementation in infants (Rahman et al. 1995; Rahman et al. 1996; Rahman et al. 1997). In one study, 61% of infants remained deficient despite vitamin A supplementation (Rahman et al., 1996). Infants whose relative dose response (RDR) was abnormal at the end of the supplementation period had an increased incidence of acute respiratory infections and increased cumulative duration of fever and cough as compared with infants whose RDR was normal. These studies suggest a high prevalence of co-existing conditions that reduce absorption and/or utilization of vitamin A supplements and the probable protection conferred against common childhood illnesses.

The investigators' hypothesis that lack of improvement of vitamin A status after supplementation is due to co-existing zinc deficiency is plausible and based on experimental evidence from animal and human studies. In most developing countries, children have low intakes of foods rich in readily absorbable zinc, such as liver, red meat, poultry, fish, oysters, and crabs (Penny and Lanata, 1995). Phytate, fiber, and lignin reduce the bioavailability of zinc in traditional staple foods such as cereals, legumes, and tubers. Evidence from animal and human studies suggests that zinc deficiency impairs synthesis of retinol binding protein (RBP) and mobilization of liver vitamin A stores. There is a clear

zinc deficiency may be a requisite to detect effects of zinc treatment on biochemical indicators of vitamin A status.

Supplementation protocol: Because zinc is not stored in the body, combined daily supplementation with vitamin A and zinc is most likely to result in a benefit in terms of improved vitamin A utilization.

Outcome measures: The vitamin A-zinc interaction will be documented by a combination of biochemical and functional indices such as growth, cell-mediated immunity, and morbidity due to respiratory infections.

Weaknesses:

Placebo control: The proposed intervention study is not placebo-controlled. As a result, the experimental design is adequate to address interactive but not independent effects of vitamin A and zinc on the outcome variables. The need for a placebo control is demonstrated by a previous study in Thailand in which elevations of plasma zinc and retinol concentrations in a placebo group were nearly as large as those produced by vitamin A and/or zinc supplementation (Udomkesmalee et al. 1992). The investigators recently completed placebo-controlled trials of vitamin A supplementation in infants at the International Centre for Diarrhoeal Disease Research in Bangladesh (Rahman et al. 1995, Rahman et al. 1996). Thus omission of a placebo control group appears to be based primarily on financial constraints.

Duration of intervention: The proposed duration of supplementation with vitamin A and/or zinc is only two weeks. This supplementation period is likely to be adequate to detect a vitamin A-zinc interaction by biochemical indices, such as serum retinol, serum RBP, and MRDR values, but inadequate to detect an interaction by functional indices such as growth and morbidity due to respiratory infections which are listed as major outcome variables. A 1979 study reported slight but significant increases in plasma vitamin A and RBP in malnourished Indian children after only 5-10 days of oral zinc supplementation (40 mg/day) (Shingewar et al 1979).

Evaluation

The vitamin A-zinc interaction will be documented by a combination of biochemical and functional indices. The duration of supplementation is adequate to detect improvements in biochemical indices such as serum retinol, serum RBP, and MRDR values, but is likely to be inadequate to detect improvements in some functional indices such as growth and morbidity due to respiratory infections.

Project Site

The proposed project is highly feasible as proposed in the specific location. If funded, this project will continue the long-term and successful collaboration of the University of Alabama at Birmingham and the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh.

Replicability

The findings of this project will be extrapolatable to other populations of children with prevalent vitamin A deficiency and protein energy malnutrition.

Summary

The proposed project addresses an important problem with a potential clear benefit in terms of ensuring adequacy of vitamin A nutriture in high-risk populations and resultant improvements in child morbidity and mortality. The duration of the intervention is adequate to detect a vitamin A-zinc interaction by biochemical indices of vitamin A nutriture and possibly by improvements in cell-mediated immunity. The duration of supplementation is likely to be inadequate to detect functional improvements in terms of growth or morbidity due to respiratory infections which are listed as major outcome variables. The experimental design would be strengthened by inclusion of a placebo control group which would allow the investigators to address independent as well as interactive effects of vitamin A and zinc on the outcome variables. However, these improvements in experimental design would markedly increase the cost of the project. Drs. Alvarez, Rahmen and colleagues are making fundamental contributions to understanding of physiological factors that precipitate vitamin A deficiency. They are extremely well-qualified to direct the proposed research which should receive a high priority for funding.

OVERALL PROJECT SCORE: 4.5

Proposal Review

Authors: Alvarez & Rahman

Title: Effects of Simultaneous Zinc and Vitamin A supplementation on the Bioavailability of Vitamin A

1. Impact on Child Health: The concurrence of Vitamin A (VA) and zinc deficiencies in some populations is a real problem. There is little doubt that the efficiency of supplementation of VA in improving health status in some children is lessened by low zinc status. It would be important to determine whether co-administration is effective in this population. One difficulty is the possible sustainability of this approach of daily supplementation of both nutrients in a large population. It could be instituted as standard practice in hospitals or clinics if prov efficacious. However, it would be more difficult outside of these environments.

2. Practical Application: See comments in #1. Also, it would be just as easy to provide VA and zinc as VA alone as a supplement although at some higher cost .

3. Funding Considerations: This is a very reasonable budget, not at all inflated.

4. Purpose and Objectives: This is a very well designed and written proposal except for two minor things. First, the title really is misleading. The work does not deal with bioavailability of VA. It deals with improvement of health with simultaneous zinc and VA supplementation. Secondly, on pg. 5 the level of zinc supplementation is listed as 40 mgs while it is 20 mgs daily elsewhere. In that regard, have the authors of the proposal considered the copper status of the population? 40 mgs daily certainly would reduce copper absorption; 20 mgs may as well. Overall, however, the purpose and aims of the work are excellent.

5. Project Design: Clearly this is a strength of the proposal. Careful consideration was given to all details needed to make this a successful work.

6. Evaluation: Excellent

7. Project Site: The investigators have an excellent track record at working together. This is a most appropriate site.

8. Replicability: Yes, I believe it is replicable.

9. Summary: This research team has an excellent track record of producing meaningful results from an appropriate population in Bangladesh. The details of the design of the work and methods are excellent. This work is important, and the results will be of interest to areas outside Bangladesh. The only minor concern is the effects of zinc supplementation on copper status of the children. The overall project score is 4.7.

Response to reviewers' comment

Reviewer 1

1. **Placebo group**

We agree with the reviewer that a placebo group is needed to strengthen the design. In fact, in the initial proposal (concept paper) which was submitted to Thrasher had 4 groups; one of those was placebo. Later one of the reviewer of the concept paper suggested to eliminate the placebo group to reduce cost and also on the ethical ground. Therefore, according to the suggestion of the reviewer we have proposed the study without a placebo. The word 'placebo' was not delated from the protocol layout on page 5 and in the lay abstract section by mistake. We, feel that the reviewer has rightly suggested to include a placebo group. We will revise our proposal by adding a placebo group.

2. **Duration of supplementation**

There are several studies with zinc supplementation of various duration starting from 2 weeks to several months. However, data for combined vitamin A and zinc supplementation is not available. The main purpose of zinc supplementation is to replete the zinc deficiency to normal which as we hypothesized will increase the availability of vitamin A. Zinc supplementation for 2 weeks will be adequate to improve the zinc status and subsequently increase serum retinol and RBP. Zinc supplementation alone might not be adequate to improve the growth rate or to reduce morbidity. But the combined effect of zinc and vitamin A might exert a beneficial effect (interaction or synergistic effect). The purpose of our study will be to evaluate whether the addition of zinc can overcome the failure of vitamin A supplementation alone. So, long term supplementation will not be a practical approach too.

3. **Dose of supplementation**

Vitamin A : A dose of 200,000 IU is recommended for children aged 1 year and above by WHO. There is no evidence of adverse effect with 200,000 IU vitamin A in a one-year old child.

Zinc: We will give 20 mg of elemental zinc (in one place 40 mg is a printing mistake) which is 2 times of RDA. This dose for two weeks should not be toxic to children of this age, particularly in malnourished children.

4. **Exclusion criteria**

Presence of infections and hospitalization are exclusion criteria. In exclusion section (a) "absence" is a printing mistake. It will be "presence of systemic infection .....

As we have mentioned (d) wt/age refers to % of weight/age median NCHS median.

5. **Urine collection**

A metabolic balance study is indicated for measuring the exact losses stool or/and urine. However, a balance study is expensive and is very difficult to perform in children who are not sick. Further more, we do not expect loss of retinol and zinc in urine of apparently healthy children. Excretion of retinol was reported in children with diarrhea and pneumonia (Alvarez et al. 1995, Stephensen et al. 1994). In normal children there should be virtually no loss of retinol. We proposed to measure retinol and zinc in urine to investigate whether there is any loss at all. If there is loss further balance studies will be needed for quanlification. Eight hour sample collection although is not accurate, will be a surrogate measure of loss of vitamin A and zinc if any in urine. We will collect urine using a sterile PUC bag which should not contain any zinc and after collection we will store it in a zinc free container (test tube).

The male children will be enrolled to ensure urine collection.

6. **Blood samples**

Collection

Two ml venous blood (from antecubital vein) will be drawn in a zinc free container (test tube). The samples will be kept in a box containing ice and will be transported to ICDDR,B Biochemistry laboratory. The blood will be centrifuged at the LAB and serum will be preserved at -70 0 C until analysis. The time interval between blood collection and centrifugation will be less than an hour.

Two ml blood will be adequate for serum retinol, RBP and zinc. One ml for retinol and RBP and 1 ml for zinc. We can collect in 2 tubes. However, for convenience we will collect blood in one tube (zinc free) and transport it to the laboratory immediately.

Sample Analysis

Samples will be analyzed at ICDDR,B Biochemistry Laboratory. The ICDDR,B biochemistry Lab has established techniques to analyze many macro and micronutrient/trace minerals including retinol, zinc and iron. The ICDDR,B Biochemistry Lab has been measuring zinc and retinol since 1977 and has been participating in the quality control program of NIST, USA. NIST has evaluated ICDDR,B as one of the excellent Laboratories (one of the top tens).

Atomic absorption spectrophotometric method (AOAC) is available at ICDDR,B and will be used for analyzing serum for zinc.

Radial immunodiffusion kits for RBP are available at ICDDR,B and are being used for measuring retinol binding protein.

7. Omittimg the assessment of CMI will not reduce the sample size because for weight

gain variable the sample in each group is 94 (95 for CMI). As mentioned by the reviewer, the cost of CMI kit is $ 25/kit. However, previously we bought it for $8/kit. So, we feel that by spending only $ 8000 we will be able to examine the effect of zinc/vitamin A interaction effect on cell mediated immunity as measured by skin test reactogenicity.

8. **Sample size**

For calculating sample size we assumed 25% increase in retinol and RBP concentrations. We think that this is a reasonable estimate. We can even find a much higher increase. A 25% morbidity reduction although is a high expectation is possible.

9. **Budget**

Travel cost for follow-up is estimated as $2/visit. The subjects will not be paid. It is an average estimate. Some visits will cost less and some will more.

The cost of buying a computer is approximately $3500 as we needed in the past.

10. The reviewer has rightly mentioned that diarrhea and respiratory infection morbidity will be good functional indices to measure the zinc-vitamin A interaction. Diarrhea morbidity will double the sample size and cost. So, we will not be able to study that many subjects. We will have the respiratory infection morbidity. Also, we will be collecting information on diarrhea morbidity without extra cost and effort and we will be able to examine to see whether there is any trend of difference in reduction of diarrhea morbidity.

11. **Evaluation**.

We will be able to measure the biochemical parameters at ICDDR,B with accuracy. However, whether measuring zinc levels in blood is a good indicator for zinc status is questionable. Serum zinc levels will be a proxy indicator of baseline and post dose zinc status.

12. **Data on zinc deficiency**

As we have mentioned in our proposal, prevalence data on zinc deficiency in Bangladesh is not available particularly at community level. Clinic-based data shows that 45% children attending ICDDR,B hospital (coming from urban and peri-urban community of Bangladesh for treatment of diarrhoea) are deficient of zinc when serum zinc was taken as an indicator (SK Roy, personal communication).

13. **Fasting**

It is very difficult to keep small children over night fasting. However, we can draw blood 2-3 hours after last feed.

14. **Who will record morbidity data ?**

The study health workers will collect and record morbidity data weekly.

15. The reviewer has rightly mentioned that other micronutrient (s) deficiency may confound the results. This is a real problem in such a study. However, our hypothesis is that zinc deficiency is the most limiting factor for vitamin A metabolism and interaction of vitamin A with zinc more than any other micronutrient.

Reviewer # 2.

Subject enrollment

As mentioned above (reviewer 1, item 4) subjects with infection at the time of enrolment will be excluded from study.

Reviewer # 3

1. Inclusion of a placebo group
   As discussed above in response to reviewer # 1 (item 1) we will add a placebo group.

2. Duration of supplementation has been discussed above (reviewer 1, item 2).

Review # 4

As we have mentioned above (reviewer 1, item 3) the dose of zinc will be 20 mg (2 x RDA). A dose of 20 mg zinc should not interfere with copper absorption.

UAB SCHOOL OF PUBLIC HEALTH

Department of International Health

April 29, 1997

RE: Dr. Mujibur M. Rahman

To Whom It May Concern:

This is to confirm that Dr. Rahman successfully defended his dissertation proposal entitled *Effect of Simultaneous Supplementation of Vitamin A and Zinc on the Bioavailability of Vitamin A* for the Doctor of Public Health degree in the Department of International Health in February 1997.

If additional information is necessary, please feel free to contact me.

Sincerely,

Jose O. Alvarez

Jose O. Alvarez, PhD
Professor and Chairman

sp

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