Attachment I. (FACE SHEET)

Library

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date Dec 10, 1996

(6a)

Principal Investigator George Fuchs, MD Sastia Osendarp

Trainee Investigator (if any) _____

Application No. 96-024

Supporting Agency (if Non-ICDDR,B) US-AID

Title of Study Effect of zinc supplementation during pregnancy and infancy on the immune response to vaccines in Bangladeshi 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 N.A
   (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 N.A
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
   - √ 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.

S Osendarp — S Osendarp
Principal Investigator

Trainee

ENTERED 2 1 JUN 1996

CHECK-LIST FOR SUBMISSION OF PROPOSALS TO THE RESEARCH REVIEW COMMITTEE (RRC)

[Please tick (✓) the appropriate box]

1. Has the proposal been reviewed, discussed and cleared at the Division level ?

   Yes [✓]

   No [ ]

   If 'No', please clarify the reasons: _____

2. Has the proposal been peer reviewed externally ?

   Yes [✓]

   No [ ]

   If the answer is 'NO', please explain the reasons: _____

3. Has the proposal scope to address gender issues ?

   Yes [ ]

   No [✓]

   If the answer is 'YES', have these been adequately incorporated in the proposal. Please indicate: _____

4. Has a funding source been identified ?

   Yes [✓]

   No [ ]

   If the answer is 'YES', please indicate the name of the donor: US-AID

5. Whether the proposal is a collaborative one ?

Yes [√]

No [ ]

If the answer is 'YES', the type of collaboration, name and address of the institution and name of the collaborating investigator be indicated:

Dr. Manthu Santhosham, Dr Robert Black, Dr Mohamed Hasher
Johns Hopkins University, Department of International Heal
School of Hygiene & Public Health - 615 North Wolfe Street
Baltimore MD 21205-2179
USA

6. Has the budget been cleared by Finance Division ?

Yes [√]

No [ ]

If the answer is 'NO', reasons thereof be indicated: _____

_____

_____

7. Does the study involve any procedure employing hazardous materials, or equipments ?

Yes [ ]

No [√]

If 'YES', fill the necessary form.

Dec 10, 1996
Date

Signature of the
Principal Investigator

1. Title: Effect of Zinc supplementation during pregnancy and infancy on the immune responses to vaccines in Bangladeshi children.

2. Principal Investigators: Saskia J.M. Osendarp[1]
George J. Fuchs, MD[1]

Co-Principal Investigator: Mathuram Santosham MD, MPH[2]
Robert E. Black MD[2]

Co-Investigators: Mohamed Hashem, MD[2]

[1]International Center for Diarrheal Disease Research, Dhaka, Bangladesh

[2]Johns Hopkins University, Department of International Health, School of Hygiene and Public Health

3. Starting Date: January 1997

4. Completion Date: December 1998

5. Total Direct Cost: $ 99,891

Source of Funding: USAID

6. Signature of the Division Director: _____

7. Abstract Summary:

It has been demonstrated that infants in developing countries, have lower immune responses to vaccines such as rotavirus vaccine and oral polio vaccine, compared to the responses of infants from developed countries. One explanation may be malnutrition. Several micronutrients are known to affect the immune system in characteristic ways; of which vitamin A and zinc exert the most significant effects. In the proposed study we would like to evaluate the humoral and cell mediated immune responses of infants supplemented with zinc from birth until six months of age and of children born to mothers supplemented with zinc during the last five months of their pregnancy.

Pregnant women at 12-16 weeks of pregnancy were randomized to one of 2 groups (A or B). Group A women has been supplemented daily with tablets of 30 mg (2 x RDA) of zinc (as acetate) until delivery and group B women received a placebo. Infants less than four weeks of age, born to a separate cohort of mothers who were not involved in the pregnancy zinc supplementation study, will be randomized to one of two groups (C or D). Group C infants will be supplemented daily with 10 mg of elemental zinc until six months of age and group D infants will receive a placebo. Infants born to women who were supplemented with zinc or placebo (groups A & B), and groups (C & D) infants will receive the routine childhood immunization as recommended by the Expanded Program on Immunization (EPI). In addition they will receive three doses of the combined diphtheria, tetanus toxoid, and pertussis (DTP)-Haemophilus Influenza type b (Hib) vaccine (Diphtheria CRM197 Protein Conjugate) TETRAMUNE (Lederle-Praxis), at four weeks intervals beginning at six weeks of age. Blood samples will be obtained at 6 and 24 weeks of age. The sera obtained at six and 24 weeks will be assayed for antibodies to DTP-Hib vaccine serotypes, and to all three serotypes of the trivalent oral polio vaccine (TOPV). Tuberculin testing will be performed with purified protein derivative (PPD) at 24 weeks of age as a measure for cell mediated immune response to BCG.

A. INTRODUCTION

1. Objectives

1. To evaluate the effect of zinc supplementation of mothers during pregnancy on infants immune responses to the Haemophilus Influenza type b, oral polio, and BCG vaccines.

2. To evaluate the effect of zinc supplementation of infants beginning at 4 weeks of age on their immune responses to the Haemophilus Influenza type b, oral polio, and BCG vaccines.

2. Background:

The responses to childhood vaccinations especially to certain antigens are known to vary in different populations. In many developing countries, the immunogenicity of three doses of live attenuated, trivalent oral polio vaccine (TOPV) is lower than industrialized countries. Whereas, rates of sero-conversion following the administration of TOPV approach 100% in industrialized countries, only 73% (range, 36%-99%) and 70% (range, 40%-99%) of children in developing countries have detectable antibody level to poliovirus type 1 and 3 respectively after receiving three doses of TOPV[1]. Recently, a study conducted in Bangladesh[2] strongly suggests that concurrent diarrheal illness contributes to the less than optimal humoral immune response to TOPV. Similarly, the immune responses to Haemophilus influenzae type b (Hib) vaccines of some populations such as, certain American Indian populations are known to be lower than the general U.S. population[3], and the immune responses to rotavirus vaccines are known to be lower in infants from developing countries compared to infants from developed countries.[4,5]

There may be various reasons for the depressed immune responses to vaccines among infants in developing countries. One explanation may be malnutrition[6,7]. Undernourished infants are known to have depressed immune responses. Several micronutrients are known to affect the immune system in characteristic ways, vitamin A and zinc exert the most significant effects.

The zinc atom has a unique combination of physical and chemical properties that render it useful in biological systems[8]. Zinc appears to have distinct roles in the mammalian metabolism and being a component of zinc-containing metaloenzymes, zinc is important for nearly all aspects of cellular metabolism[9].

Zinc and immunity: The following evidence suggests that zinc plays an important role in immunity, zinc deficiency: (i) impairs cell regeneration (due to its role in metalloenzymes, and other metabolic pathways); (ii) impairs immunocompetence with reduced cell mediated immune response, decreased T-lymphocytes, and abnormal T- Helper and/or suppressor cell functions[10], (iii) impairs macrophage function thus depressing the delayed hypersensitivity reaction[11]; (iv) reduces the natural killer cells and antibody dependent cytotoxicity[12], (v) impairs epithelial barrier functions, (both skin lesions and mucosal defects are common findings); and (vi) impairs linear growth. Zinc supplementation has been shown to improve growth, indeed poor growth and diarrhea are cardinal features of acrodermatitis enterohepatica, the phenotypic expression of which is attributable to severe zinc deficiency[13].

Several studies have demonstrated a reduction in infant morbidity due to infections in response to dietary zinc supplementation. Schlesinger et al.[14] observed that zinc fortified formula improved linear growth and immune response in 7 month old Chilean infants. In a study among preschool children in rural Mexico, supplementation with 20 mg zinc/day for 12 months reduced the number of episodes of diarrhea and respiratory diseases[15]. Recently, Sazawal et al., demonstrated that dietary supplements of zinc along with selected vitamins, resulted in clinically important reductions in the duration and severity of diarrhea among preschool-age children[16]. In rural Bangladesh, normal fullterm infants showed significantly better growth and less diarrheal morbidity after zinc supplementation[17].

Furthermore, there are obvious interactions between zinc and vitamin A deficiency[18]. For instance, zinc deficiency decreases the rate of production of retinol-binding protein. It may also hinder absorption of vitamin A.

3. Rationale

In the proposed study we would like to evaluate the effect of zinc supplementation of mothers during the last five months of pregnancy and the effect of zinc supplementation during the first six months of life on the humoral seroresponses of infants, using Hib and TOPV vaccines as markers. The cell mediated immune response will be evaluated using PPD skin reaction to BCG as a marker.

B. SPECIFIC AIMS

Hypothesis

Zinc supplementation of infants beginning at 4 weeks of age, or zinc supplementation during the last five months of pregnancy will enhance infants immune responses to Haemophilus influenzae type B, oral polio, and BCG vaccines.

C. METHODS OF PROCEDURE

Study design

A prospective, four cell, double-blind design is proposed. Women (between 12 and 16 weeks gestation) and infants (less than 4 weeks old) will be enrolled and assigned to one of two groups, zinc or placebo. Pregnant women were randomized to groups (A or B). Group A women were supplemented daily with tablets of 30 mg of zinc (2 x RDA), as acetate, until delivery, and group B women received a placebo. Infants born to mothers in group A and B will be prospectively followed without additional zinc supplementation and their responses to various immunization will be evaluated. A separate cohort of infants will be enrolled at birth and randomized to receive 10 mg (2 x RDA) of zinc or a placebo (group C and group D) beginning at 4 weeks of age. Infants in groups C and D will also have their responses to various immunization evaluated.

Inclusion Criteria

Pregnant Women

All pregnant women, living in the study area (see section V) in Dhaka, Bangladesh were eligible for the study provided they met the following criteria:

1. Were identified by the surveillance system of the International Center for Diarrheal Diseases, Bangladesh (ICDDR,B)
2. Met the criteria of residence
3. Were 12-16 weeks of gestation
4. Agreed to participate
5. Remained at/near Dhaka for the delivery
6. Gave a written informed consent.

Infants

Infants, living in the study area will be eligible for the study provided they meet the following criteria:

1. Age less than 4 weeks
2. In good general health without known underlying illness.
3. Parents give written informed consent.

Exclusion Criteria

Pregnant women with abnormal pregnancies or other significant illness were excluded from the study.

Infants will be excluded if they have one or more of the following:

1. Known or suspected hypersensitivity to one of the vaccine antigens or components
2. Presence of immune system disorder
3. Recipients of immunosuppressive therapy, blood products, corticosteroids or immunoglobulin
4. History of prior vaccination with OPV and/or DPT vaccine
5. Previous invasive Haemophilus Influenza disease (in the first 15 days of age)
6. Acute febrile illness (temperature $>38.1°C$) within 72 hours.
7. Major congenital malformation or serious chronic illness.

8. Subject involved in any other vaccine trial.

Randomization

This will be a four cell double blind controlled safety and immunogenicity trial (table 1). Pregnant women at 12-16 weeks of pregnancy, who meet the inclusion criteria, were randomized to one of 2 groups (A or B). Group A women was supplemented daily with tablets of 30 mg of zinc (zinc acetate) until delivery. The amount of 30 mg of zinc/day is twice the RDA for zinc during pregnancy and there is no evidence of adverse effects for either the mother or fetus[19]. Previous investigators have used daily oral dose of up to 30mg with no adverse effects[20]. Zinc acetate has been used because salts of zinc acetate generally result in less nausea and gastrointestinal irritation than other zinc salts[21]. Group B women received a placebo, containing an inert substance (sucrose, maize starch, cellulose). A separate cohort of infants less than four weeks of age, whose mothers did not participate in the zinc trial and who meet the inclusion criteria, will be randomized to one of 2 groups (C or D). Group C infants will be supplemented daily with 10 mg of elemental zinc which is twice the RDA, until 6 months of age. Group D infants will receive a placebo. The zinc supplement and the placebo will be indistinguishable in appearance and taste.

Table 1

STUDY SCHEMA

Group	Pregnancy*	Infancy#
A	Zinc	-
B	Placebo	-
C	-	Zinc
D	-	Placebo

*From 12-16 weeks of gestation until delivery

#From 4 weeks of age until 6 months of age

Data Collection

Women have been recruited through the identification system (described in section V) before 16 weeks of pregnancy and written informed consent has been obtained. At 16 weeks gestation, a standard questionnaire has been administered to the women. Women were randomly assigned to one of the two treatment groups (A or B), and supplementation begun at 16 weeks gestation until delivery. Health workers delivered a one week supply of the zinc supplement or placebo tablets at the houses weekly. They instructed pregnant women to consume the tablets preferably together with a meal or a drink and not together with other vitamins/mineral. Compliance has been checked by counting the remaining tablets at the following visit.

The zinc and placebo preparations for infants will look identical and will be dispensed in bottles in the liquid form. A one week supply of zinc or placebo will be delivered to the home once a week. Each dose will be dispensed in a separate vial. Compliance will be checked by measuring the remaining amount of liquid in the bottles.

Any vomiting or diarrhea that occurs in the pregnant women or infants will be recorded. Anthropometrics (weight, length, midupper arm circumference, head circumference, chestcircumference) will be measured at monthly intervals.

Immunization and Serology

Infants born to women who were supplemented with zinc or placebo (groups A & B), and supplemented infants groups (C & D) will receive three doses of the combined diphtheria, tetanus toxoid and pertussis (DTP)- Haemophilus Influenza type b (Hib) vaccine (Diphtheria CRM, 97 protein conjugate) Tetramune, (Lederle- Praxis) at 6,10, and 14 weeks of age, at the same time as trivalent oral polio vaccine (TOPV). The BCG vaccine will be administered at birth.

Table 2						
Schedule for Immunization and Testing						
Age (weeks)	Birth	4 weeks	6 weeks	10 weeks	14 weeks	24 weeks
BCG	X					
TOPV			X	X	X	
DPT-Hib			X	X	X	
Blood testing*		X				X
Skin test						X

TOPV = Trivalent Oral polio vaccine, DPT-Hib = Diphtheria, pertussis and tetanus-Haemophilus Influenza type b vaccine, BCG = Bacillus Calmette-Guerin, Skin test = Mantoux test 5 TU-PPD.

*At 4 weeks of age, a base-line blood specimen for plasma zinc and pre-vaccination DPT-Hib & polio serologies and at 24 weeks of age, post-vaccination DPT-Hib, polio serologies and plasma zinc.

Blood specimens (2.5ml of whole venous blood) will be obtained from all infants, at 4 weeks (baseline), and 24 weeks (10 weeks after third doses of pneumococcal and polio vaccines) The pre-vaccination specimen will be obtained at 4 weeks instead of 6 weeks of age since a blood sample is obtained at 4 weeks of age as part of the ongoing study. Sera will be assayed for antibodies to H. influenza b polysaccharide and TOPV (all three serotypes).

Cell-mediated immune response of each infant will be determined by tuberculin testing with purified protein derivative (PPD) at 6 months of age. Tuberculin testing and reading will be performed by trained health workers. They will apply tuberculin solution (PPD) (0.1ml) to the volar surface of the forearm using an Omega glass PPD syringe with platinum needles. After 72 hours the size will be read in millimeters as the transverse diameter of induration developed. An induration > 2mm will be considered positive. Health workers will be extensively trained and standardized in the administration and interpretation of PPD skin testing.

Safety Monitoring

Post-vaccination safety surveillance will be carried out from day 1 through day 5 post-vaccination. The following data will be collected and recorded daily: (i) evening rectal temperature; (ii) local swelling and redness; (iii) generalized rash; (iv) seizures; (v) any unexpected reactions.

Sample Size:

The sample size (Table 3) was calculated to detect differences between treatment groups with 80% power and a type 1 error of 5% (i.e. 95% significance). In previous immunogenicity trials in the UK. With DTP-Hib vaccine the sero-response to PRP was 82% (22). There are no prior data available using H.Influenza type B vaccines in developing countries. We have assumed that approximately 30% of Bangladeshi infants (20% less than the general U.S. population) will have an antibody level of ≥ 0.15 μg/ml to PRP if they are given three doses of the vaccine. We have also assumed that with zinc supplementation, their immune responses will be the same as the U.S. infants.

Similarly, after three doses of TOPV 73% of children in developing countries have detectable antibody level to poliovirus type[1,2,] thus we have assumed that with zinc supplementation, their immune responses will be the same as industrialized countries, where rates of conversion following the administration of three doses of TOPV approaches 100%.

In developing countries, the percentage of anergic children to tuberculin testing approaches 70%[23]. We assume that with zinc supplementation the cellular immune response will improve and anergic children will decrease from 70% to 50%.

In order to allow 15% for attrition, we anticipate recruiting approximately 122 infants per group during the study period.

Table 3				
ASSUMPTIONS FOR SAMPLE SIZE CALCULATIONS				
Outcome Variable	Control Group	Expected Outcome	No. required in each cell*	No. to be enrolled in each group†
% with antibody level above 0.15 μg/ml to PRP	309%	98%	103	113
% with detectable antibody to poliovirus type 1	70%	95%	38	44
% anergic for tuberculin testing	70%	50%	103	119

* 80% power (Type 2 error of 20%) and Type 1 error of 5%
† Assuming that 15% will not complete the follow up

Description of the site

The study population will be from households of five thanas of the former ICDDR,B Urban Surveillance System (USS) area in Dhaka. These households form a representative sample of Dhaka's slum population. The Urban Surveillance System was operated by Urban MCH-FP Extension Project (UEP) of the International center of Diarrheal Disease Research Bangladesh (ICDDR,B). The target population consists of five thanas (Mohammadpur, Lalbagh, Kotwali, Sutrapur and Demra) of Dhaka city which contains about 800 slums with a total population in these five thanas of approximately 400,000.

An identification system to identify pregnant women was established in the former USS areas for a recent study on the determinants of low birth weight, gestational age, and perinatal morbidity. In this system, a community worker is assigned by the UEP in selected clusters. In these clusters, the community worker identifies all pregnant women in their neighborhood. In the recent study of low birth weight in this population, a mean number of 150 pregnancies were identified monthly.

ETHICAL IMPLICATIONS, INFORMED CONSENT AND CONFIDENTIALITY

Signed informed consent will be obtained from each participating woman after through explanation of the purpose of the study, requirements of participation, risks and benefits to the participant. Another person present (relative/neighbor) will be acting as a witness.

Women will be supplemented with 30 mg zinc/day. This amount of zinc is based on twice the RDA and there is no evidence of any teratogenic effects for either the mother or fetus, due to this amount of zinc[19]. Previous investigators have used daily oral zinc doses of up to 30 mg with no side effects[20]. Zinc acetate will be used because, according to the Consensus statement on zinc nutrition as prepared by UNICEF[21], salts of zinc acetate in general results in less nausea and gastro-intestinal irritation then other salts.

All women and infants requiring medical treatment will be referred to the nearest health care facility with whom collaborative arrangements for such referral have been made during the previous studies. All mothers and infants who are referred will be accompanied by an interviewer so that they are received and cared for in a timely manner. Arrangements will be made for treatment at the nearest hospital whenever necessary. Women defined anemic (Hemoglobin < 9 g/dL) will supplied with weekly irontablets.

Some of the information collected might be considered sensitive in nature. Therefore, to protect each participant's privacy, interviewers will conduct all interviews in private, exceptions only being made if the respondent insists on the presence of others. Each questionnaire and data form will be given an identification code. The linkage between the code and the study participant's name will be kept in a locked register at UEP office. All staff and interviewers will be counseled about the sensitive nature of some of the information collected, and on the need for strict confidentiality of the records.

D. SIGNIFICANCE

There is no published data from developing countries regarding the immune responses of infants to the combined DTP-H. Influenza type B vaccines. If this vaccine is to be used routinely in developing countries it is important to know if the immune responses in these countries are similar to infants in developed countries. It is well known that the immune responses of infants in developing countries to certain antigens, e.g. polio is lower. If the antibody responses are found to be lower, it is important to know if the impaired immune responses can be overcome with micronutrient supplementation.

E. CAPACITY BUILDING AND COLLABORATIVE ARRANGEMENTS

This project will provide an opportunity for investigators at the ICDDR,B to work closely with JHU investigators. The H.Influenza type b antibodies assays are currently not performed at the ICDDR,B. During the course of the study, we will attempt to provide training for an individual from ICDDR,B to obtain the necessary expertise for setting up such assays. In addition Dr. Santosham and Dr. Black have extensive experience in conducting field studies, they will assist the ICDDR,B investigators in all aspects of the study and in development of additional protocols related to this topic.

ZINC AND IMMUNE RESPONSE

LOCAL SALARY:

POSITION	GRADE	#	RATE	MONTH	SUB-TOTAL	TOTAL
Research Investigator	NOC	0.5	$ 1200	12	7200	
Study Physician	NOA	1	750	1	750	
Senior Interviewer	GS4	1	265	1	265	
Nurses	GS5	3	374	1	1122	
Extra nurses	GS5	3	374	7	7845	
Field Research Officer	GS5	3	374	4	4488	
Data Entry Assistant	GS3	1.3	239	7	2175	
Statistician	GS6	0.5	482	12	2892	
Intrviewer (FRA)	GS3	6	239	1	1434	
Extra Interviewer (FRA)	CS3	5	239	7	8365	
Female Helper	CSA	6	31	5	930	
Extra Female Helper	CSA	3	31	3	279	37,745
TOTAL LOC SALARY						

INTERNATIONAL SALARY

POSITION	GRADE	#	RATE	MONTH	SUB-TOTAL	TOTAL
Research Investigator		1	1500	5	7500	7500

LOCAL TRAVEL

	RATE	MONTH	SUB-TOTAL	TOTAL
Int Fellow/RI	54	1	54	
Field Research Officer	18	1	18	
Nurses/Interviewers	350	7	2450	
Travel reimbursement	345	7	2415	
Female Helper	46	1	46	4983

INTERNATIONAL TRAVEL

	#	RATE	DAYS	SUB-TOTAL	TOTAL
Per diem	1	185	10	1850	
Air fare (to present study results-	1	2500	0	2500	4,350

SUPPLIES AND MATERIALS

	RATE	#	SUB-TOTAL	TOTAL
Syringes & Needles	0.25	2000	500	
PPD	300		300	
Zinc and Placebo suppl	600		600	
Miscellaneous	1595		1595	
Office supplies	800		800	3,795

E. Hai
10/12/96

TOTAL SUPPLIES OTHER				
DIRECT COSTS				
MISCELLANEOUS:				
Office Rental (Mdpur & Lalbagh)	750	1	750	
Printing cost(questionnaires)	1000		1000	
Fax	200		200	1,950
TOTAL OTHER DIRECT				
INTERDEPARTMENTAL	RATE	#	SUB-TOTAL	TOTAL
Laboratory				
Blood Zinc	$3	$160	$1,380	
Polio serologies	$15	$920	$13,800	
Xerox	750	750	$750	15,930
TOTAL DIRECT COSTS				76253
OVERHEAD (31%)				23638
TOTAL COSTS				99891

S. Hon
10/12/96

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ANNEX

TIME FRAME OF THE STUDY

January 1997	- Procuring supplies recruiting and training study personnel
February 1977 - October 1997	- Enrollment, data collection, and laboratory studies.
November 1997- December 1997	- Completion of collection and processing serum samples
January 1998- December 1998	- Laboratory studies, data analysis and publication.

WRITTEN CONSENT FORM FOR PARTICIPATION IN THE STUDY:

Effect of zinc supplementation during pregnancy and infancy on the immune response to vaccines in Bangladeshi children

Clinical Science Division of ICDDR,B (Cholera Hospital) is conducting a special study in newborns. The purpose of this study is to find out whether a daily zinc supplement or a placebo will help to improve the health of your baby. We want to do this by asking you to give your baby a special drink every day until your baby is 6 months old. You will be able to participate in this study if your baby is younger than 4 weeks of age.

If you agree to participate in this study, I will ask you to come to Cholera Hospital two times together with your baby. The nurse will take a very small amount (2.5 ml) of blood from your baby's vein. Then we will visit your baby at home and inject him/her with two vaccines. We will check your baby in the days following the vaccination to see if the baby is doing all-right. We will visit you at home every week to give you a weekly supply of the supplement drink and we will ask you to give the drink to your baby every day. We will ask some questions on your baby's health during these visits. Every month we will measure the weight, length and arm size of your infant and ask you some questions about feeding practices.

There is no possibility of harm coming to your newborn as a result of participating in this study, rather the measurements we perform will help us to assess how healthy your baby is. The zinc drink will not harm your baby's health and the small amount of blood we will take from your baby will not cause any harm. The vaccines we will provide will prevent your baby from getting polio, diphtheria, tetanus, pertussis and Haemophilus influenza. We will give you some money for your travel to the hospital. If your baby is ill we will help to seek treatment at the nearest hospital.

We know that some questions will be personal in nature. Therefore, we will keep all of your answers secret. If there are any questions which you do not want to answer, that is okay. If you don't want us to take blood from your baby, you will not be able to participate but you will always be able to use the services at the Cholera Hospital as usual. Also, if you decide not to participate in this study, you will always be able to use the services at the Cholera Hospital as usual. If you have any questions about the study, you can contact the principal investigator Saskia Osendarp, or the ICDDR,B, at the telephone number 871751-8, extension 2519.

Do you agree to participate in this study?

_____ _____

Signature of participant Date of consent

_____ _____

Signature of Interviewer Signature of Principal Investigator

WRITTEN CONSENT FORM FOR MOTHERS ALREADY PARTICIPATING IN THE ZINC IN PREGNANCY STUDY FOR PARTICIPATION IN THE STUDY:

Effect of zinc supplementation during pregnancy and infancy on the immune response to vaccines in Bangladeshi children

You are participating in a study on zinc in pregnancy conducted by the Urban MCH-FP Extension Project of ICDDR,B (Cholera Hospital). In this study we have asked you to take a daily zinc supplement or placebo during your pregnancy and we have visited you weekly to check whether you and your baby were doing fine. Now, the Clinical Sciences Division of ICDDR,B wants to ask you to participate in an additional study in which we are going to vaccinate your baby with two vaccines and checking the health of your baby after vaccination. You will be able to participate in this study if your infant is younger than 8 weeks of age.

If you agree to participate in this extra study, I will ask you to come to Cholera Hospital as usual together with your baby. The nurse will take a very small amount (2.5 ml) of blood from your baby's vein. Then we will visit your baby at home and inject him/her with two vaccines. We will check your baby in the days following the vaccination to see if the baby is doing all-right. We will visit you at home every week as we did before, and all other procedures or questions will not be different from the procedures we are already following.

There is no possibility of harm coming to your newborn as a result of participating in this extra study. The small amount of blood we will take from your baby will not cause any harm. The vaccines we will provide will prevent your baby from getting polio, diphtheria, tetanus, pertussis and Haemophilus influenza. As usual, we will give you some money for your travel to the hospital. If your baby is ill we will help to seek treatment at the nearest hospital.

We know that some questions will be personal in nature. Therefore, we will keep all of your answers secret. If there are any questions which you do not want to answer, that is okay. If you don't want us to take blood from your baby, you will not be able to participate in this extra study but you will still be able to continue in the current study. If you decide not to participate anymore in any of the studies, you will always be able to use the services at the Cholera Hospital as usual. If you have any questions about the extra study, you can contact the principal investigator Saskia Osendarp, or the ICDDR,B, at the telephone number 871751-8, extension 2519.

Do you agree to participate in this extra immunization study?

_____ _____

Signature of participant Date of consent

_____ _____

Signature of Interviewer Signature of Principal Investigator

Summary of protocol and progress of the ongoing study: Effect of zinc supplementation during pregnancy on infant birth weight, growth, morbidity and response to BCG.

Investigators: Saskia Osendarp, Dr. G. Fuchs, Dr. A.H. Baqui, Dr. Shams El Arifeen, Mr. M.A. Wahed, Dr. J.M.A. van Raaij
International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B); Department of Human Nutrition, Wageningen Agricultural University, The Netherlands

Rationale

Most research and intervention efforts are concentrated on the prominent micronutrient deficiencies of vitamin A, iron and iodine. Deficiencies of other nutrients, however, may be equally prevalent and harmful and are now in the focus of attention with the trace element zinc as one of the most important ones. Because of its fundamental role in gene replication, nucleic acid metabolism and mediator of growth hormone action, zinc deficiency has been implicated in low birth weight, pregnancy complications, reduced immunocompetence and growth failure in infants and children.

Little is known about the effects of zinc deficiency during pregnancy on infant birth weight, growth, morbidity, and immunity. Although observational studies suggest a positive association between zinc status during pregnancy and pregnancy outcome, so far, no controlled supplementation trials have been performed in developing countries, where zinc intakes are habitually very low and where zinc deficiency is likely to be a problem. This study is examining the effects of zinc supplementation during pregnancy on pregnancy outcome in a very poor urban community where LBW is highly prevalent.

Aims

This study aims to determine the importance of zinc supplementation during the last six months of pregnancy on infant birth weight, health and growth during the first six months of life. The study will be performed in the slums of Dhaka.

The specific objectives are:

1. Determine the effect of zinc supplementation during the last six months of pregnancy on infant birth weight.

2. Determine the effect of zinc supplementation during pregnancy on infant growth during the first six months of life.

3. Determine the effect of zinc supplementation during pregnancy on infant cell-mediated immunity (CMI) as measured by response to BCG vaccination during the first six months of life.

4. Determine the effect of zinc supplementation during pregnancy on infant morbidity from infectious diseases during the first six months of life.

5. Determine the effect of zinc supplementation during the last six months of pregnancy on maternal and infant zinc status.

Study design and methods

The study follows a prospective double-blind intervention design. Women from selected clusters of Dhaka city slums, were enrolled between 12-16 weeks gestation, stratified by parity and randomly assigned to two treatment groups, receiving either 30 mg elemental zinc/day (as zinc acetate) or a placebo. Supplementation continues until delivery and compliance is being assessed weekly. The women are prospectively followed from 4 months gestation until birth and their newborns from birth until 6 months postpartum.

Serum zinc and Haemoglobin levels are estimated at baseline and again at 7 months gestation during hospital visits, while anthropometry and dietary intake of the women is assessed monthly and morbidity weekly at home.

Gestational age, birth weight, anthropometric measurements and BCG vaccination are carried out within 72 hours after birth. The infants are followed up weekly for morbidity assessment while infant serum zinc will be determined from a venous blood sample during hospital visits at 1 and 6 months postpartum. Immune response for BCG will be tested by skin reaction on PPD (TBC microbacteria), when the infant is 6 months old.

Current status

After a delay in activities in the month of February 1996 due to external causes (political unrest, elections) data collection actually started from March 1, 1996. Enrollment of women was slowed down in March because of political actions but as soon as the situation went back to normal (by April 1, 1996) we were able to catch up and completed enrollment by the end of June 1996. By the end of June, a total of 559 pregnant women had entered the study. Data collection is expected to be completed by the end of June 1997.

To date, 401 women delivered, including 378 live births, 12 still births and 11 early neonatal deaths (within 7 days after birth). So far, the birth notification system has been very efficient: 371 infants were visited within 72 hours. For 114 (31%) of these infants birth weights were measured on the day of delivery. In 6 cases birth weight assessments exceeded 72 hours due to late notification (n=3) and delivering outside Dhaka (n=3). Forty three women remain pregnant in the follow-up and are expected to deliver in the next month. By the end of December 1996, the first infant will reach the age of six months and will be released from the study.

Significance

As far as we are aware, this study would be one of the first zinc supplementation trials among pregnant women in a very poor urban community with a high prevalence of low birth weight. Information obtained from the study will increase the understanding of the importance of zinc supplementation during pregnancy on infant birth weight, growth, immune response and morbidity in urban populations in developing countries.

Information derived from this study will be essential for planning and implementing health intervention programs aimed at reducing the incidence of low birth weight, improving maternal health, and improving infant growth and immunity. The information can also be used to make existing programs more cost-efficient.

December 9, 1996

Study ID: __/__/__/__/__/__

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH (ICDDR,B)

CLINICAL SCIENCE DIVISION

IMMUNIZATION INTERVIEWS

ROUND:

[COMPLETE FOLLOWING FACE SHEET INFORMATION PRIOR TO INTERVIEW]

Head of Household's Name: _____

Husband's Name: _____

Mother's Name: _____

Child's Name: _____

Address: _____

_____

[THIS FACE SHEET TO BE FILED SEPARATELY BY THE PRINCIPAL INVESTIGATOR]

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH (ICDDR,B)

CLINICAL SCIENCE DIVISION

IMMUNIZATION INTERVIEWS

ROUND:

[COMPLETE FOLLOWING FACE SHEET INFORMATION PRIOR TO INTERVIEW]

Stratum No.:___ Cluster No.:___ Struc. No.:___ HH No. ___

Date of Visit: (1):___/___/___ (2):___/___/___ Time of Visit: _____ a.m./p.m.

(3):___/___/___ (4):___/___/___

(5)___/___/___

Interviewer:_____ Interviewer Code:_____

ELIGIBILITY/FOLLOW-UP STATUS

___ Woman has left the cluster — DETERMINE WHERE THE WOMAN WENT

___ IF MOVED OUT OF DHAKA, SHE IS NOT ELIGIBLE TO CONTINUE THE STUDY

___ IF MOVED WITHIN DHAKA, GET INFORMATION ON WHERE MOVED TO AND DISCUSS FOLLOW-UP PLANS WITH FRO

___ Woman temporarily absent — DETERMINE WHEN WOMAN IS EXPECTED TO RETURN AND RECORD DATE ON FOLLOW-UP REGISTER

SECTION 0: IMMUNIZATION (DAY O)

IM01a. Trivalent oral polio vaccine has been given to infant:

Yes 1
No . . . . . 0 [GO TO QIM01c]
Yes, through EPI 2

IM01b. Date and time of oral polio vaccination; [GO TO Q IM02a]:

Date: ____/____/____/ Time:_____ a.m./p.m.

IM01c. Reason for not giving oral polio vaccine:

(1):_____ CODE:_____

(2):_____ CODE:_____

(3):_____ CODE:_____

IM02a. DPT-HiB vaccine has been given to infant:

Yes 1
No . . . . . 0 [GO TO Q IM02C]
Yes, DPT through EPI 2

IM02b. Date and time of DPT-HiB vaccination; [END OF INTERVIEW]:

Date: ____/____/____/ Time:_____ a.m./p.m.

IM02c. Reason for not giving DPT-HiB vaccine:

(1):_____ CODE:_____

(2):_____ CODE:_____

(3):_____ CODE:_____

END OF INTERVIEW DAY 0

SECTION 1: DAY 1 OF FOLLOW-UP:

IM11 How is your baby (body/health) now?
- Ill . . . 1
- Some what okay . . . 2
- Well . . . 3

IM12 Did your baby cry more than usual since yesterday?
- Yes . . . 1
- No . . . 0
- Don't know . . . 9

IM13 Did your baby eat usual, less than usual or more than usual since yesterday?
- Less than usual . . . 1
- More than usual . . . 2
- Normal, as usual . . . 0
- Don't know . . . 9

IM14 Has your baby been more, less or normal active since yesterday?
- Less than usual . . . 1
- More than usual . . . 2
- Normal, as usual . . . 0
- Don't know . . . 9

[EXAMINATION OF INFANT BY STUDY NURSE]:

IM15 Temperature (Rectal) . . . |__|__|.|__| °C

IM16 Infant has local swelling and/or redness at injection site:
- Yes . . . 1
- No . . . 0

IM17 Infant has rash all over its body:
- Yes . . . 1
- No . . . 0

IM18 Infant has\ has had seizure:
- Yes . . . 1
- No . . . 0

IM19 Condition of infant:
- Restless, Irritable . . . 1
- Lethargic . . . 2
- Well Alert . . . 0

IM110 Other comments/observations:

	__ __ __ __ __ __

END OF INTERVIEW DAY 1

SECTION 2: DAY 2 OF FOLLOW-UP:

IM21 How is your baby (body/health) now?
- Ill . . . 1
- Some what okay . . . 2
- Well . . . 3

IM22 Did your baby cry more than usual since yesterday?
- Yes . . . 1
- No . . . 0
- Don't know . . . 9

IM23 Did your baby eat usual, less than usual or more than usual since yesterday?
- Less than usual . . . 1
- More than usual . . . 2
- Normal, as usual . . . 0
- Don't know . . . 9

IM24 Has your baby been more, less or normal active since yesterday?
- Less than usual . . . 1
- More than usual . . . 2
- Normal, as usual . . . 0
- Don't know . . . 9

[EXAMINATION OF INFANT BY STUDY NURSE]:

IM25 Temperature (Rectal) . . . |__|__|·|__| °C

IM26 Infant has local swelling and/or redness at injection site:
- Yes . . . 1
- No . . . 0

IM27 Infant has rash all over its body:
- Yes . . . 1
- No . . . 0

IM28 Infant has\ has had seizure:
- Yes . . . 1
- No . . . 0

IM29 Condition of infant:
- Restless, Irritable . . . 1
- Lethargic . . . 2
- Well Alert . . . 0

IM210 Other comments/observations:

	__ __ __ __ __ __

END OF INTERVIEW DAY 2

ECTION 3: DAY 3 OF FOLLOW-UP:

M31 How is your baby (body/health) now?
- Ill 1
- Some what okay 2
- Well 3

M32 Did your baby cry more than usual since yesterday?
- Yes 1
- No 0
- Don't know 9

M33 Did your baby eat usual, less than usual or more than usual since yesterday?
- Less than usual 1
- More than usual 2
- Normal, as usual 0
- Don't know 9

M34 Has your baby been more, less or normal active since yesterday?
- Less than usual 1
- More than usual 2
- Normal, as usual 0
- Don't know 9

EXAMINATION OF INFANT BY STUDY NURSE]:

M35 Temperature (Rectal) . . . . . |__|__|.|__| °C

M36 Infant has local swelling and/or redness at injection site:
- Yes 1
- No 0

M37 Infant has rash all over its body:
- Yes 1
- No 0

M38 Infant has\ has had seizure:
- Yes 1
- No 0

M39 Condition of infant:
- Restless, Irritable 1
- Lethargic 2
- Well Alert 0

M310 Other comments/observations:

END OF INTERVIEW DAY 3

SECTION 4: DAY 4 OF FOLLOW-UP:

IM41 How is your baby (body/health) now?
- Ill . . . 1
- Some what okay . . . 2
- Well . . . 3

IM42 Did your baby cry more than usual since yesterday?
- Yes . . . 1
- No . . . 0
- Don't know . . . 9

IM43 Did your baby eat usual, less than usual or more than usual since yesterday?
- Less than usual . . . 1
- More than usual . . . 2
- Normal, as usual . . . 0
- Don't know . . . 9

IM44 Has your baby been more, less or normal active since yesterday?
- Less than usual . . . 1
- More than usual . . . 2
- Normal, as usual . . . 0
- Don't know . . . 9

[EXAMINATION OF INFANT BY STUDY NURSE]:

IM45 Temperature (Rectal) . . . |__|__|.|__| °C

IM46 Infant has local swelling and/or redness at injection site:
- Yes . . . 1
- No . . . 0

IM47 Infant has rash all over its body:
- Yes . . . 1
- No . . . 0

IM48 Infant has\ has had seizure:
- Yes . . . 1
- No . . . 0

IM49 Condition of infant:
- Restless, Irritable . . . 1
- Lethargic . . . 2
- Well Alert . . . 0

IM410 Other comments/observations:

	__ __ __ __ __ __

END OF INTERVIEW DAY 4

SECTION 5: DAY 5 OF FOLLOW-UP:

IM51 How is your baby (body/health) now?
- Ill 1
- Some what okay 2
- Well 3

IM52 Did your baby cry more than usual since yesterday?
- Yes 1
- No 0
- Don't know 9

IM53 Did your baby eat usual, less than usual or more than usual since yesterday?
- Less than usual 1
- More than usual 2
- Normal, as usual 0
- Don't know 9

IM54 Has your baby been more, less or normal active since yesterday?
- Less than usual 1
- More than usual 2
- Normal, as usual 0
- Don't know 9

[EXAMINATION OF INFANT BY STUDY NURSE]:

IM55 Temperature (Rectal) . . . . . |__|__.|__| °C

IM56 Infant has local swelling and/or redness at injection site:
- Yes 1
- No 0

IM57 Infant has rash all over its body:
- Yes 1
- No 0

IM58 Infant has\ has had seizure:
- Yes 1
- No 0

IM59 Condition of infant:
- Restless, Irritable 1
- Lethargic 2
- Well Alert 0

IM510 Other comments/observations:

	__ __ __ __ __ __

END OF INTERVIEW DAY 5

External review #1

Name of proposal: Effect of zinc supplementation during pregnancy and infancy on the immune responses to vaccines in Bangladeshi children.

Name of investigators: Fuchs, GJ, Santosham M, Black RE, Hashem M.

Reviewers' comments:

The proposed study is important and the design to be used is appropriate. The institutions involved have all the expertise necessary to carry out the study.

Abstract: Indicate the sample sizes for the 4 groups.

<> in program, sufficient

Introduction

The point made about the lower sero-conversion found among children from developing countries should be expanded to indicate what this represents in terms of overall childhood morbidity and mortality; what is the potential public health significance of this problem.

Second paragraph: define 'malnutrition/undernutrition'. What type of malnutrition has been associated with depressed immune responses: kwashiorkor and marasmus or marginal nutritional status such as low weight-forage (< -2 SD), etc. Please expand.

The review of the literature on how zinc supplementation reduces infant morbidity due to infections is incomplete, it should include two other published studies: one by Ninh et al. in Vietnam (AJCN, 1996) and the study by Bates (1993) in the Gambia.

The discussion about zinc supplementation and immunity should include the study by Schlessinger et al. (I 993 Acta Paediatr.), which actually showed that zinc **supplementation** impaired monocyte function.

Vitamin A is mentioned twice in the introduction, first for its role in the integrity of the immune system and then because of its interactive effect with zinc. It is not clear then, why the authors do not plan to measure vitamin A status as well (before and after supplementation). This would definitely help interpret the results and understand mechanisms of action.

The introduction does not mention anywhere why the authors hypothesize that zinc may be a limiting factor in this population. Is there any evidence of deficient diet, poor maternal zinc status, poor weaning diet, poor child growth, etc. Why zinc? Why not vitamin A?

Objectives

Do the authors plan to relate Objectives 1 and 2, i.e. compare the response obtained by infants who have been supplemented postnatally, with the response obtained by infants whose mothers have been supplemented during pregnancy? If so, a third objective should be stated.

Study design

The authors indicate a 'four-cell' design. The design is not a 4-cell design, but rather two 2-cells experiments. This is clearly shown by how the objectives are stated - as two separate experiments. Also, randomization is to be done separately for pregnant women and for infants, as opposed to one randomization into 4 groups.

P.8 section c: *Randomization*. What is described here is not the randomization process, but rather a description of the intervention.

No information is given on the total amount of supplement (volume) that will be given to the young infants, how it will be given, and how the supplement will be prepared to mask the strong metallic taste of zinc. In order to ensure that the infants consume all of the daily supplement offered, the total volume must be as small as possible. This, however, makes the issue of taste more difficult to handle. These are issues that need to be considered carefully and to be discussed in the proposal because of their importance for compliance to the treatment.

Giving the mother a supply of bottles for the week and counting the left-overs at the end of the week is NOT an appropriate way to measure compliance. It may be that financial and logistical constraints do not permit hiring community workers to visit the homes every day to distribute the supplement and to observe its intake, but failure to do so has important implications for the measurement of compliance to the treatment. This is particularly important for zinc supplementation trials because the bad taste of the supplement (for young children) and the potential side effects (gastrointestinal discomfort for mothers, for example), may result in a systematically lower compliance among the zinc supplemented group compared to the controls. Hence, compliance needs to be measured accurately in both groups. The authors must discuss this issue in the proposal.

Other data to be collected

The authors do not indicate whether they will be collecting any additional data on their subjects besides the main outcomes of the study and the occurrence of vomiting and diarrhea. For instance, do the authors plan to collect data on other potential benefits of zinc supplementation such as: improved growth (by measuring infant anthropometry at

birth (for the group of pregnant women) and at the beginning and the end of the supplementation trial for the group of infants); prevalence of morbidity from diarrhea and respiratory infections reduction in the incidence and
(using longitudinal monitoring of morbidity); vitamin A status, etc.? Breast milk zinc among mothers who have been supplemented during pregnancy would also be an interesting outcome to measure.

Maternal characteristics (anthropometry, age, parity, socioeconomic status, which are easy to measure) may also be useful characteristics to control for in the analysis. The authors should clarify whether or not they plan to collect any of these data.

Because the design is a double-blind experimental trial, the authors may argue that they do not need to measure any of these intermediary or potentially confounding factors. While this is true, controlling for these factors in the analyses may increase the precision of the estimates and the statistical power of the comparisons.

Analytical methodology

The authors should include a section on planned analyses and statistical testing.

Page 10 the authors indicate that the study is related to an 'ongoing study'. Please provide more information about how the two studies would be related.

Policy relevance

This section is particularly weak. Most of this section relates to the importance of documenting whether the immune response of infants to vaccines is low. For this purpose, one does not need a supplementation trial. A cross-sectional study, measuring the immune response of infants in the population of interest would be sufficient. Then the authors go on and indicate that 'if the response is found to be lower, then it is important to know if (...) [this] can be overcome with micronutrient supplementation'. The authors are not proposing to do a micronutrient supplementation trial, but rather, a 'zinc' trial. So, the study will provide information on whether or not zinc helps, but not on the role of micronutrients in general. Thus, this section is particularly weak in providing justification for the policy relevance of the study.

The point about what are the implications of the reduced immune response among children from developing countries relative to childhood morbidity and mortality should be picked up in this section. What is the global public health significance of the problem and its consequences, and given what we know about the prevalence of zinc deficiency in developing countries, how likely is it that zinc supplementation will really make a difference?

Evidence of a response to zinc supplementation is an indication of a deficiency. Are there any other studies from Bangladesh that have shown that zinc is a problem? If not, this is important information, which has policy implications for the country.

If the results indicate that zinc deficiency is a problem among this population and that supplementation makes a difference, what are the authors planning to do in ten-ns of helping policy makers think of an intervention strategy? For example, would they recommend to supplement mothers, or infants? What would they base their recommendations on? Considering the logistical difficulties associated with the need to supplement daily, how would the cost of the intervention be weighed against the benefits,' etc.

Ethical issues

The authors need to clarify what they intend to do when they encounter severely malnourished or dehydrated children, etc.

Time frame

There is a gap between October 1997 and August 1998. Probably a typo.

Response to external review # 1

Response to reviewers' comments:

1. The sample size is indicated in the methods of the protocol.

2. The issue of sero-conversion in developing countries and relevance to policy is discussed in the Policy Relevance section.

3. Immunity is compromised in severe and moderate, and perhaps in mild, degrees of malnutrition as defined anthropometrically (Pelletier, 1995).

4. The rationale section was not intended to be a comprehensive literature review, but rather reference sufficient publications to justify the hypotheses of the proposed study.

5. The proposed study is designed to assess the role of zinc, not vitamin A, for the reasons elaborated in the hypotheses of the proposal.

6. The diet of all segments of the Bangladesh population is deficient in zinc. Further, the rate of low birth weight is approximately 50%, and undernutrition (low weight for age, stunting) is recognized to be among the highest in the world. Zinc deficiency is known to be highly associated with each of these conditions.

7. There are several individual micronutrients that can, and should be studies, however, the focus of the proposed study is zinc for reasons described in the introduction. Further, previous studies in Bangladesh and other areas have studied the immune effect of vitamin A supplementation.

8. The major objectives are the two objectives described in the protocol. We intend to conduct a variety of subgroup analyses, including comparison to the groups as described.

9. As indicated in Table one, this is a four cell trial, not a two cell trial.

10. Randomization will be conducted separately for pregnant mothers supplemented with zinc or placebo and for a separate cohort of infants identified at birth born of mothers not supplemented with either zinc or placebo.

11. We plan to use (0.5 ml?) of zinc supplement we have used extensively for other previous studies of zinc.

12. We have extensive experience in conducting studies with this type of compliance monitoring. Certainly direct supervised dosing is optimal, but is not financially feasible. However, we will be conducting systematic and periodic randomized checks of compliance.

13. We plan to collect information on tolerance and acceptability of the supplements, growth (weight, length, MUAC) at monthly intervals, and morbidity (diarrhea and ARI) of the infants via weekly recall.

14. Maternal characteristics data will also be collected using a pretested questionnaire. In addition, anthropometry (weight, height, MUAC) and morbidity will be assessed in the mothers that are supplemented with zinc or placebo.

15. We will forward a copy of the ongoing protocol as soon as possible.

16. As concluded during a recent international conference at Johns Hopkins University, zinc supplementation has the potential to have a major impact on childhood morbidity, and perhaps mortality. Studies to specifically assess the effect of zinc on the immune response to childhood vaccines was identified as a major priority.

17. As described above, zinc deficiency is considered to be a important health problem in Bangladesh. The results of several intervention trials recently completed were presented at the Johns Hopkins conference.

18. We hope the results of the proposed study will provide the basis for our recommendations to policy makers.