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MATERNAL NUTRITION AND INFECTION STATUS AND INFANT SURVIVAL: ASSESSMENT AND INTERVENTION

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INTRODUCTION

Low birth weight (LBW less than 2.5 kg) is an important contributing cause to the high mortality rate among Indian infants. Determinants of LBW in India and suitable interventions to prevent it are not fully understood, but research priorities were identified by the Indian Council of Medical Research's (ICMR) Task Force on Low Birth Weight in March 1982. A review of the current state of knowledge in this field is provided in Attachment 1. USAID is interested in funding research over a six-year period in these priority areas, particularly the role of maternal malnutrition, infection, and interventions to prevent them, including better antenatal care and anthropometric assessment of fetal growth. The studies will be conducted by various Indian research institutes in collaboration with several U.S. universities and research institutes and under the coordination of the ICMR. See Attachment 2 for the list of the investigators and collaborating institutes.

In this research, an attempt will be made to clarify the separate and combined roles of malnutrition and infection during pregnancy on the growth and viability of the fetus and infant. The young child is far more vulnerable to the combined challenge of infection and malnutrition than to either isolated insult. Whether this is also true of the fetus is unknown. In India, poor pregnant women are heavily exposed to a variety of infections and many exist at the margin of nutritional adequacy. If the outcome of the proposed research is successful, causes of maternal and infant health and survival will be much better understood and the results can be applied to improve the well being of Indian women and children, especially those of the lower socioeconomic group.

It was the ICMR Task Force's opinion that these important issues should first be studied in an orderly set of projects around the separate issues of maternal malnutrition and infefction, both to deepen understanding of prevalence and effects of these separate insults in poor Indian women, but also to develop the technical skills and administrative infrastructure that will facilitate the later execution of more complex studies of joint effects.

An important secondary goal of the proposed research is to better define anthropometric and other at-risk characteristics of women in early to mid-pregnancy that are correlated with both greatest need for and responsiveness to food supplementation. Well controlled trials completed over the last generation have clarified the amount of change in birth weight among populations of poor women in both developed and developing countries, following nutritional supplementation (Attachment 1). However, several key issues remain obscure. The ultimate benefit to the infant, either in short-term survival or in long-term growth and development, that follows from these increments in intrauterine growth, is not known. Furthermore, the characteristics or at-risk factors of poor women that are associated with greater responsiveness to nutritional intervention need to be defined with precision.

In addition to testing anthropometric indicators of maternal nutrition status and fetal growth, during the initial phases of this research an attempt will also be made to determine the prevalence of certain infections likely to be associated with low birth weight based on current knowledge (Attachment 1). The infections and organisms to be studied are: malaria, syphilis, gonorrhea, urinary tract infections, asymptomatic bacteriuria, and other genito-urinary tract infections caused by Mycoplasma hominis, Ureaplasma urealyticum, chlamydia trachomatis and Group B Streptococcus.

Two reference laboratories and three study centers will be created. The chosen institutions will gain capability in important nutritional, microbiologic, and serologic techniques, which India does not now possess. They will be able to conduct standardized analyses that have many applications far beyond their immediate use for LBW research. During the latter phase of the six-year research period, this newly acquired expertise will be used in support of intervention trials, which will be conducted at these or other centers to determine the technical feasibility and cost of improving birth weights. Cost-effective interventions identified through the studies could be considered for wider application in India's health services and in programs providing maternal food supplements, such as the Integrated Child Development Services (ICDS) scheme.

Given the obstacles described in Attachment 1 to conducting the proposed research in India, the studies will be phased so that successful completion of one phase will be a prerequisite for proceeding to the next. A list of the exposure (risk) factors and outcome variables that should be studied is provided in Attachment 1. A description of the general approach and timetable for the research is provided first, followed by the detailed protocol for the initial phases, projections for the latter phases, and total cost estimates.

Phase I (Preparatory) - 6 months

During Phase I, laboratory and field study staff will be recruited and trained, laboratories equipped, and the protocol for prevalence of infection studies finalized. One technical person from each of the two reference laboratories will be sent to the United States for three months of training in isolation and serologic techniques for Mycoplasma at Channing Laboratory, Harvard Medical School, and for Chlamydia at the Center for Disease Control. Persons chosen for training will have prior experience in cell culture with viral pathogens, immunofluorescent microscopy, diagnostic bacteriology, and serologic methods. After training in the United STates, they will transfer this technology to their institutes in India to create reference laboratories, and they will train the laboratory personnel at the study centers.

The U.S. collaborators will visit India to assist with this technology transfer. A trial exchange of standardization specimens will be made between India and the United States. The questionnaire for the study of the prevalence of malnutrition and infection in pregnancy will be written by an Indian medical sociologist from one of the selected institutes, circulated to the other collaborating institutes, revised, translated, pretested, back-translated, finalized, and printed. Research staff will be hired and trained in questionnaire administration, clean-voided urine specimen collection, speculum examination, taking of anthropometric measurements, and blood collection in a hospital setting. Methods will be tested for delivery of uncontaminated specimens to the sudy center and reference laboratories.

Phase II (Prevalence Study) - 24 months

During Phase II, the laboratory methodology in the study centers and reference laboratories will be tested in studies of the prevalence of maternal malnutrition and infection in the hospital setting and in rural communities. Approximately 200 women presenting for prenatal care at each study center will have pertinent cultures and serology performed and anthropometric measurements taken in order to determine what infections may be of significance during pregnancy and the role of malnutrition. Other risk factors will also be studied as well as outcome (birth weight).

In addition, in a portion of women, placentas will be retained for histologic and mocrobiologic examination. Duplicate specimens will be sent to the reference laboratories for standardization, and random samples of these specimens will be sent to the collaborating U.S. laboratories for quality control. Anthropometric measures of fetal growth will be tested. Once the hospital prevalence study is smoothly underway, an attempt will be made to replicate the interview and exam techniques in 200 women from rural communities (rural population area of 30,000). A cold chain for transporting samples will be tested. Further transfer of technology from the reference laboratories to the study centers to increase the self-sufficiency of these laboratories will also be done at this time.

Protocol Design for Phases III and IV - 6 months

The results of the laboratory and field techniques developed in the first two phases will be analysed by ICMR and evaluated by the ICMR Task Force on LBW and the Indian and U.S. collaborators at a joint meeting to decide whether and how to proceed with Phases III and IV. The most appropriate institutes

for the next phases will be identified by the review panel. The protocol for Phases III and IV will be written at a joint meeting of the investigators from the chosen institutes and the U.S. collaborators.

Phases III and IV (Outcome/Intervention Studies) - 36 months

Phases III and IV will address the relationship between malnutrition, infection, and LBW by hypothesis testing of association or by intervention or both. The nature of these longitudinal studies will depend heavily on new knowledge acquired in the previous phases and in studies done elsewhere. One technical person from each of the reference laboratories will be sent to the United States for retraining in the latest microbiologic and serologic techniques for maternal infections. Other research staff will also be trained as necessary in the United States up to 12 person months during these phases.

The studies will take place in underprivileged urban areas and in rural communities. It is anticipated that three research institutes will be chosen to conduct the clinical trial of treatment of infection in the urban areas they serve and in approximately 24 rural villages over a two-year period at each site. The effect of food supplementation in malnourished pregnant women will be studied by a separate institution at separate sites comprising up to 100 "fed" villages and 100 control villages. Midway through Phases III/IV, the ICMR Task Force on LBW and the Indian and U.S. collaborators will meet to review progress and to determine any modifications required in the research designs. Continuous quality control checks by ICMR staff and U.S. collaborators on both epidemiologic and laboratory data will be necessary throughout Phases III and IV. At the end of Phase IV, cost benefit and cost effectiveness analysis will be made of the various interventions as well as assessment of their suitability for large scale application in the GOI's health delivery system and in ICDS.

SIX-YEAR RESEARCH SCHEDULE

On the assumption that it will take several months to obtain approvals for the study and for staff training outside India, preliminary work will begin as soon as possible and continue until approval for the research is received.

Preliminary Activities

1. Draft, modify, and finalize protocol for study of maternal nutritional status and prevalence of infection.

2. Prepare purchase orders, customs forms, and other documents necessary for acquisition and importation of equipment.

3. Identify and prepare for hiring and training of reference and study center research personnel, including clearances.

4. Develop and exchange draft of questions for questionnaire, translate and back translate.

Phase I

Month	
0	Complete approvals by USAID, Government of India, Ministry of Health and Family Welfare, Indian Council of Medical Research and study centers and sign agreements.
0-6	Hire and train senior laboratory staff in the U.S. and clinical, administrative, and other personnel in India.
0-6	Complete procurement and delivery of laboratory and other equipment.
0-6	Finish preparation and pretesting of forms and questionnaires and proceed with printing.

Phase 2

Month	
6-9	Complete pilot test of hospital specimen collection.
6-9	2 months' technical assistance by one to two of the U.S. collaborators and a one week progress review meeting at ICMR.
9-15	Enroll 200 pregnant women visiting prenatal clinic at hospital for the first time at each study center.

9-12	Reculture 100 women entering during these months at 34 weeks of pregnancy.
9-12	Test rural village study procedures.
9-18	Monitor and record required information at delivery in hospital on enrolled women.
6-15	Perform laboratory tests on specimens collected from enrolled women.
12-20	Analyze data at ICMR.
12-24	Enroll 200 village women visiting the prenatal clinic at the rural primary health center or subcenter for the first time at each study site. Recruitment will also be done during health workers' visits to villages to identify pregnant women.
12-30	Monitor and record required information at delivery on enrolled village women.
18	Data review and decision on Phase III and IV intervention trials made at meeting of ICMR, Indian, and U.S. collaborators.
18-30	Plan and make preparations for Phases III and IV.
31-35	Review final results of Phases I and II and write protocol for phases III and IV. Meeting held for ICMR, Indian and U.S. collaborators to write protocol for Phases III and IV. Protocol pretesting done.

Phases III and IV

Months

30-36	Study pregnancy outcome on a larger sample of malnourished and infected women and test antimicrobial therapy and impact of nutritional supplementation at separate sites according to anthropometric measurements of the women.
48	Progress review meeting at ICMR of Indian and U.S. collaborators.
61-72	Analyze data and write final report.
72	Hold meeting at ICMR of Indian and U.S. collaborators to review final results.

Detailed Protocol for Phases I and II

During Phases I and II, the anthropometric measurements and the prevalence of the infections listed in the introduction will be studied in pregnant women served by the hospitals and rural health centers of: Post Graduate Institute of Medical Education and Research (PGI), Chandigarh; King Edward Memorial (KEM) Hospital, Pune; and Christian Medical College (CMC), Vellore.

Recruitment and study will begin in the hospitals and then proceed to the rural areas. All pregnant women attending the hospital prenatal clinics for the first time will be registered, using a registration form. On each clinic day, up to three randomly selected women will be recruited for the study, given a brief standard description of the purpose and explanation of all procedures, and asked for their consent to participate. Enrollment will proceed until 200 women from each of the hospitals at the three study sites have been recruited.

A separate roster of study subjects and a manual of the study's prenatal clinic requirements and procedures will be available in the clinic at all times.

Prenatal Examination

Urine specimen. A clean voided midstream urine specimen will be obtained and processed according to standardized methods (Attachment 3).

Physical examination. The following information will be collected and recorded on a prenatal clinic visit form: standardized anthropometric measurements, including the woman's height, arm circumference, triceps skinfold, weight, abdominal girth, fundal height, and subscapular skinfold; and standardized blood pressure measurement.

An examination of the vaginal introitus and canal will be made using an unlubricated speculum without antiseptic. Findings and notation of specimens obtained will be recorded on a prenatal clinic visit form. Swabs will be taken from the cervical os (1 Ca Alginate, 3 cotton), the anterior vaginal canal (1 cotton), and the rectum (1 cotton). Swabs will be processed in the study center clinic according to standardized methods (Attachment 3).

Blood Sample. Blood (15 cc) will be drawn by standard venipuncture technique into vacutainer tubes (silicon plugged) and processed according to standardized methods (Attachment 3).

Questionnaire. A standard, pretested questionnaire will be administered by a trained health professional in the subject's own language. It will contain information on socioeconomic status, dietary habits, morbidity, pregnancy history, etc.

Instructions/Rescheduling. The first 100 subjects enrolled at each center will be given appointments for return visits at 34 weeks of pregnancy and identified as participants. They will be instructed to notify the clinic or delivery personnel whenever they return that they are study subjects.

Follow-up visit. When the subject returns for the second appointment, the information collected will be recorded on a new prenatal clinic visit form. She will be reexamined, following the procedures specified above for her first visit.

Delivery phase

Delivery area procedures. A separate roster of study subjects and a manual of the study's delivery requirements and procedures will be available in the delivery area at all times. Upon arrival for delivery, each woman will be asked whether she is a study participant. Her response will be compared with the roster. At the time of delivery, placentas of subjects who have been delivered by cäesarean section prior to rupture of membranes will be obtained and processed according to standardized details.

Delivery Log Surveillance. The delivery log will be reviewed once each day to determine whether any subjects have delivered in the previous 24 hours or were expected to have delivered. Special efforts will be made to trace subjects on whom there is no record of delivery one week past the expected date of delivery.

Postpartum Procedures. For each delivery of a study subject, selected information from the intake/admission record on the time of birth, neonatal measurements, and complications of pregnancy and delivery will be abstracted onto a delivery form. Blood (15 cc) will be obtained by standard venipuncture technique and handled according to standardized details. Neontal measurements will be taken of the infant's length, weight, and head circumference.

Standardized methods for specimen processing and laboratory analysis for these phases are shown in Attachment 3. Reference laboratories will be established at CMC-Vellore and PGI-Chandigarh to do this work. Pune will send its specimens to Vellore for processing. There will be an exchange of random samples of duplicate specimens between Vellore and Chandigarh for quality control and between these two institutes and the

collaborating U.S. institutes for quality control throughout the study. The Government of India will expedite this exchange of random samples of biological specimens between the United States and India. Toward the end of Phase II, Vellore will begin to train the lab staff at Pune and transfer the necessary technology so that they become self-sufficient for the processing of specimens collected in their area.

A sample of some of the forms and questionnaires to be used is shown in Attachment 4. These are by no means complete and will need to be carefully designed by an Indian medical sociologist, distributed to the participating research institutes in India and the United States, revised, translated, pretested, back-translated, and finalized. Data analysis for the study will be done at ICMR. A sample of some of the survey data pertaining to the biological specimens sent to the United States will also be sent to the National Institute of Allergy and Infectious Diseases (NIAID) for quality control.

It is expected that it will take nine months to complete the hospital study. Three months after beginning the hospital study, collection of similar information will begin on 200 pregnant women from the rural area surrounding each study site. This will be done in part by mobile teams and by staff at the primary health centers and subcenters.

PROJECTIONS FOR PHASES III AND IV

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PROJECTIONS FOR PHASES III AND IV

During Phases III and IV two different interventions to improve birth weight will be studied: (1) the treatment of prevalent maternal infections associated with low birth weight; and (2) the provision of food supplements to pregnant women and taking of anthropometric measurements to identify which women are most at-risk and can benefit most from supplementation.

It is believed that it will not be possible to study both of these interventions in the same sample. Therefore, it is preferable that the studies of infection treatment and food supplementation be conducted in different areas by different researchers. It would seem most logical for the infection control studies to be done in the same areas and by the same researchers as used in Phases I and II. The areas for the food supplementation study should be villages in which extensive and intensive maternal feeding programs are already in place and for which similar control areas exist where no feeding is taking place. The institutes selected to conduct the nutrition studies should have experience in doing rural nutrition field survey work, such as the National Institute of Nutrition. Study variables to be measured in both in the infection control and food supplementation studies will now be described.

Outcome (Dependent) Variables

The variable on which sample size will be predicated is birth weight. The duration of gestation, stillbirth, morbidity, and mortality rates of the infants will also be carefully recorded. Duration of gestation is reported to be as much as one and a half weeks shorter in Indian and other poor populations than for middle-class women. Thus, as much as 200 g of the birth weight discrepancy due to poverty, disease, and malnutrition may be mediated by shortened gestation. While differences in mortality may not be detected in these studies (a larger number of subjects would be needed and the effect of increased birth weight on decreased mortality might be contingent on effective preventive and curative health services for mothers and children), it would be folly to pursue these studies without assessing effects on mortality. Every attempt will be made to account for all identified pregnancies in the study villages. Measurement of birth length and head circumference will also be made. These measurements can be obtained during the first week of life and the value at birth can be estimated in analysis. Other secondary outcome vaiables will include growth, morbidity (from fevers, diarrhea, and respiratory illness), and mortality during the first year of life.

Independent Variables

Usual maternal diet and anthropometry will be assessed. A practical method will be devised to weigh women (all village women of reproductive age will be weighed during the initial phase of the studies), and to get at least one, and preferably three, weights later in pregnancy. Arm circumference, height, and in midpregnancy, abdominal girth, fundal height, triceps skinfold, and sub scapular skinfold will be measured. These anthropometric data will be used to determine whether responsiveness to dietary supplements can be predicted in early or midpregnancy. They will also be used to determine whether malnourished, infected women show greater or lesser response to antimicrobial therapy than normally nourished women.

Covariates

Some covariates will be collected because they are historically and traditionally included in most surveys and studies of reproduction. Most, though, are necessary because they could potentially confound the relationship between intervention and outcome. Confounding could occur when some factor is related both to use of nutritional supplements, maternal health status, and to birth weight. This underlying factor could be the cause of the increased birthweight that might falsely be ascribed to supplementation or antimicrobial treatment, unless the factor were controlled in analysis. Thus, better educated or informed women might be healthier or use more supplements and bear heavier infants. This relationship could be an indirect one, caused by some other correlated factor, such as better use of health services or less active physical labor near term. Unless data on these potential confounding factors are gathered in the most comprehensive and careful way, there is a danger of falsely concluding that infection control or nutritional supplementation is causally related to outcome.

All inquiries on the study must be phrased unambiguously, posed in terms easily understood by the investigator, questioner, and informant, and asked in carefully standardized ways. The questions must relate directly to the lives and culture of Indian village women (and these may have different implications, therefore, for different subgroups). They should be questions, if possible, that have been developed, standardized, and tested in past empirical Indian biomedical and social research. Some of the broad categories of covariates have been mentioned here. Others will need to be identified during the design of Phases III and IV through intensive review of past social and biomedical research efforts in India and consultation with the relevant investigators. Some lessons can be learned from efforts outside India, such as the current U.S. nationwide evaluation of the Special Supplemental Food Program

for Women, Infants, and Children, (WIC) directed by Dr. Rush, and studies in India, such as the ICMR sponsored Collaborative Studies on High Risk Pregnancies and Maternal Mortality. Extensive further preliminary development on questionnaire design for the projected studies must be undertaken.

Health care. A meticulous assessment of both the services available to the women in any study site and her use of these services is imperative. Some issues are the numbers and level of training of available health personnel, distances, accessibility, range of services provided, and usual patterns of use of available facilities, such as time of first seeking of care for the index pregnancy, frequency of care, and compliance with appointment scheduling and with prescribied preventive and therapeutic regimens.

Age, parity, past reproductive and health history, and medical progress of current pregnancy. These issues are relatively straightforward and likely to be similar to past research in and out of India. Other limitations will have to be resolved such as those inherent in gathering data from relatively unsophisticated subjects by previously inexperienced research workers, and those related to issues about which the women may be less open and frank, such as sexual practices or the relatively common practice of illegal abortion.

Social status, caste, education, literacy, past family planning behavior, income, aspirations, occupational status, possessions and attitudes to pregnancy, child bearing, family planning, etc. No set of issues is more important and more dependent on past empirical research among the population to be studied or similar village Indian women. Given the work of J. Cravioto in Mexico and P. Graves in West Bengal on the maternal behavioral determinants of infantile malnutrition, some actual observation of the mothers behavior toward older children, or the index infant after birth could be of immense value, but probably technically impossible under the conditions of this field research. Some information is needed on whether there is community prejudice against female infants, and the subject's own aspirations and views (as well as the relative survival, growth, and provision of benefits, such as schooling and health care to her male and female children). Expectations of family size and family planning practices might also be good indicators of social mobility. Family possessions and the tidiness of the house may be important. Housing, sanitation, water supply, and other amenities should be assessed in this category.

Physical work and activity. Evidence has mounted that heavy work and extensive, tiring travel in late pregnancy are associated with preterm labor. Given that work also influences caloric and other nutrient needs, a detailed history of duration and level of usual physical exertion and whether this is modified with progress of pregnancy is important.

Logistics of Outcome/Intervention Studies

Development of instrumentation and training of staff. This will include questionnaires, coding forms, equipment choice, and manuals of procedure to be developed and pretested. Equipment would include tape measures, scales for mothers and infants, calipers for measuring triceps and subscapular skinfold and fundal height, and laboratory equipment as specified in the attached budget. Training manuals and procedures will be developed. Training of supervisory staff will precede trainning in the field.

Village census. A complete census of each village will be necessary with name, age, sex, marital status, contraceptive practice, and relationship to head of household for each household member. All women in the reproductive age range (15-45 years) will be weighed.

Ongoing survey of reproductive-age women. Every woman of reproductive age should be contacted periodically (preferably monthly but at least every three months) to determine whether contraception is being used and the date of onset of last menses. All women who have missed a menstrual period will be placed on a register of possible pregnancies and initial survey data collected. Special forms will be used to follow each such woman monthly, with complete anthropometric resurvey and questions on use of dietary supplements or examination for infection around 20, 30 and 36 weeks gestation. At 30 weeks, special survey qestionnaires will assess progress of pregnancy and use of health services.

After 30 weeks, all women should be visited weekly. If birth has taken place, the date of birth and sex of the infant(s) will be noted and anthropometry performed. If the infant is not in the hospital or has died, these data will be noted.

Quality control, retraining, and surveillance. All forms will be reviewed by the local supervisor for completeness and for predetermined consistency checks. All survey procedures will be periodically rechecked.

Postpartum followup. It would be preferable to follow up each child and mother to assess adequacy and duration of lactation; infant health, growth, and survival; and maternal weight. This might be done at four weeks, six months, and one year of life.

Data analysis. An appraisal of analytic capacity would be helpful. Since large amounts of complex data will be analyzed, moderately large computers with software packages for multivariate analysis, such as SAS, SPSS, BMDP, or comparable

programs, will be needed. Data analyses will consist of progress reports of process issues to assess management procedures in the field and of interim outcome results. Stopping rules should be developed for the study.

Cross-tabular and multivariate analyses will be used. Covariates will probably be subjected to factor or cluster analysis. Multiple linear reqression analysis will be used and logistic regression for infrequent dichotomous outcomes. By the time of final analysis and interpretation, it is likely that more refined techniques for analysis of sequential data will be available.

The following are specific projections and assumptions for the separate infection control and food supplementation studies.

Antimicrobial Treatment of Maternal Infections

These studies will be designed based on the findings of the prevalence of various infections in Phases I and II and their association with birth weight. The plan will be to work in the same populations served by the three study site hospitals and rural health centers. The most prevalent maternal infections previously found associated with low birth weight will be treated and improvement in birth weight and infant survival measured. Projections for the clinical trial in which appropriate antimicrobial therapy will be provided for maternal infections have been based on a number of assumptions, most of which are arbitrary, worst-case expectations. For example, the maximum number of women who would need to be treated has been estimated, based on the need to treat the organism with the lowest prevalence. This and all other assumptions have purposely led to a budget at the upper limit of the range. The projections are as follows:

1. The intervention regimen, if and when effectively followed, will lead to a 100 g increase in the average birth weight of the babies of treated mothers.

2. One of the organisms requiring antimicrobial eradication is Chlamydia, whose prevalence rate in this population is 10 percent.

3. Two organisms requiring eradication will be examined in a placebo-controlled trial for either, two groups of women for each of two therapeutic regimens or four groups.

4. The study will test the null hypotheses, i.e., that intervention will not achieve a 100 g reduction. A two-tailed test will have 80 percent power to reject the null hypothesis at the 0.05 significance level with a sample size of 400 per group.

5. Two drug regimens will be used. The most expensive is a six-week course in the pregnant woman with a two-week course in her partner (Rs.224 erythromycin), and the next most expensive is a two-week course in the pregnant woman with a one-week course in her partner (Rs. 84 erythromycin).

6. All culture-positive women will be recultured at 34 weeks gestation.

7. Lack of compliance and attrition from other causes will not exceed 50 percent.

8. There will be 30 births/1,000 women in the villages, on average.

9. The trial will be divided so that two-thirds of the subjects will be recruited from urban hospitals and one-third from rural villages.

10. In addition to assessment of the infant's status at birth, there will be three followup assessments at 28 days, 6 months, and 1 year.

11. The study could require as many as 12,000 women to be screened with prenatal evaluation and appropriate cultures done. This would mean 2,600 women recruited at the hospital and 1,400 women recruited in 24 rural villages over a two-year period at each study site.

Food Supplementation for Pregnant Women

In reviewing possible designs for studying the impact of nutrition supplements during pregnancy, the difficulty of execution, the security of inference that can be derived from the results, and the applicability of these results--both to the advance of scientific understanding and to the urgent and practical problem of improving the health and well being of Indian mothers and children--have been balanced. These goals conflict: more complex designs may lead to stronger inference, but are less likely to be efficiently executed and completed and may bear little relationship to potential applied programs. A number of approaches to randomized controlled trials have been ruled out as impracticable in rural Indian villages, including randomization by individuals and matching designs by group. See

David Rush's consultancy report to USAID/India, May 1983, for a more detailed discussion. It is believed that the preferred approach would be to do an observational study utilizing existing supplementation programs. In such a study, a comparison would be made of the average birth weights in supplemented and unsupplemented communities.

This design can be inferentially strong, efficient in cost, and likely to produce results that can be directly generalized to large numbers of potential recipients. The inference is reasonably strong, because the design does not centrally depend on historical measures of supplementation, nor is it confounded by individual self-selection since entire communities with supplementation programs would be compared with communities without such programs. (There does exist the possibility of confounding by community, since villages with feeding programs may have more resources, such as health care, than villages without supplementation programs. Such confounding must be guarded against by careful site selection and extensive community description that will allow for control by analysis.

The cost of mounting new feeding programs would not be incurred, and results achieved by existing programs are clearly more easily applicable to wider populations than very expensive ad hoc programs created for intervention trials. However, the caveats to the implementation of this design are important. First, the supplementation program must be extensive and intensive enough to be likely to make real improvements in women's diets. If too few women are served, or are served by inadequate type or amounts of food, a fair test of supplementation cannot be made. Second, control sites must be identified that are similar to study sites, if possible, in food production and availability in the market, sanitation, economic resources, culture, and health care. If baseline data are available, then perinatal outcome before the onset of the programs shold be similar in the fed and control villages. In addition, it must be possible to mount a comparable research effort in the control sites as in the supplementation sites.

Birth weight is the central variable on which the power of the study can be calculated. Among populations of poor women, nutritition supplements over the last two trimesters of pregnancy are likely to be able to raise birth weights by 60 to 80 grams, but almost certainly not over 100 g. There is greater statistical power in calculating change in mean birth weight, than the proportion of low birth weight infants; the two indices are interdependent. If it is assumed that adequate supplementation programs ought to reach at least half of eligible pregnant women, a community increase in birth weight of 35 g might reasonably be expected. The cell size required for detecting a 35g difference in bith weight following a two sides test at $p=0.05$ and $p=0.20$, and assuming the standard deviation

of birth weight to be 600 g, would be 3,085. The design effect due to the clustering of women in treatment or control villages cannot be calculated, but a reasonable assumption might be a doubling of the necessary sample size to approximately 6,000 subjects and an equal number of controls.

If villages have an average of 1,000 inhabitants and 30 births a year, 200 village years of observation data collection for supplemented and another 200 control villages could be needed. One hundred villages would be needed each for supplemented and control groups if followed for two years, or 67 villages if followed for three years. Design effects would be more important for longer followup in fewer villages. For the purpose of the budget estimated for these studies, it has been assumed that the studies would run for two years in 100 fed and 100 control villages.

A possible location for the fed villages might be one of the ICDS blocks in Bharuch district, Gujarat. The health services in this district are being upgraded to the level of the GOI's Model Plan with assistance from USAID's Integrated Rural Health and Population Project. Furthermore, USAID has proposed to assist with ICDS in this same district and fund some innovations that could greatly improve the feasibility of the proposed study. These include paying incentives to dais to identify and recruit pregnant women early for ICDS and health services, use of women's charts to monitor all women 15-45 years of age to detect pregnancy and to regularly record weight gain during pregnancy, and the provision of portable scales to all dais and auxiliary nurse midwives for measuring birth weight. A similar control block would have to be identified from another area not covered by any maternal food supplementation programs. An appropriately qualified institute would have to be selected to carry out the study, e.g., National Institute of Nutrition or M.S. University of Baroda, Food and Nutrition Department. Work has already been done in Gujarat on development of food supplements with great appeal to pregnant women, known as Matruahar, which could be used in the supplemented villages.

The key independent variable will be whether the woman resides in a village with or without a nutritional supplementation program for pregnant women. Measurement of regular diet, i.e., diet from routine sources exclusive of supplements, will be both an independent variable and an important covariate. All other things being equal (body weight, reproductive history, etc.), it must be assumed that level of regular diet will influence the acceptability, utility, and effectiveness of supplementation. Given the limitations on data gathering at the village level, such techniques as quantitative diet recall or food diaries (either historical or using weighing of portions) are impractical. Some modified food frequency, household consumption, or household purchasing technique might

be possible, as might repeated 24-hour recall on a subset of women by occasional investigators periodically brought into some study villages. Further inquiries into currently used techniqes must be undertaken. The adequacy of mother's breast milk supply to sustain normal growth of the child to a certain age should also be measured as a secondary outcome variable.

While the studies suggested are difficult, lengthy, and expensive, the current investment in food supplementation programs warrants that they be given serious consideration. They are, in theory, methodologically sound and can be carried out at reasonable levels of field technology.

Further Studies

The early results of the outcome/intervention studies of infection during pregnancy can be considered for application in some of the nutritional study villages to determine whether effects of malnutrition and infection act synergistically. A final stage of both the infection control and food supplementation studies might be the extension of a less rigorous and expensive study to a larger population in order to assess effects on infant survival.

Staff, Equipment, and Budget Estimates

Staff projections and cost by institution are shown in table 1. These costs will be borne by ICMR. A summary of the total costs of the research over a six-year period and the portions to be borne by USAID and ICMR are shown in tables 2 and 3. The annual budget requirements by institution for each category of expenditures are shown in table 4. The cost of vehicles, POL, drugs, and staff will be met by ICMR. All other costs will be met by USAID. Budget worksheets describing the equipment, transport, and supply needs of each institution are found in Attachment 5. All dollar costs of the research will be managed by agreements between USAID and the appropriate U.S. research institutions as described in tables 5, 6, and 7. All USAID-funded rupee costs will be managed by a grant to ICMR.

Other comments on the budget estimates and equipment needs are that shipping costs for imported items have been estimated at 21 percent of the cost of the equipment and supplies; a shipping rate of 10 percent has been estimated for locally purchased equipment. It is expected that most imported equipment would be shipped by air freight.

CMC-Vellore can import all equipment duty free through the Indo-U.S. Agreement using the CMC Board, New York, NY, as the shipping agent. For other equipment, ICMR will have to assist the institutes with securing appropriate customs certificates,

etc. Brand names for some imported items have been specified for the following reasons: Compatibility with other equipment available at each center, ease of repair, and known high quality. An inflation rate of 10 percent per year has been applied on all costs.

Supply, media, and reagent costs include glassware, maintenance of equipment, specimen collection materials, autoclaving, etc., for the Group B Streptococcus (GBS), Gonococcus (GC), Chlamydia, Mycoplasma, and urine cultures. For CMC, instead of media, reagent, and manpower costs for the GBS, GC, and urine cultures at RS.30 per culture (4 per woman = Rs.120 per woman per culture) have been estimated. Since the assessment of the impact of maternal food supplementation will be carried in areas with ongoing food distribtuion programs, it is expected that food for these programs will be supplied from existing sources, i.e., CARE or the state governments.

Collaboration with the U.S. Research Institutes

Since some of the proposed research on infection has never been done in India and many difficulties will be faced in transferring the needed technology, continued collaboration with experienced U.S. researchers will be essential. Several institutes and individuals with extensive experience in research on LBW in the United States have been identified to serve this role as listed Attachment 2. Assistance from experts at other U.S. institutes will also be sought as required. The collaborating institutes will assist with training, experimental design, standardization of random samples of duplicate specimens through laboratory analysis and exchange, data analysis, procurement and shipping of supplies and equipment from the United States, and other technical services.

At least four meetings will be held in India with the Indian and U.S. collaborators at key stages over the life of the project to jointly write protocols and review progress. The mechanism for the collaboration will be agreements between USAID and the U.S. institutions. Of these institutions, NIAID will take the lead role for coordinating the U.S. response. Funding will be provided by USAID for training in the United States of Indian researchers, periodic meetings of U.S. and Indian collaborators, technical assistance, data analysis, and procurement and shipping of equipment, supplies, and specimens within India and between the U.S. and India.

TABLE 1

Number of Staff Required and Cost

Staff Position	Annual unit cost (salary TA/DA	Year 1-3 CMC	Year 1-3 KEM	Year 1-3 PGI	Year 4-6 CMC	Year 4-6 KEM	Year 4-6 PGI	Other Nutrition sites	Total Cost Rs.'000
Research officer	20,000	1	1	1	1	1	1	1	420
Assitant research officer	17,000	2	2	2	7	7	7	10	1,887
Senior research fellow	20,000	2	0	2	2	2	2	0	600
Senior lab technician	24,000	2	0	2	2	2	2	0	720
Senior stenographer	13,000	1	1	1	1	1	1	1	273
Lab attendant (male)	6,000	1	1	1	1	1	1	1	126
Auxiliary nurse midwife	8,000	3	3	3	8	8	8	20	1,272
Medical social worker	17,000	3	3	3	8	8	8	20	2,703
Nutritionist	17,000	0	0	0	0	0	0	20	1,020
Driver	8,000	1	1	1	2	2	2	7	384
Dais/Incentives	600	20	20	20	30	30	30	200	630
Subtotal									10,035
Inflation (@ 34 percent)									3,402
GRAND TOTAL									13,437

TABLE 2

Low Birth Weight Research Summary Cost Estimate (US$'000)

Items	AID Grant LC*	AID Grant FX*	ICMR	Entity Managing Funds
Equipment	149	280		Imported-NIAID/NIH Indian-ICMR
Supplies, media, Reagents	350	103		Imported-NIAID/NIH Indian-ICMR
Drugs			24	ICMR
U.S. collaborators (travel, per diem, salary)		319		CDC NIAID/NIH Harvard
U.S. lab work/ quality control		161		Harvard CDC
U.S. data analysis		134		NIAID/NIH Harvard
Training of Indian researchers in U.S.		89		Harvard
Local transport				
Vehicles			173	ICMR
POL			262	ICMR
Indian staff			1,344	ICMR
Other contingencies	253			ICMR
Total	752	1,086	1,803	
Total USAID	1,838			
Grand total Research cost	$3,641			

*LC = Local currency or rupees

*FX = Foreign exchange or $

TABLE 3

Low Birth Weight Research Summary Cost Estimate
(Rs.'000)

Items	AID Grant LC*	AID Grant FX*	ICMR	Entity Managing Funds
Equipment	1,490	2,800		Imported-NIAID/NIH Indian-ICMR
Supplies, media, & Reagents	3,500	1,030		Imported-NIAID/NIH Indian-ICMR
Drugs			240	ICMR
U.S. collaborators (travel, per diem, salary)		3,190		CDC NIAID/NIH Harvard
U.S. lab work quality control		1,610		Harvard CDC
U.S. data analysis		1,340		NIAID/NIH Harvard
Training of Indian researchers in U.S.		890		Harvard
Local transport				
Vehicle			1,730	ICMR
POL			2,620	ICMR
Indian staff			13,440	ICMR
Other contingencies	2,530			ICMR
Total	7,520	10,860	18,030	
Total USAID LC + FX	18,380			
Grand total Research cost	Rs.36,410			

*LC = Local currency or rupees

*FX = Foreign exchange or $

TABLE 4

Low Birth Weight Research
Annual Cost Estimates in (000) $ & Rs

Item	Year I		Year II		Year III		Year IV-VI	
	$	Rs	$	Rs	$	Rs	$	Rs
Equipment								
CMC	45.1	276.6	-	-	18.5	425.0	-	-
KEM	42.5	329.2	-	-	2.5	34.0	-	-
PGI	45.7	63.5	-	-	18.5	25.0	-	-
ICMR	50.0						8.0	200.0
Shipping	38.5	66.9	-	-	8.3	48.4	1.7	20.0
Subtotal	221.8	736.2			47.8	532.4	9.7	220.0
Supplies, media, maintenance, reagents, drugs, etc.								
CMC	2.6	70.0	2.7	79.0	0.2	30.0	23.0	1,193.0
KEM	2.6	33.0	2.7	47.0	0.2	27.0	23.0	923.0
PGI	2.6	34.0	2.7	44.0	0.2	22.0	23.0	923.0
Shipping	1.6	13.7	1.7	17.0	0.13	7.9	14.5	280.0
Subtotal	9.4	150.7	9.8	187	.73	86.9	83.5	3,319.0

TABLE 4, continued

Item	Year I		Year II		Year III		Year IV-VI	
	$	Rs	$	Rs	$	Rs	$	Rs
Transport								
CMC (Vehicle)	-	97.0	-	-	-	-	-	128.0
(POL)	-	40.0	-	44.0	-	48.4	-	342.0
KEM (Vehicle)	-	97.0	-	-	-	-	-	128.0
(POL)	-	40.0	-	44.0	-	48.4	-	342.0
PGI (Vehicle)	-	97.0	-	-	-	-	-	128.0
(POL)	-	40.0	-	44.0	-	48.4		342.0
Nut-study Sites (Vehicle)		-	-	-	-	-	-	896.0
(POL)	-	-	-	-	-	-	-	1,197.0
Shipping	-	29	-	-	-	-	-	128.0
Subtotal (Vehicle)		320.0	-	-	-	-	-	1,408.0
(POL)		120.0	-	132.0	-	145.2	-	2,223.0

TABLE 4, continued

Item	Year I		Year II		Year III		Year IV		Year V		Year VI	
	$	Rs	$	Rs	$	Rs	$	Rs	$	Rs	$	Rs
Staff costs												
CMC	-	256.0	-	256.0	-	256.0	-	480.0	-	480.0	-	480.0
KEM	-	168.0	-	168.0	-	168.0	-	480.0	-	480.0	-	480.0
PGI	-	256.0	-	256.0	-	256.0	-	480.0	-	480.0	-	480.0
Other nutrition sites	-		-		-		-	1,225.0	-	1,225.0	-	1,225.0
10% annual inflation	-		-	68.0	-	136.0	-	800.0	-	1,066.0	-	1,333.0
Subtotal	-	680.0	-	748.0	-	816.0	-	3,465.0	-	3,731.0	-	3,998.0

TABLE 4, continued

Item	Year I		Year II		Year III		Year IV-VI	
	$	Rs	$	Rs	$	Rs	$	Rs
Other contingencies								
CMC	-	75.0	-	82.5	-	90.75	-	550.0
KEM	-	75.0	-	82.5	-	90.75	-	550.0
PGI	-	75.5	-	82.5	-	90.75	-	550.0
Nutrition study sites	-		-		-		-	140.0
Subtotal	-	225.0	-	247.5	-	272.25	-	1,790.0
Total (without vehicle)	231.2	1,918.0	9.8	1,314.5	47.0	1,875.4	93.2	18,746.1
(with vehicle)	-	2,238.0	-					20,154.1
Grand total (without vehicle)					$381.2		Rs.23,854.0	
(with vehicle)							Rs.25,582.0	

TABLE 5

Budget for U.S.$ Costs of LBW Research

Centers for Disease Control (Participating Agency Support Agreement)		Cost ($)
Per diem	: 60 days New Delhi @ $90	5,400
	: 60 days outside NDLH @ $66	3,960
Air fare	: Atlanta - New Delhi - 8 round trips @ $2,168	17,344
Lab supplies, work and shipping for quality control & training		20,000
Subtotal		46,704
Inflation @34%		15,879
Grand total		62,583

TABLE 6

Budget for U.S.$ Costs of LBW Research

National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIAID/NIH) (Participating Agency Support Argument)		Costs ($)
Per diem :	60 days New Delhi @ $90	5,400
	60 days outside NDLH @ $66	3,960
Air fare :	Washington D.C.-NDLH - 8 round trips @ $1,982	15,856
Data analysis		50,000
Subtotal		75,216
Inflation @ 34%		75,216
Inflated Subtotal		100,789
Procurement of equipment, lab Supplies, reagents and computer software in U.S. and shipping to India (including inflation)		382,730
Grand total		483,519

TABLE 7

Budget for U.S.$ LBW Research

Harvard University, Channing Laboratory and School of Public Health, Department of Nutrition (Cooperative Agreement)		Costs ($)
Per diem :	225 days New Delhi @ $90	20,250
	225 days outside Delhi @ $66	14,850
Air fare :	Boston - Delhi 18 round trips @ $1,842	33,156
Consulting fees :	375 days @ $242 maximum	90,750
	Overhead @ 30%	27,225
Lab supplies, work and shipping for quality control and training		100,000
Data Analysis (food supplementation study)		50,000
Training of Indian researchers		
Per diem in Boston and Atlanta 540 days @ $75		40,500
Air fare :	New Delhi - Boston - Atlanta 10 round trips @ $2,568	25,680
Subtotal		402,411
Inflation @ 34%		136,220
Total		539,231

ATTACHMENT 1: ANALYSIS OF THE LOW BIRTH WEIGHT PROBLEM IN INDIA AND RESEARCH NEEDS

Definition, Nature and Magnitude of the Problem

Low birth weight (LBW - less than 2.5 kg) is an important contributing cause to the high infant mortality rate in India. It affects 30 percent of the infants, in contrast to only 7 percent in the United States and Europe.

More than 50 percent of infant deaths occur in the first month of life and those with the lowest birth weights (in India, 2 kg) experience the greatest risk. Unless birth weights can be substantially increased, it is unlikely that infant mortality can be lowered. Thus the GOI in its strategy for Health for All by 2000 AD is striving to reduce the prevalence of LBW to 10 percent.

The average birth weight of the Indian infant, 2.7 kg, has not changed over the past 30 years. The problem is most acute among the poor and illiterate. Infants born to wealthy, educated women weigh 3.4 kg on average, which compares favorably with the average birth weight of 3.2 kg in the United States and Europe. The most favorable birth weight range in terms of infant survival has been found to be 3.5 - 4.0 kg. Only 5 percent of Indian infants have weights in this range, versus 26 percent in the United States. Infants with LBW fit into one of three categories: (1) preterm with appropriate weight for dates; (2) preterm with intrauterine growth retardation; and (3) full term with intrauterine growth retardation. It is important to distinguish between these categories because the prognosis differs in each case. The LBW problem in India is largely confined to categories 2 and 3, whereas in the United States, category 1 predominates. A preterm infant is a baby born too early with a gestation of less than 37 weeks. Intrauterine growth retardation (IUGR, also referred to as small-for-dates or small-for-gestational age) is defined as a birth weight less than the 10th percentile or 2 standard deviations below the mean birth weight for gestational age and may occur in babies born preterm or full term.

Fetal growth in India is similar to the United States until the last 4 to 6 weeks of pregnancy, when the weight gain of Indian infants dramatically slows. Preterm deliveries represent 10-20 percent of all deliveries in India. The average length of gestation in India is 38.5 weeks versus a normal term of 40 weeks. With 1 1/2 weeks longer gestation, the average weight of the fetus can increase 150-200 g.

Determinants and Interventions

In order to define research priorities, an extensive review of world and Indian literature was commissioned by USAID to elucidate the causes of preterm deliveries and IUGR, and interventions to increase birth weight. Maternal factors most commonly associated with LBW in India are: malnutrition, poor antenatal care, bad obstetric history, current obstetric problems (toxemia, vaginal bleeding), young age, short interbirth interval (less than 2 years), and physical exertion. Genito-urinary and other maternal infections have not been considered a very significant factor in LBW because the prevalence and incidence of maternal infections which have been implicated in LBW elsewhere, is unknown in India and their effect on the outcome of pregnancy has not been studied.

In developed countries, heavy smoking and alcohol consumption have been associated with LBW, but the extent of these practices including chewing of tabacco and betel leaf and their role in LBW are unknown in India. Family planning programs have had some success in raising the age at first pregnancy, lengthening the interbirth interval, and reducing completed family size. Of the other factors for which preventive or therapeutic interventions exist, studies in India and elsewhere have focused most on maternal malnutrition and least on infection and workload. A great deal more research needs to be done, however, on the role these factors play and suitable interventions. It is likely that the high prevalence of both malnutrition and infection in the same group of women in India leads to many LBW infants. The synergistic effect of malnutrition and infection in pregnancy has not been studied.

Maternal Malnutrition

The major nutritional problems during pregnancy result from inadequate food, iron, and folic acid intake prior to and during pregnancy, manifest by low prepregnancy weight (38 kg.) and height (145 cm), anemia, and inadequate weight gain during pregnancy. Deficiencies of zinc, copper, and other nutrients may also play a role but few studies have been done. Dietary restrictions markedly decrease birth weight, and reductions in birth weight of 550 g have been recorded in famines. Poor pregnant Indian women consume 540 kcal less than they should daily and gain only 6 kg over the course of pregnancy when they should gain 12 kg. This inadequate weight gain leads not only to LBW but also to insufficient fat stores to meet the energy demands of nursing. More than half of all nonpregnant and two-thirds of all pregnant women are anemic. Consumption of iron and folic acid tablets during pregnancy has been shown to effectively prevent anemia. These tablets are distributed at health centers throughout the country as part of a national program, but still do not reach most women.

Provision of food supplements to at-risk pregnant women is a part of the ICDS and other national feeding programs. Studies on the impact of maternal food supplementation in India and elsewhere with a 150-250 Kcal increase in daily intake have found small, but statistically significant increases of 50 g in birth weight on average. Increases have been larger in male infants.

Supplementation has been most effective in the second and third trimesters. Furthermore, in Colombia birth weight increases were greater in infants of women chosen for supplementation on the basis of being underweight rather than by the usual criteria of poverty or inadequate diet. Birth weights increased 181 g in malnourished women with weight for height at the sixth month of pregnancy less than 360 g per cm. No studies have been done in India to determine the maternal weight for height threshold, below which food supplements will have greater impact on birth weight. The functional significance of small increases in birth weight (50 g) to reduced perinatal morbidity and mortality or improved long-term growth and development have also not been studied.

The supplementation studies have shown that maternal calorie deficiency is greater and plays a more important role in IUGR than protein deficiency. High-protein supplements may actually depress birth weight. The impact of food supplements on birth weight has not differed much between developed or developing countries. Attempts to reduce sharing of the supplement by providing enough for the whole family have not increased impact, nor has impact been greater with a supplement fitted to individual needs rather than a uniform ration for all women. However, acceptability trials in Gujarat of a food supplement named Matruahar, which was developed by the Protein Foods and Nutrition Development Association of India expressly to meet the traditional tastes of pregnant women, have shown reduced sharing of the supplement with the rest of the family and greater consumption by pregnant women. An essential review of the state of the art of maternal supplementation is "Effects of Changes in Protein and Calorie Intake during Pregnancy on the Growth of the Human Fetus" by David Rush, March 1982.

Maternal Infection

The treatable infections and organisms most commonly associated with LBW are malaria, syphilis, gonorrhea, urinary tract infections, asymptomatic bacteriuria, and other genito-urinary infections, caused by Mycoplasma hominis, Ureaplasma urealyticum, Chlamydia trachomatis and Group B Streptococcus. These infections are most detrimental in the last three months of pregnancy and probably lead to LBW through stimulating preterm delivery rather than IUGR. Most studies of

the relationship between these maternal infections and pregnancy outcome have been done recently in developed countries and not in India. The prevalence of these and other infections among pregnant Indian women and the role they play in LBW are virtually unknown.

Few attempts have been made anywhere to increase birth weight by screening for these infections during pregnancy and providing chemotherapy. One study in the United States found that women infected with Mycoplasma gave birth to babies 200 g lighter than those of noninfected women. When infected women were treated for 6 weeks with 1 g daily of erythromycin in the last trimester, birth weights increased 144 g over those of infants of infected, nontreated women. The prevalence of LBW was 3 percent in the treated versus 12 percent in the nontreated group. This is one of the largest effects seen in any controlled treatment trial.

Other observations in the United States on the prevalence of previously suspected organisms and adverse pregnancy outcome have yielded varying results due to failure to control confounding factors in the relationship. Recent advances in microbiologic description of the genital tract flora in and outside of pregnancy leave open the possibility that pathogenic agents not previously considered (e.g., Gardnerella vaginalis or anaerobic organisms) may play a role. There is evidence that an immune response demonstrating current or even recent primary infection with Mycoplasma, Ureaplasma, or Chlamydia may correlate better with LBW than mere presence or absence of the organisms. Very preliminary data from studies in San Diego suggest that different bacteria produce varying amounts of phospholipase A, an enzyme involved in metabolism of a precursor for prostaglandin, a potent stimulant of uterine contraction. This finding lends support to the theory that infections lead to LBW through stimulating preterm delivery. Given the current state of knowledge, prospects are uncertain, even under the best circumstances of scientific research, for early resolution of whether and which infections are major causes of LBW.

Research Priorities

Through the above literature review and consultations with Indian researchers and GOI officials, USAID has been able to identify research priorities for determining the technical feasibility and cost of improving birth weights of Indian infants. Studies need to be conducted to:

1. Test simple athropometric measures of women to accurately predict or detect normal fetal growth or IUGR, e.g. weight gain, height, fundal height, abdominal girth, triceps skinfold, subscapular skinfold, arm circumference and weight for height prior to and during pregnancy.

2. Determine the prevalence in pregnant Indian women of infections suspected to play a role in LBW, and to correlate these infections with fetal outcome, birth weight, maternal nutritional status, and other factors.

3. Assess the impact on pregnancy outcome and cost of chemoprophylaxis or chemotherapy for the infections identified in study 1 and correlate with maternal nutritional status and other factors.

4. Assess the impact and cost of food supplementation on pregnancy outcome in women with anthropometric signs of malnutrition and IUGR, using measures developed in study 1 and correlate with infections during pregnancy and other factors.

5. Assess the synergistic impact on pregnancy outcome and cost of food supplementation and infection control in malnourished, infected women.

6. Follow the growth and survival in the first year of life of children of women receiving interventions 3, 4, and 5.

Below is a list, although not necessarily exhaustive, of the kinds of exposure (risk) factors and outcome variables that should be sought under the best of circumstances.

Demographic/Social: Age, caste/religion, income, work habits, dwelling type, cigarette and betel use, family type.

Medical & Obstetric History: Last Menstrual Period (LMP); number of pregnancies, number of children (living and dead), number of abortions, number of stillbirths; past LBW children, recognized complications, perinatal mortality; symptoms in past pregnancies - genito-urinary, fever.

Current Pregnancy: Fever, productive cough, skin lesions, diabetes, abnormal lie or presentation; sexual activity - habits by week, partners.

Examination:

- Edema
- Temperature
- Blood Pressure and Glucose
- Smear for malaria where history of fever/chills or current fever

- Hemoglobin, hematocrit,weight, height, fundal height, triceps skinfold, subscapular skinfold, arm circumference, abdominal girth, and other nutritional measures

- Vaginal fluid culture for: Mycoplasma, Ureaplasma, Group B Streptococcus.

Cervical culture - Chlamydia, Gonococcus,
Urine albumin, and sugar

- Quantification and identification of usual organisms, especially E. coli, Klebsiella, Enterobacter, Proteus, Pseudomonas, Enterococcus

Serum specimens (2-3 cc) - paired
Syphilis - STS
Mycoplasmacidal Ab, other
Chlamydia - microimmuno - fluorescence
Malaria

<u>Pregnancy Outcome</u>
Eclampsia
Spontaneous abortion
Stillbirth
Sepsis
Preterm prolonged rupture of membranes
Preterm delivery

<u>Infant</u>
Gestational age

Birth weight, length, and head circumference
Perinatal, neonatal, and post neonatal mortality, morbidity, and growth in first year of life correlated with birth weight.

Ongoing Research in India

There are no studies underway in India that address the research priorities identifed by USAID. However, several studies are relevant to the proposed research. Of most interest is an ICMR-sponsored national collaborative study to identify high-risk pregnancies and follow their outcome with particular reference to maternal nutrition, low birth weight, perinatal and infant morbidity and mortality in seven rural and urban slum areas, covering total population of 30,000 each. The rural areas being studied are Chandigarh, Hyderabad, Varanasi, and Trivandrum. The urban slums are Calcutta, Delhi, and Madras. The study commenced in 1981 and the last babies of enrolled women will be born in September 1983 and followed till September 1984. Dr. S.K. Bhargava of Safdarjang Hospital is one of the principal investigators and overall coordinator.

Each of the seven centers was expected to generate data on 1,000 pregnant women based on prevailing birth and infant mortality rates. However, actual enrollment has been only about 40 percent of this target. The importance of this study is that careful design, execution, and analysis should assure high quality and comparability and thereby provide an accurate picture of adverse pregnancy outcome in both urban and rural settings. Additionally, all children will be followed for one year after birth, so relatively detailed information on growth and development, major illnesses, and causes of death should become available. No maternal infection data will be collected.

The study is intended to describe the existing situation and as such no interventions are included. After the results have been analyzed, the ICMR plans to follow up with appropriate intervention trials in 1984-85. Intensive neonatal care units will be established to provide regional perinatal care at Safdarjang Hospital, Delhi; B.J. Medical College, Ahmedabad; and JIPMER Hospital, Pondicherry. Interventions could be tested at these sites.

Other observational as well as intervention studies on maternal nutrition, infection, and LBW are under way at the National Institute of Nutrition, Hyderabad. They are studying the effects of anemia and supplementation with iron and folic acid on LBW. They have studied the prevalence of common infections, i.e., respiratory, gastrointestinal, skin, and vaginal, in pregnancy and found no effect on birth weight. Fetal growth monitoring measurements such as abdominal girth, fundal height, and maternal weight gain are being tested by NIN.

Studies on anemia, maternal malnutrition, supplementation, and IUGR are underway at Banaras Hindu University. The M.S. University of Baroda, Department of Food and Nutrition, is doing acceptability trials on specially formulated maternal food supplements.

Capability for Conducting Proposed Research

The LBW problem is great in India and the stakes are high. However, a number of obstacles that could threaten the feasibility or validity of conducting the proposed studies need to be reviewed. The hurdles to be overcome are especially great for the study of maternal infection, a research area which is not settled by the studies that are underway in the United States. There are probably a greater number of cofactors contributing to preterm delivery and IUGR in India then in the United States, making it more difficult to determine cause and effect.

There are few investigators in India with experience in the design, execution, and analysis of large-scale population studies of the type proposed. Biostatistical support in ongoing studies is frequently weak. Interrelationships and confounding variables that should be analyzed, frequently are not. Microbiologic and serologic capabilities for Mycoplasma exist only at the laboratories at AIIMS and at the Post Graduate Institute of Medical Education and Research (PGI), Chandigarh. No Indian laboratories possess these capabilities for Chlamydia. Current serologic laboratory methods for these microorganisms are difficult and slow, but newer and simpler methods are evolving in the United States. Indian researchers would need to be trained at experienced laboratories in the United States and to be retrained in newer methods through continual collaboration during the study. A reference laboratory would need to be established in India for quality control. It is not clear how easily paramedical workers can be trained to perform interviews or to obtain vaginal, cervical, or blood samples outside the hospital setting. High turnover of research staff is also a problem.

Logistical difficulties arise from lack of transport, equipment, and cold-chain capacity for preserving biologic specimens. Losses of specimens could occur at several points. Local ground transportation can be uncertin, particularly in the wet season. A reliable supply of dry ice is not always available. Multiple transfers between airplanes and ground vehicles may account for losses in submissions to the reference laboratory. Certain laboratory supplies and equipment are not available in India and will need to be imported from the United States. Inexpensive, portable scales are not available in India or elsewhere for weighing pregnant women in field studies. The

scales used in the seven-center study of high-risk pregnancy weigh 12 kg and were imported from the United Kingdom at a cost of $1,800 each. Applications for customs waivers and clearance for importation due to nonmanufacture in India can take many months to obtain.

A number of problems also result from lack of cooperation and low response rates of pregnant women. It is difficult to enroll women until late in pregnancy (after 28 weeks), because they conceal the fact that they are pregnant for superstitious and other reasons. Many rural women seek no professional antenatal care. Movement of village women within a study area to a place outside during the latter part of pregnancy, e.g. to the parents' home (15-70 percent), reduces followup completeness, although it may not significantly bias results. This is particularly true of the first delivery and is less common in urban areas.

Perhaps the most serious response obstacle is the refusal of women outside the hospital or health center to permit an internal examination by speculum, or blood sampling by venipuncture or finger prick, especially on more than one occasion. The problem appears to be more or less uniform throughout India and overcoming it will require a rather intensive educational effort. Similarly, there may be important questions of medical history that the women cannot answer accurately (e.g., date of last menstrual period, prepregnancy weight, previous LBW outcome) or will not answer accurately (e.g., sexual practices). A local events calendar may need to be developed to help assess gestational age. It has been difficult in some studies to get clean-voided urine specimens.

In the absence of a reference laboratory in India on the microorganisms being studied and during the early stages of establishing one, quality control and standardization will need to be achieved by sending random samples of duplicate specimens to the United States. In a similar fashion, a sample of epidemiologic data should also be independently analyzed. Restrictions against exchange of biologic samples and data between Indian and U.S. collaborators will need to be overcome.

Whether these and other obstacles can be circumvented or eliminated without seriously compromising the value of the research will have to be carefully assessed from the earliest stages of the project and reassessed at each phase. It is preferable that these studies be rural community-based. However, if the obstacles to conducting rural studies cannot be overcome, urban and hospital-based studies are a second best alternative.

The current cost of treating a Mycoplasma infection with erythromycin for six weeks of Rs. 168 (@ Rs.4.0/g) may be prohibitive given the current budget for drugs at the PHC. Food

supplementation programs during the last trimester are likely to cost Rs. 100. The cost benefit and effectiveness of various interventions to prevent LBW will have to be calculated as an integral part of the research.

Capability of Various Institutes

Fourteen Indian institutes were visited and their capability for the proposed research was assessed, based on the following criteria:

1. Research - History of commitment to research accomplishments, including specific references in laboratory or field projects relevant to the proposed studies.

2. Laboratory - Capabilities in routine bacteriology, anaerobic bacteriology, and other special studies; experience in *Mycomplasma* isolation; for *Chlamydia* isolation including virology; experience in serologic diagnostics; and current facilities and staffing.

3. Field - Longevity of work in community, access to hospital and community population, village projects; organization, accomplishments, and goals of community projects; field staff and facilities, center-base project staff; and relationships apparent on site visit.

4. Clinical - Background and potential time demands among obstetrical and pediatric professionals; thinking about the issues of proposed study.

5. Interest/Commitment - Collective response to the idea of the study, willingness to be part of a collaborative effort, control of resources and environment.

Three institutes were identified at which the potential for conducting studies on the prevalence of maternal infections, malnutrition and pregnancy outcome is good: (1) Post Graduate Institute of Medical Education and Research (PGI), Chandigarh; (2) Christian Medical College (CMC), Vellore; and (3) King Edward Memorial Hospital (KEM), Pune. Two of these institutes could serve as reference laboratories for the infection studies (PGI, Chandigarh and CMC, Vellore); and all three could serve as sites for field studies. A description of these institutes and the reasons why they were chosen follows:

Post Graduate Institute of Medical Education and Research (PGI), Chandigarh. This modern sophisticated facility is academically oriented. The clinical staff in the critical departments are actively involved in research and can probably devote a greater portion of time to it because of fewer service obligations. They seemed receptive to the proposed research as outlined, remaining appropriately skeptical about any study that would not thoroughly examine the major variables.

Their microbiology laboratories are extremely well organized and equipped compared to the other laboratories visited. They are busy but relatively well staffed, and research obviously occupies a substantial portion of their time. For example, they are doing Mycoplasma cultures now and are planning Chlamydia work for the near future.

The field and community activities are also well established through participation in the ICMR seven-center study and in separate, longer lived projects, (e.g., on intervention for high-risk mothers and infants). Although the community program was not personally observed, it did not appear that the field activities had quite the ample support that the hospital-based research had. The uncertain capabilities here are not so much greater than at the other centers as to offset the obvious advantages. Good transportation access by air and land would make PGI a suitable reference laboratory as well as study center.

Christian Medical College, Vellore (CMC). The creative leadership and experience of Dr. Jacob John, the director of the ICMR Virology Laboratory, coupled with long-standing capabilities of introducing new lab techniques into India, made CMC attractive as a reference center. Although the laboratory has the basic necessities, some of the equipment is outdated and in need of repair. Also, the need for additional laboratory space might require some remodeling. None of these needs is likely to entail any major capital expenditures.

From discussions with people in the other sections of the microbiology laboratory, it appears that they would be capable of serving the reference function for other microbiologic studies, although they did not express any special commitment to this function. One limitation to CMC serving as a reference laboratory is that there are no air connections to other major cities and all samples would have to be transported overland. There are direct rail connections between Pune and Vellore. On the plus side, CMC has a good working relationship with both the KEM Hospital and the National Virological Institute in Pune and could serve as reference laboratory for them.

As for the other elements of a clinical investigation, the field and research activities of the hospital and university are fairly well developed. However, it was not clear how easy it would be to graft a laboratory-based, epidemiologic research project onto the exisisting community outreach program. The interest or commitment in the population aspects of the project on infection and LBW among the key professionals in the pertinent disciplines other than microbiology did not seem to be as great as elsewhere.

King Edward Memorial (KEM) Hospital, Pune. The enthusiasm about the LBW problem and the proposed research was higher than

elsewhere. Moreover, the research orientation, commitment, and mentality are quite strong under the dynamic directorship of Dr. Banoo Coyaji. Experience in field and clinical investigation on the part of the professionals who would be involved is another advantage. Although their five years of experience in both research and service in the 19 rural villages in the VADU project certainly favors the accomplishment of the most difficult field components of the study, even there the formidable obstacles common to all the institutions will challenge their capabilities.

The weakness of the institution, they are frank to admit, is the microbiology laboratory. Minimal equipment and absent research expertise would make them dependent for training and technology transfer on another institution. Proximity to the National Virological Institute, with which working relationships are already established, may offset this deficiency. Microbiologic technology transfer would not be a major difficulty. Air connections to Bombay facilitate transport of samples, and they regularly send specimens back and forth to England without much trouble.

Research institutes for intervention trials to be conducted during the latter years of the research program will be identified, based on the outcome of the prevalence studies and the types of interventions proposed. Institutes to be considered would most logically be the three prevalence study centers, but the seven centers in the ICMR study of high-risk pregnancy and the regional perinatal care centers to be established by ICMR could also be considered.

ATTACHMENT 2: Investigators and Collaborating Institutes

India

Christian Medical College, Vellore

Dr. Jacob John	Department of Microbiology
Dr. Mark Steinhoff	Department of Microbiology
Dr. Sulechana Abraham	Community Health and Development Center (CHAD) Obstetrics Division
Dr. J. Prakash	Community Health and Development Center (CHAD) Obstetrics Division
Dr. Prabha Jairaj	Department of Obstetrics and Gynaecology
Dr. Balasubramaniam	Department of Obstetrics and Gynaecology
Dr. Lalitha	Department of Bacteriology
Dr. G. Sridharan	Department of Bacteriology

King Edward Memorial Hospital, Pune

Dr. Banoo Coyaji	Obstetric and Medical Director
Dr. Anand Pandit	Department of Pediatrics
Dr. Rao	Research Director
Dr. Kulkarni	Vadu Rural Health Project Director
Dr. K.B. Niphadaker	Department of Microbiology

Post Graduate Institute of Medical Education and Research, Chandigarh

Dr. G.I. Dhall	Department of Obstetrics and Gynaecology
Dr. Kamla Dhall	Department of Obstetrics and Gynaecology
Dr. K.C. Agarwal	Department of Microbiology and Parasitology

Dr. N.K. Ganguli	Department of Microbiology and Parasitology
Dr. R.C. Mahajan	Department of Microbiology and Parasistology
Dr. Vijay Kumar	Department of Community Medicine
Dr. B.M.S. Walia	Department of Pediatrics
Dr. Bakoo	Department of Pediatrics

United States

Albert Einstein College of Medicine, New York, NY

Dr. David Rush	Division of Pediatric and Perinatal Epidemiology

Centers for Disease Control, Atlanta, Georgia

Dr. Russell Alexander	Operation Research Branch Venereal Diseases Control Division

Harvard University, Boston, Massachusetts

Dr. Julia Walsh	Channing Laboratory
Dr. Ed Kass	Channing Laboratory
Dr. M.G. Herrea	Department of Nutrition, School of Public Health

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Dr. Richard Kaslow	Epidemiology and Biometry Section

ATTACHMENT 3: STANDARDIZED METHODS FOR STUDY OF MATERNAL INFECTIONS (PHASES I AND II)

Specimen Processing Details - Hospital

Urine - Within 30 minutes of collection in a sterile container, urine will be taken to the laboratory.

Swabs - endocervical swabs will be inserted as follows:

1. Ca Alginate - immediately into 2.0 ml of phosphate buffered sucrose, placed in icebox and, within 6 hours, taken to the laboratory, aliquoted X4, and frozen at minus 70 degrees centigrade, until inoculation.

2. Cotton - immediately into 2.0 ml of trypticase soy broth with BSA and penicillin, placed in icebox, aliquoted X4, and frozen until incoluation.

3. Cotton - immediately into 2 ml of Todd-Hewitt broth with nalidixic acid, polymyxin, and crystal violet; aliquoted X4, and frozen until inoculation.

4. Cotton - immediately onto Thayer-Martin agar and held for inoculation.

Vaginal swab will be inserted immediately into 2.0 ml of supplemented trypticase soy broth as in 2 above.

Rectal swab will be inoculated immediately into Todd-Hewitt broth (see 3 above).

Blood - will be separated from the cells within 1 hour, or as soon as possible, aliquoted into a 3 cc and at least three 0.5 cc vials, and frozen.

Placentas - detail to be provided.

Laboratory Methods

Chlamydia trachomatis

Endocervical swabs (calcium alginate) will be placed in 1.0 ml. of 0.2 m. sucrose in 0.02 m phosphate buffer (25 p) and frozen at 70 degrees C until inoculation. At inoculation vials will be thawed, specimen vortexed, all liquid expressed from swab, and 0.1 ml per vial inoculated on to Mccoy cells in a cycloheximide microtiter system. Initial antigen recognition in tissue culture will be by iodine stain, but this will be replaced during the study by use of C.trachomatis-specific monoclonal antibody. If current evaluation suggests that the direct slide test could replace cultures for antigen recognition, this technique will be introduced.

Serologies will be performed using the simplified microimmunofluorescent method of Wang. In women with detectable anti-chlamydial antibody, both IgG and IgM titres will be determined.

M. hominis/U.urealyticun culture

Endocervical swabs are placed in transport broth (trypticase soy broth with 0.5 percent bovine serum alubumin and added penicillin) and frozen at -70 degree C until inoculation. At that time 0.2 ml of transport broth is inoculated into 1.0 ml of urea broth for Ureaplasma urealyticum and into 1.0 ml of arginine broth for M.hominis. Specimens in which the ph rises are subcultured to mycoplasma agar. Sensitivity disks impregnated with antisera to the three major M. hominis strains and to erythromycin are placed at either end of streaks through the colonies. Plates are incubated at 37°C in a nitrogen/carbon dioxide atmosphere and examined after 2-4 days. Ureaplasma are identified by their characteristic colonial morphology, inhibition of growth around the disk containing ergthromycin, and persistence of growth around the disk imypregnated with antiseram to M. hominis. M. hominis is identified by its colonial morphology and by inhibition of growth around the disk containing specfic antiserum.

Mycoplasma antibody, at least at the start of the study, will be determined by mycoplasmicidal determination of Lin. The method consists of mixing serial dilutions of the patients's serum with a standard inculum of mycoplasma and a constant amount of complement. The medium is deficient in urea for ureaplasma, and in arginine for M. Hominis. After 30 minutes of incubation, the specific substrate is added and color change is read on day 5. Killing is read as absence of color change.

Group B Streptococcus

Cervical, vaginal, and rectal swabs will be placed in 2 ml NPC broth and deep frozen at 70°C. This same broth is used for culture. It is a selective medium which consists of Todd-Hewitt broth with nalidixic acid, polymyxin, and crystal violet. Broth cultures will be subcultured into NPC agar, which consists of trypticase soy agar base with 5 percent sheep blood, plus nalidixic acid polymixin and crystal violet. B hemolytic colonies will be serogrouped.

On a subsample, group B streptococcal serologies will be performed, initially by Dr. Dennis Kasper of Channing Laboratory, although this may be transferable later. The method consists of a radioactive antigen binding assay (Baker). Antibody to all for capsular polysaccharides is determined.

Gonocceus

Swabs will be streaked into Thayer-Martin media in the clinic. In the rural portion of the study, either transport methods may be evaluated (e.g., culturette). Plates will be transported from the field in a candle jar or equivalent. A sample of gonococci will be serotyped.

Urine cultures

Urine will be transported to the laboratory in sterile containers and inoculated onto blood agar and McConkey using calibrated loops.

ATTACHMENTS

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REGISTRATION FORM

NAME _____ AGE _____

ADDRESS/SECTION/VILLAGE _____

PARITY _____ LMP _____ EDC _____

WHERE PLANNING TO DELIVER _____

(Copy of this information in delivery area.)

PRENATAL CLINIC VISIT FORM

NUMBER OF WEEKS

GESTATION _____

STUDY NO. _____ NAME _____ AGE _____

FIRST VISIT _____ SECOND VISIT _____ THIRD VISIT _____

1. URINE SPECIMEN: TAKEN _____ NOT TAKEN _____
2. BLOOD PRESSURE _____ / _____ ; _____ / _____
3. HEIGHT _____ WEIGHT _____
4. ARM CIRCUMFERENCE _____
5. TRICEPS SKINFOLD SUBSCAPULAR SKINFOLD _____
6. FUNDAL HEIGHT _____ ABDOMINAL GIRTH _____
7. PELVIC EXAMINATION & CULTURES

   a. Vagina

   1. Redness Check all applicable

   2. Discharge

      a. Scant a. Yellow a. Thin

      b. Moderate b. White b. Creamy

      c. Heavy

   3. (Holmes protocol modified)

   4. Swabs (1) (describe) - Taken Not Taken

   b. Cervix

      Swabs (4) (describe) - Taken Not Taken

8. URINE SPECIMEN: TAKEN _____ NOT TAKEN _____

REGISTRATION QUESTIONNAIRE

Demographic

Education - head of household, woman, literacy, sources of information

Occupations - male, female head

Income # 23

Possessions

Type of house - e.g. # 27

Rooms # 28

Families

Family structure

Sanitation

1. Water facility - protected/unprotected #32 & Source e.g. # 33
2. Waste Disposal - where, type # 29-31

Maternal History

Diet - milk/meat/fruit/vegetables

Habits - tobacco (smoking/chewing), betel, alcohol

Illness - fever, chills, cough, diarrhea, pain or burning on urination, discharge, amount, color, odor, unusual for her

Complications - check list and other open ended

From prenatal visits

Drugs/Therapy for what?

Age at Menarche _____

Age at Marriage - Remarriage _____

Age at First Intercourse _____

Partners other than Current Husband _____

LIST OF PREGNANCIES:

Date of delivery	Cause of Death of child	Date of Death of child	Outcome	Major compli-cations

Date of Last Menstrual Period _____

DELIVERY QUESTIONNAIRE/RECORDINGS

A. Interval Record from ANM/Clinic Notes

Last Clinic Visit:

Date

Weight

Total # of visits recorded

Findings/Diagnoses, e.g. anemia - Treatment or lowest Hg.

1. Edema, proteinuria

2. Fever

3. Chills

4. Cough

5. Diarrhea

6. Urinary burning pain

7. Vaginal bleeding

QUESTIONNAIRE

Had edema

Fever

Chills

Vaginal bleeding

Other

Measure of physical activity

Plan to breast feed?

Sexual history (frequency of intercourse in previous two weeks)

B. Delivery

Date

Time labor began

Time membranes ruptured

Date and Time of delivery

Tyupe of delivery - c/s, vaginal, forceps, presentation

Anesthesia

Episiotomy

C. Neonate

Date & time of birth, # _____ , Sex

Weight , Head Circumference _____, Length

Resuscitation] from

Respiratory distress] notes

Congenital anomalies

ATTACHMENT 5

BUDGET WORKSHEET

PGI - CHANDIGARH

Source and Estimated Cost

Item	Year	Quantity	Unit Cost US $	Unit Cost Rs.	Total US $	Total Rs.
Epifluroscent microscope, Zeiss, West Germany, with 2 filters	I	1	13,000		13,000	
Standard freezer (-20 degree C)	I	1		2,500		2,500
Ultra freezer (-70 degree C) - Revco	I	1	7,000		7,000	
Refrigerated centrifuge - IEC	I	1	7,000		7,000	
CO2-Incubator with 3-way gas intake	I	1	5,000		5,000	
Two Temp water bath	I	1	1,500		1,500	
Biohazard hood with laminar flow and air filter	I	1	7,000		7,000	
Airconditioners - 1 1/2 ton	I	2		18,000		36,000
Voltage regulator-large central	I	1		5,000		5,000
1 room for equipment	I	1		10,000		10,000
Liquid nitrogen container - large	I	2	600		1,200	
Electronic precision Balance - Mettler	I	1	3,000		3,000	
Adult scale] Baby Scale] Length/Weight measuring device]	I	3 of each			1,000	10,000
Skin fold calipers] Tape measure]	III IV	5 of each			2,500	25,000
Serology Equipment - ELISA T/or RIA ELISA plates -800 plates Calculator	III		16,000		16,000	
TOTAL Year I					45,700	63,500
Year III-IV					18,500	25,000

K E M - PUNE

Source and Estimated Cost

Item	Year	Quantity	Unit Cost US $	Unit Cost Rs.	Total US $	Total Rs.
CO_2 Incubator (mid size approximately 1.5 ml. high	I	2	5,000		10,000	
Hot Air Oven	I	1		2,500		2,500
Water bath 2 temperature	I	1		1,500		1,500
Colony Counter	I	1		1,000		1,000
Autoclave	I	1		8,000		8,000
Universal centrifuge - floor model	I	1		5,000		5,000
Table top centifuge	I	1		2,000		2,000
Refrigerator (+4 degree C)	I	2		10,000		20,000
Deep freeze (-20 degree C)	I	1		2,500		2,500
Low temp freezer (-70 degree C) - Indian made	I	1		40,000		40,000
Binocular microscope (4 oculars - 10X, 40X, 100X, oil) - Carl Zeiss-Jena (available in Bombay with Rupee payment)	I	2		30,000		60,000
Disecting microscope - Carl Zeiss-Jena (available in Bombay with Rupee payment)	I	1		15,000		15,000
Inverted microscope - Carl Zeiss-Jena (available in Bombay with Rupee payment)	I	1		15,000		15,000
Vortex	I	2		600		1,200
Magnetic Stirier	I	2		600		1,200
Vacuum pump & gauge	I	1		3,500		3,500
Seitz filter assemply	I	3		1,200		3,600
Analytical balance	I	1		5,000		5,000

K E M - PUNE

Source and Estimated Cost

Item	Year	Quantity	Unit Cost US $	Unit Cost Rs.	Total US $	Total Rs.
Electronic Precision balance (Microwa)	I	1	3,000		3,000	
Generator - 20 KV	I	1		15,000		15,000
Cold Boxes	I	4		500		2,000
Central voltage regulator - servo type	I	1		5,000		5,000
Liquid nitrogen containers						
Large	I	1	500		500	
Medium	I	1	500		500	
Small	I	2	300		600	
Gas tank valves	I	4		300		1,200
Gas mixer	I	1		5,000		5,000
Room remodeling	I	2		15,000		30,000
Refrigerated Centrifuge - IEC	I	1	7,000		7,000	
Epifluorescent microscope - Zeiss, West Germany	I	1	13,000		13,000	
Airconditioners - 1 ton	I	4		18,000		72,000
Biohazard Hood - Laminar Flow & air filters	I	1	7,000		7,000	
Adult scale] Skinfold calipers] Length/height measuring devise (adult & baby)] Scale for weighing infants] Tape measure]	I	3 of each			1,000	10,000
	III-IV	5 of each			2,500	25,000
Extra Media for Bacteriology (GBS, GC, urine)	I-III	1		2,000		2,000
	IV	1		9,000		9,000
Total Year I					42,600	329,200
III & IV					2,500	34,000

CMC - Vellore

Source and Estimated Cost

Item	Year	Quantity	Unit Cost US $	Unit Cost Rs.	Total US $	Total Rs.
Epifluorescent microscope - Leitz	I	1	14,000		14,000	
Inverted microscope - Leitz	I	1	1,200		1,200	
Dissecting microscope - Leitz with carriers for plates	I	1	1,300		1,300	
Refrigerated centrifuge - IEC	I	1	6,000		6,000	
CO_2 incubator (approximately 4 ft. high - mid size)	I	2	5,000		10,000	
Electronic balance - Mettler	I	1	3,000		3,000	
Biohazard hood with laminar flow and airfilter	I	1	7,000		7,000	
Refrigerator (+4 degree C)	I	1		10,000		10,000
Standard freezer (-20 degree C)	I	1		2,500		2,500
Ultra freezer (-70 degree C)	I	1		40,000		40,000
Water bath - 2 temp	I	1		1,500		1,500
vacuum pump and gauge	I	2		3,500		7,000
Gas mixer	I	2		5,000		10,000
Liquid nitrogen transport	I	2	500		1,000	
Container large + small	I	2	300		600	
Generator - 20 Kv	I	1		14,000		14,000
Central Voltage regulator servo-type	I	1		3,000		3,000
Gas tank valves	I	4		300		1,200

CMC - Vellore

Source and Estimated Cost

Item	Year	Quantity	Unit Cost US $	Unit Cost Rs.	Total US $	Total Rs.
Glass Dessicator with Evacuation tubes/catalyst	I	1		500		500
Cold boxes	I	4		125		500
PH meter	I	1		3,000		3,000
Magnetic stirrers	I	2		600		1,200
Vortex	I	2		600		1,200
Air conditioners 1 1/2 tons	I	4		18,000		72,000
Remodelling room for culture	I	1		15,000		15,000
Remodelling room for dark room	I	1		5,000		5,000
Serology equipment - ELISA +/or RIA - ELISA reader plate calculator	III	1	16,000		16,000	
Skin-fold calipers Baby scales Adult scales	I	3 of each			1,000	10,000
Height & length measuring devices Tape Measure	III-IV	5 of each			2,500	25,000
Bacteriology cultures Rs.120 per person + 10% inflation	I+ II+ III	1		79,000		79,000
	III+ IV	1		400,000		400,000
Total	I				45,100	276,600
	III				18,500	425,000

Nutrition Study Sites

Source and Estimated Cost

Item	Year	Quantity	Total US $	Total Rs.
Adult Scale	IV	22		
Baby Scale	IV	22		
Baby Scale (Spring)		220		
Height & Length Measuring Device	IV	22		
Tape Measure	IV	22		
Skin Fold Caliper	IV	22		
TOTAL			8,000	80,000
Other Supplies, Questionnaires, etc.	IV			140,000
TOTAL	IV		8,000	220,000

ICMR - New Delhi

Source and Estimated Cost

The following spare parts and peripheral equipment are required for the H.P.1,000 RIE IV B Computer System at ICMR:

Item	Year	Quantity	Total US $
Data entry terminals	I	2]	
Multiphaser	I	1]	50,000
Additional 64 K memory	I	1]	
Disc Drive 20 MB	I	1]	

MEDIA, REAGENTS AND GLASSWARE -- EACH CENTER

Total Year I + II Import from U.S.A.	PPLO Broth] Pilfro PPLO Agar] Horse serum Fetal calfserum Monoclonal antibodies Fluorescein - conjugated antibodies ELISA plates Selicon plugged tubes Dacron - Aluminium swabs MES buffer Ice packs - (koolit) 113 Adams ft. Medfield, MA 02052	$ 5,000
Other reagents available in India	Total each center Year I-III	Rs. 60,000
Transport (including petrol and vehicle rental and maintenance)		Rs. 40,000
	Annual Cost Year I + II + III Year IV + V + VI	Rs.100,000
Cost of vehicle (Matador - Diesel - 16 seats)	Year I	Rs. 97,000
	Year IV additional vehicle	Rs.128,000
Other: Contingencies	Printing, local travel, international and local transport of specimens, stationery, training of personnel, repair & maintenance Annual Cost	Rs. 75,000

DRUG COSTS FOR THE INTERVENTION STUDY YEARS 4-6

	Weeks of Drug
800 women treated for 2 weeks	1,600
800 women treated for 6 weeks	4,800
800 partners of chamydia positive women treated for 1 week	800
800 partners of mycoplasma positive women treated for 2 weeks	1,600
	8,800

Drug taken qid X 7 days per week = 28 pills/week
Therefore 8,800 (28) = 246,400 pills
(half erythromycin and half placebo)

Erythromycin costs Re.1/pill = Rs.123,200

Estimate placebo costs Re.0.5/pill = Rs. 61,600

= Rs.184,800

Estimate 30% loss of pills and non compliance.
Therefore treat 30% additional women + partners
= 184,800 (1.3) Rs 240,200