Attachment 1.

Date 2.9.86. 2/3/10/86

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. S.K.Roy.

Application No. 86-033 (Revised)

Title of Study INTAKE & ABSORTION OF NUTRIENTS IN PERSISTENT DIARRHOEA (PHASE I)

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B) WHO.

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

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

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

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

(PTO)

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

S.K.Roy

Principal Investigator

OCT 29 1986

Trainee

86-033(Revised)
23/10/86

SECTION 1 - RESEARCH PROTOCOL

1. TITLE: NET INTAKE AND NUTRIENT ABSORPTION FROM DEFINED DIETS IN PERSISTENT DIARRHOEA (PHASE I)

2. PRINCIPAL INVESTIGATOR: Dr. S.K. Roy

   CO-INVESTIGATORS: Dr. R. Haider, A.N. Alam, and Dr. F.C. Patra

   CONSULTANT: Prof. R. Eeckels, Dr. A.M. Molla

   COLLAB. INVESTIGATOR: Prof. M.S. Akbar, Dhaka Shishu Hospital

3. STARTING DATE: October 1986.

4. COMPLETION DATE: September 1987

5. TOTAL DIRECT COST: US $ 32,540

   (POSSIBLE SOURCE OF FUNDING) : WHO

6. SCIENTIFIC DIVISION: This protocol has been approved by the Head, Clinical Sciences Division

Signature of the Acting Head,
Clinical Sciences Division

Date: 22.9.86.

7. ABSTRACT SUMMARY:

Persistent diarrhoea is directly responsible for growth faltering, malnutrition and mortality in children. The cornerstone of successful management of this critical syndrome lies on adequate nutrient absorption by the gastrointestinal tract. Selection of dietary ingredients depends upon exact knowledge of absorption during persistent diarrhoea. For the above purpose, 48 children with persistent diarrhoea will be studied in the metabolic ward of ICDDR,B. Exact amount of nutrient intake and absorption will be measured from familiar diet of defined composition. After rehydration, charcoal marker will be fed; followed by the diet offered at libitum. Tests of absorption for dietary carbohydrate, fat and protein will be performed and coefficient of absorption of the nutrient will be calculated. There will be 2 groups of children, each having a different diet during diarrhoea. A control group of children will be matched for age, sex and nutrition from the hospitalized nondiarrhoeal patients in Dhaka Shishu Hospital.

Comparison of absorption coefficients and other variables among diet groups and controls and their determinants also will be looked for. Available data will be used for formulation of appropriate therapeutic diets for better management of persistent diarrheoa.

8. REVIEWS:

(a) Ethical Review Committee : ----------

(b) Research Review Committee : ----------

(c) Director, ICDDR,B : ----------

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objective.

To estimate net intake and nutrient absorption in persistent diarrhoea using metabolic balance techniques in phase I to be followed by study of pathophysiology in phase II by the next protocol.

2. Background.

Though acute diarrhoea is a major health problem in many developing countries, there maybe hope now for its successful management mainly because of implementation of knowledge obtained from extensive research works during the past decades. Persistent diarrhoea is a life threatening challenge to growth and child survival in both developed and developing countries, mostly due to inadequate understanding of its pathophysiology and nutrient utilization by the gastrointestinal tract. Although ranging from irritable bowel syndrome to rare inborn errors of metabolism all types of long continued diarrhoea may fall under the term 'chronic diarrhoea', a vast majority of persistent diarrhoea in children with failure to thrive can begin with an acute episode of gastroenteritis. Though a definition is still to be agreed upon, a duration of diarrhoea for at least 3 weeks will be acceptable as persistent diarrhoea (Merson MH, 1986). The negative impact of diarrhoea on growth and nutrition is now well documented but this effect is probably more deleterious during persistent diarrhoea. There have been suggestions to define the persistent diarrhoea of acute infective origin as 'persistent postenteritis diarrhoea syndrome in children, (PPDC)

(Sack RB, 1984). The basis of the problem lies in the fact that nutrient absorption is impaired leading to malnutri-tion which in turn affects physiological process of digestion, absorption and metabolism, entering into a vicious cycle. This over simplification of the fact is far from the complex underlying derangements but besides further understanding of this process it is mandatory to formulate some therapeutic and dietary intervention for control of diarrhoea.

Magnitude of the problem

Although prevalence of persistent diarrhoea is lower than that of acute diarrhoea, significant impact of persistent diarrhoea on subsequent malnutrition and mortality has been identified quite early and is being more recognised now. A number of indepth studies have been reported from the developed parts of the world (Avery et al., 1968, Cohlan et al., 1956). On the otherhand several epidemiological studies from developing countries have indicated higher prevalence of persistent diarrhoea in those parts of the world than that of developed ones. It is not unlikely that 5-20% of children suffering from acute gastroenteritis may go on to persistent diarrhoea (Black et al., 1982, Snyder JD et al., 1982).

The persistent diarrhoea of infective origin must be dealt separately from other specific chronic diarrhoea syndromes such as, tropical sprue, tropical enteropathies, intractable and chronic non-specific diarrhoea of infancy, coeliac disease, inflammatory bowel diseases, blind loop syndrome, post surgical syndrome and necrotizing enterocolitis.

Although the complete understanding is far from clear, a considerable amount of information on persistent diarrhoea is now available from both developed and developing countries (Gordon JE, 1971, Gracey M, 1972). The

fact becomes clearer that there are significant differences in aetiology of persistent diarrhoea observed in different geographical and socioeconomic settings. In studies from London (Larcher, 1977), carbohydrate intolerance and milk protein intolerance were shown to be common cause of persistent diarrhoea in children below two years. Reports from Ethiopia, Bangladesh, and Guatemala showed that 4-7% of acute diarrhoeal patients became chronic (Gordon et al., 1968, Black et al, 1982, Mata et al., 1976). From surveillance studies on diarrhoeal diseases in two separate communities in Bangladesh, it has been reported that dysenteric type of illnesses continued to be persistent diarrhoea (Bari et al., 1986) and repeated attacks of acute diarrhoeal episodes lead to persistent diarrhoea (Rahman MM et al., 1986).

Prevalence of carbohydrate malabsorption causing persistent diarrhoea is reported to be common. Malabsorption of lactose was found in 12% of children in U.K. (Larchar et al., 1977), 31% in Australia (Halliday et al., 1982) 22% in Peru (Perez Flores 1972) and 80% in India (Ansari Z et al., 1979). Malabsorption of sucrose is also reported in chronic diarrhoea (Davidson et al., 1982, Chandra RK et al., 1968, Ansari Z et al., 1979). Several studies reported secondary monosaccharide intolerance in persistent diarrhoea (Kilby AM, 1977, Gracey M et al.,1969). Malabsorption of glucose has been successfully managed by replacing glucose with fructose in the diet (Lifshitz 1970, Jalili et al., 1982, Kilby AM, 1977). It can be mentioned that mild degrees of glucose malabsorption may occur in Rotavirus diarrhoea (Sack et al., 1982). Milk protein intolerance has been found in persistent diarrhoea where lactose intolerance might be associated (Lui et al, 1968). B lactoglobulin, fraction of milk protein has been postulated to be the cause of enteropathy, which now has been well recognized (Walker Smith JA, 1978). Similarly, soya protein intolerance is a recognized entity in childre. hitington et al., 1977).

During acute or persistent diarrhoea, there is significant change in mucosal permeability which is more severe in persistent diarrhoea (Ford RPK et al., 1985). It has been seen that antigenic protein uptake is increased during this state while cow's milk protein uptake causes to enteropathy and intolerance (Jackson D et al., 1983) Severe protein loss during diarrhoea can lead to persistent diarrhoea while endogenus protein from serum is reflected by - antitrypsin in stool. Molla et al have found very low co-efficient of absorption for dietary protein probably because of not taking account of endogenus protein loss from increased mucosal permeability, mucosal damage and bacterial protein in stool.

It has been demonstrated in a number of studies that small intestinal mucosal structure and enzymatic function vary significantly between wel-nourished and malnourished children (Chandra RK et al.,1968, Alleyene GAO et al., 1977). Pancreatic enzyme activity also varies according to nutritional status (Jain MK et al., 2986). Further studies on abnormal bacterial overgrowth in small intestine has been reported with significant difference between welnourished and severely malnourished children (Mehta AP et al., 1986). The above phenomena most likely further reduce digestion and absorption during persistent diarrhoea. A comparison of nutrient absorption during persistent diarrhoea with that in absence of diarrhoea needs to be controlled for nutritional status of the children.

Inability of nutrient digestion and absorption by the gastro-intestinal tract per se, may be interrelated with several other intermediate factors. Early supplementation with formula feeds, complex carbohydrates of plant origin, excess undigestable cellulose, and contaminated food continue to be common ingredients for feeding young infants in developing countries.

Although several studies from different parts of the world have reported carbohydrate malabsoroption in persistent diarrhoea, there is no available information on quantitative and qualitative estimates of nutrient intake and utilization in persistent diarrhoea. Such report from Bangladesh is totally lacking and the only insight in this aspect has been studied in ICDDR,B in acute diarrhoea recently (Molla A et al., 1982, Sarker SA et al., 1982). It becomes evident from the information available that, depending on the extent and degree of pathophysiological derangement, gastrointestinal tract fails to handle dietary ingredients leading to a condition of persistent diarrhoea and malnutrition.

To understand this particular aspect we propose to undertake a balance study in young children with persistent diarrhoea to estimate nutrient intake and absorption in the ICDDR,B metabolic ward from defined familiar diets. Control value will be estimated in the similar way from matched non diarrhoeal children admitted to Dhaka Shishu Hospital in a collaborative study.

3. Rationale

A vast majority of malnourished children with persistent diarrhoea need dietary intervention which is still not welunderstood. Bangladesh is highly endemic for diarrhoea and persistent diarrhoea is a threatful challenge to health workers, it thus appears to be an area of priority. The study will be a pioneer one to exactly quantitate nutrient utilization in chronic diarrhoea and will enable to formulate dietary intervention to control persistent diarrhoea. Formulation of appropriate diet will reduce malabsorption and prevent subsequent risk of mortality from persistent diarrhoea.

B. SPECIFIC AIMS

1. To quantitate nutrient and energy intake in children during persistent diarrhoea and to assess the impact of specific diets on the stool output and duration of diarrhoea.

2. To estimate fat, carbohydrate and protein absorption during persistent diarrhoea from defined diet.

3. To carryout the above tests in an age, sex and nutrition matched control group of children without diarrhoea for purpose of comparison.

C. METHODS AND PROCEDURE

Selection of study children

Male children of 3-24 months of age, having diarrhoea with acute onset (excluding bloody mucoid stool) for more than 21 days (PPDC), will be eligible. Those with a component of growth faltering (less than 80% wt. for age compared to NCHS standard) will be selected. Informed consent will be obtained from the legal guardian before recruiting for the study.

INVESTIGATIONS

Investigations will be done after obtaining the full clinical, feeding and socioeconomic information as follows:

- Measurement of nutritional status using recumbent length, body weight, age and mid arm circumference.
- Stool M.E. with formal ether concentration method for identification of Giardia lamblia, Sudan III stain for fat and modified Zehl Neelson stain for cryptosporidium.
- Stool culture for E.coli, ST, LT, EPEC, enterobacteriace, Salmonella, Shigella, and Cholera.

- ELISA for rotavirus

  antitrypsin clearance in stool. (Radial Immunodiffusion method)
- Stool thin layer chromatography for sugar qualitative and quantitative
- Stool pH and glucose
- Permeability test (described briefly in appendix)
- D-Xylose absorption test (" ")

Urine Routine analysis and culture

Blood CBC, platelet, electrolytes and culture if indicated

X-ray chest AP view if indicated

Balance Study

Study diet will be selected according to the scheme outlined in the page 13 which is presently used in ICDDR,B for management of persistent diarrhoea. Patients will be put on study diet 2 days before the balance study. 1st marker will be given as charcoal tablet to be followed by study diet. A 2nd marker will be given after 48 hours at the same time of 1st marker. Time of appearance of 1st marker in stool will be taken as 0 hour. Collection of stool and urine will be started with the appearance of 1st marker untill second marker comes out. 5 ml of acetic acid will be given in the collecting buckets. Forty-eight hours dietary intake will be measured and recorded. All vomitus will also be collected in the same way. These samples will be kept in deep freeze at -20 ^{o}C.

1st marker	2nd marker
e.g., at 10 a.m. on 21/5	10 a.m. on 23/5
marker out	2nd marker out
collection started	48 hr collection
	stopped.
all marker stool will be saved.	

Aliquots of stool samples from homogenized 48 hours collection will be taken and will be analysed for fat (by Vandekamer's method), nitrogen by Microkjheldal method (Henry RJ, 1964) and carbohydrate by subtracting those from total energy. Total energy will be determined by adiabetic Bomb Calorimetry. Dietary contents for fat, carbohydrate and protein will also be analysed. Total daily urinary nitrogen excretion will be estimated. Absorption will be estimated by substracting loss in stool from intake.

$$\text{Absorption co-efficient} = \frac{\text{intake-loss} \times 100}{\text{intake}^{0.75}}$$

Gross energy intake per W kg (metabolic intake) will be calculated to overcome the effect of body size on intake. Absorption per metabolic body size will also be calculated to assess exact utilization of nutrients.

Dietary management

The child's usual home diet will be given till changes are made on dietary regimen on the basis of investigations. Two dietary groups will be made from the existing defined diets in current practice for managment of persistent diarrhoea in ICDDR,B. Rice suzi diet has been used with success in ICDDR,B and comminuted chicken is used in London and other places.

Diets to be used:	Composition/liter	Indication for diet selection
1. Milk, soya and sucrose free diet		
Rice suzi:		Cow's milk protein, Lactose and sucrose intolerance >4 months age >6 months age in presence of grade III PEM
Rice powder	60 g	
Egg	2	
Oil	30 g	
Glucose	20 g	
Kcal	70/100 ml	
2. Milk, soya, sucrose and complex carbohydrate free		
Comminuted chicken:		
Minced chicken	180 g	Severe PEM with intractable diarrhoea Failure with rice suzi <4 months age
Glucose	25 g	
Oil	30 g	
Kcl	1 g	
Papaya	20 g	
Kcal	60/100 ml	

After recovery from diarrhoea, transition home-diet will be given as test feeds. Based on the results of the balance study, investigations and clinical response dietary changes may be made.

Management of cases

Rehydration will be done with I.V. fluid or ORS and 8 hourly intake and output measures will be taken. Vital signs will be checked every 8 hours while study physicians care for 24 hours will be ensured. Appropriate infection control and regular assessment on improvement of diarrhoea will be monitored as shown in appendix.

Safety of the study children and exclusion

Any child developing sepsis, convulsion, enterocolitis, or any other lifethreatening condition will be immediately transferred to the intensive care unit and will be excluded from the study.

STATISTICAL CALCULATION:

Sample size

Assuming the hypothesis that nutrient absorption will be at least 80 percent and unchanged in healthy children, which maybe reduced 25% in acute diarrhoea (Molla A, 1982). There may be at least equal reduction in persistant diarrhoea. A difference of 20% can be expected to be malabsorbed in children with persistent diarrhoea.

As there is no exact information on standard deviation of nutrient malabsorption in chronic diarrhoea an estimate can be assumed from data on acute diarrhoea (Molla A et al., 1982).

Using formula $n \geq \frac{2 \times S^2 \times 7.9}{d^2}$ with 95% confidence limit

(taking care of sensitivity or type I error) and with power 80%, number in each group becomes 16. For 3 groups (2 treatment and 1 control) 16 X 3 = 48 cases will be required for this study.

Variables:

1. Age, nutritional status
2. Previous home feed
3. Nutrient/Energy intake
4. Absorption co-efficient:

   (a) Carbohydrate (2) fat (3) protein (total nitrogen)

5. Days required for recovery
6. Xylose absorption
7. Stool volume

Analysis of data

Data generated from the study will be entered into micro computer and appropriate tests will be selected. Analysis of variance for comparibility, cross tabulations, Chi-squared test, or Student's 't' test as required, Mann Whitene U test, Wilcoxson's rank sum/sign test for comparison,and Regression analysis will be considered.

D. SIGNIFICANCE:

This study has been proposed to increase understanding of nutrient absorption (quantitative and qualitative) in chronic diarrhoea which will help to develop better care in this vulnerable group of patients. This body of knowledge will be of special help to those concerned with pediatric nutrition and feeding programmes.

E. FACILITIES REQUIRED:

1. Office space: The existing office space will be utilized.
2. Laboratory space: The existing lab. space will be utilized.
3. Hospital beds: Present metabolic ward with 2 beds will be occupied.
4. Rent, Communication and Utilities: ICDDR,B transport will be used.
5. Collaborative arrangement with Dhaka Shishu Hospital.

Abstract Summary for Ethical Review Committee

1. The project aims at caring for the most vulnerable children of persistent diarrhoea through defined measures and the investigations will be directly useful to formulate appropriate dietary regimes.

   Children less than 2 years are most vulnerable and will receive standard care at hospital. The investigations will not cause any risk to the child.

2. Informed consent will be obtained from parents or legal guardian of each child. They will be identified by code number to ensure confidentiality.

3. There maybe immediate benefit that persistent diarrhoea in young children will be treated with knowledge of investigations and ICDDR,B general hospital service cannot provide every detailed investigations. The cases will have all necessary dietary intervention and treatment of pathogens.

4. The samples required for investigations are 4 ml blood for Dxylose and routine examination. Stool and urine will be collected as required.

5. There will be record of the patients for use in the study. No other record will be necessary.

REFERENCES

Alleyene GAO, Hay RW, Picou DI, Stanfield JP, Whitehead RG. The pathology of protein energy malnutrition. Edward Ernold. London.1977;pp 244-245.

Ansari Z, Malik AS, Dutta AK, Ahmed SH and Saiahuddin. Prevalence of sugar intolerance in diarrhoea of infancy and childhood. Indian Pediat. xvi;1979,10:879-885

Avery GB, Villavicencio L, Lilly JR, Randolph JG. Intractable diarrhoea in early infancy. Pediatrics. 1968;41:712-722

Balistreri WF. Bile acid induced intestinal dysfunction: implications to protracted infantile diarrhoea and malnutrition. In: Chronic diarrhoea in children. ed. Labenthal E. Nestle, Raven press. New york,1984;pp:347-364,

Bari A. Chandpur project, ICDDR,B (Personal communication) 1986

Behrens R, Docherty H, Elia M, Neale G. A simple enzymatic method for the assay of urine lactulose. Clin Chem Acta. 1983;137; 361-367.

Black RE, Brown KH, Becher A, Alim ARMA, Huq I. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. AM J Epid. 1982;115:315-324,

Chandra RK, Pawa RR, Ghai OP. Sugar intolerance in malnourished infants and children. Brit Med J. 1968;4:611-613

Davidson GP, Robb TA. Children with chronic diarrhoea and altered disaccharidase ratio who respond to sucrose withdrawal. Lancet 1982;2:325,

Ford RPK, Menzies IS, Phillips Ad, Walker-Smith JA, Turner M. Intestinal sugar permeability: Relationship to diarrhoeal disease. J Ped Gastroenterol Nutr.1985;7:468-474.

Gordon JE. Diarrheal disease of early childhood - worldwide sign of the problem. Ann N.Y. Acad Sci. 1971;176:9-15.

Gordon JE. Nutrition and infection field study in guatemalan villages. Arch Environ Hlth.1968;16:423-437.

Gracey M, Burke V, Anderson CM. Association of monosaccharide malabsorption with abnormal intestinal flora. Lancet 1969;2,384.

Gracey M, Stone DE. Small intestinal microflora in Australian aboriginal children with chronic diarrhoea. Aust N Zeal J Med. 1972;3:215-219.

Halliday K, Edmeades R, Shepherd R. Persistant postenterilities diarrhoea in childhood. Med J Aus. 1982;1:18-20.

Henry RJ. Clinical Chemistry: Principles and Technics. Harper and Two. New York, 1964

Jackson D, Walker-Smith JA, Phillips AD. Macromolecular absorption by histologically normal and abnormal small intestinal mucosa in childhood: An invitro study using organ culture. J Ped Gastroenterol Nutr.1983;2:235-248.

Jain MK, Bhui PS, Mehta NJ, Taskar SP, Sane SY, Mehta AP. The role of pancreas: Pancreatic function in malnourished children. In Walker-Smith JA and McNeish AS eds. Diarrhoea and malnutrition in childhood. Butterworths, London, Boston, Sydney. 1986;142-146.

Jalili F, O'Brian Smith E, Nichols VN, Mintz AA, Nichols B. Comparison of acquired monosaccharide intolerance and acute diarrheal syndrome. J Ped Gastro & Nutr. 1982;1:81-89.

Kilby AM, Dolby JM, Honour P, Walkersmith JA. Duodenal bacterial flora in early stages of transient monosaccharide intolerance in infants. Arch Div Childhood. 1977;52:228.

Larchar VF, Shepherd R, Francis DEM, Harries JT. Protracted diarrhea in infancy. Analysis of 82 cases with particular reference to diagnosis and management. Arch Dis Child. 1977;52:597-605.

Lifshitz F, Coello-Ramirez P, Gutierrez-TopeteG, Gutierrez MLC. Monosaccharide intolerance and hypoglycemia in infants with diarrhea. I. Clinical course of 23 infants. J Peds.1970;77:595-603.

Lui H, Tsao MU, Morre B, Giday Z. Bovine milk protein-induced intestinal malabsorption of lactose and fat in infants. Gastroenterology.1968;54:27-34

Mata LJ, Kronmal RA, Garcia B, Butler W, Urrutia JJ, Murillo S. Breast-feeding, weaning and the diarrheal syndrome in a Guatemalan Indian village. In: Acute Diarrhea in Childhood. Ciba Foundation Symposium 42 (new series) June, 1976, Elsevier/North Holland.

Mehta AP, Doiphode Sh, Dhar AP, Jain MK. Bacterial overgrowth in the small intestine in malnourished children. In Walker-Smith JA and McNeish AS eds. Diarrhoea and malnutrition in childhood. Butterworths, London, Boston, Sydney.1986;43-49.

Merson MH, Director. Diarrhoeal Disease Control Programme, WHO, Geneva (personal Communication) 1986.

Molla A, Molla AM, Rahim A, Sarker SA, Mozaffar Z, Rahaman M. Intake and absorption of nutrients in children with cholera and rotavirus infection during acute diarrhoea and after recovery. Nutr Res.1982;2,233-242.

Perez Flores CA. Diarrhea cronica infantil. Doctoral Thesis, Universidad Peruana Cayetano Heredia, Lima, Peru, 1972

Rahaman MM, Aziz KMS, Rahman M, Alam N. Do replated attacks of acute diarrhoea cause chronic diarrhoea? In Walkersmith JA and McNeish AS, ed. Diarrhoea and Malnutrition in Childhood. Butterworths, London, Boston, Durban, Singapore, Toronto, Wellington. 1986;PP 103-106.

Roe JH, and Rice EW. Photomatic method of determination of free pentoses in animal tissues. J Biol Chem. 1948;173;507-512.

Sack DA, Rhoads M, Molla A, Molla AM, Wahed MA. Carbohydrate malabsorption in infants with rotavirus diarrhoea. Am J Clin Nutr. 1982;36: 1112-1118.

Sack RB. Chronic Diarhoea of children living in the developing world : A review. WHO, Geneva, 1983

Sarker SA, Molla AM, Karim AKMM, Rahaman MM. Calorie intake in childhood diarrhea. International Centre for Diarrhoeal Disease Research, Bangladesh, Scientific Report 57, Dhaka, Bangladesh, July 1982

Snyder JD and Merson MH. The magnitude of the global problem of acute diarrhoea disease: a review of active surveillance data. Bull WHO. 1982;60:605-613.

Van de Kamer JH, Ten Bokkel Huinik H, and Weyers HA. Rapid method of determination of fat in feces. J Biol Chem. 1949;12;177-507.

Walkersmith JA, Harrison M, Kilby A, Phillips A, and Francee NE. Cows milk sensitive enteropathy. Arch Dis Childhood. 1978;53:375-380.

Whitington PE, Gibson R, Soyprotein intolerance, four patients with concomitant cow's milk intolerance. Pediatrics. 1977;59:730-732.

Yamanaka K. D-Mannitol dehydrotgenase from leukonostocmesente-roides. In. W. Wood ed. Methods in enzymology, Academic Press, New York, XLI part B,1975; P 138-142.

BUDGET

Personnel

Name	Level No.	%	Time(month)	Rate/m	Annual US$
Dr. S.K. Roy	NO-B	25	12	470	1,410
Dr. A.N. Alam	P-3	-	-	-	-
Dr. R. Haider	NO-A	25	12	360	1,320
Mrs. N. Majid (Dietation)	GS VI	25	12	300	300
Dr. F.C. Patra	-	-	-	-	-
Prof. M.S. Akbar	-	-	-	-	-
Physician	NO-A	100	12	250	3,000
Research Officer	GS-V	50	12	200	1,200
Study Clerk	GS-IV	75	12	190	1,710
Secretary	GS-V	25	12	200	600
					10,140

Supplies and materials

Glass ware	250
Hospital supply	200
Stationery and office supply	300
Chemicals and media	300
Laboratory supply	500
Non-stock supplies	500
	2,350
Rent, communication, utilities	1,000
Printing and publication	500

Interdepartmental services:

	Computer (Programmer 1 for 2 months)	1,000
	Xerox	300
Pathology:	Stool M.E.	200
	Urine R.E.	200
	CBC	200
Microbiology:	Stool C.S., LT, ST	500
	ELISA	350
	Campylobacter	450
	Blood culture	500
Biochemistry:	Fat estimation	750
	Protein estimation	200
	Carbohydrate (TLC)	100
	Energy	200
	Blood electrolyte	400
	D-xylose test	500
X-ray		100
Patient hospitalization		10,000
Maintenance charges		1,000
	Sub Total :	18,850

Budget Summary

Personnel	$10,140	(Conversion rate US$1=Taka30)
Supplies	$ 2,050	
Other	$ 1,500	
Interdepartment	$18,850	
	$32,540	

(Total Direct Cost = US$:33,760)

ICDDR,B CONSENT FORM

(Mechanism of nutrient absorption in persistent diarrhoea)

(Will be read and explained clearly before consent is obtained.)

Your child is suffering from a diarrhoea which usually takes longer period for recovery. In fact there are deficit in knowledge about malabsorption of dietary nutrients in this type of disease.

We are undertaking a study to know this problem more clearly by which better management may be outlined. This study will most likely help your child for earlier recovery and then will be helpful for many other children. There is no risk in this study.

If you agree to put your child in this study, the following steps will be applicable to him/her.

1. He/she will be kept in Metabolic ward and stool, urine vomitus will be collected.

2. An amount of 4 ml blood will be obtained during the study.

3. Your child will be given two harmless tablet and stool collection for 48 hours will be required.

4. Small amount ofSugar solution will be fed and will be examined from urine.

5. He/she will receive intravenus solution as and when required.

6. We shall examine his/her health at hospital regularly.

You will always be at liberty to withdraw from study yet the child will receive treatment in this hosptial.

Signature of the Principal Investigator

Signature of the guardian/ left thumb impression

Date

APPENDIX I

Procedure for D-xylose test

This test will be done within 48 hours of balance study by 4 hours fasting followed by feeding 5 gram D-xylose dissolved in 100 ml of water. After one hour of drinking D-xylose solution, 0.5 ml of venous blood will be drawn for estimation of serum xylose level (Roe JH et al., 1948).

APPENDIX II

Procedure for permeability test

Under supervision of investigator, infants will be given a freshly prepared drink containing 5g lactulose with 0.5g lactose (7.5 ml Duphalac, Duphar Ldd., Southampton, UK) and 1g mannitol in 20 ml 1% Chloroform water. Breast feeding and fluid intake will be encourraged during the test. Urine samples will be collected for 5 hours into adhesive uribags. One drop of 20% v/v chlorhexidine gluconate will be added to each urine collection. Urine volume will be measured and recorded. Aliquots (5 ml) will be taken and stored at -20 ^{o}C. Lactulose and lactose can be measured using an automated enzyme assay system (Behrons R. 1983). Mannitol can be assayed by using a similar assay based on the oxidation of the sugar by mannitol dehydrogenase prepared from Leuconostomesenteriodes (y amanaka K. 1975).

সম্মতি পত্র

দীর্ঘস্থায়ী ডায়রিয়ায় খাদ্য গ্রহণ পরীক্ষা (আই.সি.ডি.ডি.আর,বি)
(সম্মতি লওয়ার পূর্বে বোঝানো হইবে)

আপনার শিশু এমন ডায়রিয়া রোগে ভুগিতেছে যাহা সহজে সারে না। নাড়ীতে খাদ্য হজম এবং ইহার ব্যবহার সম্বন্ধে পূর্ণ জ্ঞানলাভ করিতে এখনও অনেক দেরী আছে। ইহা আরও উত্তমরূপে জানিবার জন্য এবং তদ্দ্বারা আরো ভালো চিকিৎসা পদ্ধতি উদ্ভাবনের জন্য আমরা এই গবেষণা হাতে নিয়াছি। খুব সম্ভবতঃ আপনার শিশু আরো দ্রুত ভালো হবে এবং অন্য শিশুরাও এর দ্বারা উপকৃত হবে। ইহাতে আপনার শিশুর কোন ক্ষতি নেই।

আপনার শিশুকে যদি এই গবেষণায় অন্তর্ভুক্তি করেন তবে নিম্নোক্ত বিষয়গুলি তাহার জন্য প্রযোজ্য হইবে।

১। আপনার শিশুকে আলাদা ঘরে রাখা হইবে এবং মলমূত্র, বমি পরীক্ষা করা হইবে।

২। আপনার শিশুর শরীর হইতে ৪ সিসি রক্ত চিকিৎসার জন্য পরীক্ষা করা হইবে রোগের প্রথমে এবং আরোগ্যের পরে।

৩। আপনার শিশুকে দু'টি নির্দোষ বড়ি খাওয়ানো হইবে এবং মলমূত্র পরীক্ষা করা হইবে।

৪। এক প্রকার চিনির শরবত (৩০ মি: লি:) খাওয়ানো হইবে ও মূত্র পরীক্ষা করা হইবে।

৫। তাহার সার্বিক সুচিকিৎসার জন্য শিরার জন্য বা খাবার স্যালাইন দেওয়া হইবে।

৬। আমরা দৈনিক তাহার দেহ পরীক্ষা করিয়া উপযুক্ত যত্ন নিব।

৭। আপনার শিশুর খবর অন্যকে জানানো হইবে না।

আপনি ইচ্ছা করিলে আপনার শিশুকে এই পরীক্ষা হইতে প্রত্যাহার করিতে পারেন, তবুও তাহার চিকিৎসার নিশ্চয়তা থাকিবে।

প্রধান গবেষকের স্বাক্ষর

অভিভাবকের স্বাক্ষর/টিপসই

তারিখ

ZINC SUPPLEMENTATION IN DIARRHOEA

APPENDIX IV

Date: _____

(MORBIDITY)

Patient No.: _____

Type	Duration	Severity	Treatment
1. Diarrhoea			
2. Dysentery (Blood)			
3. Mucoid			
4. R T I			
5. Fever			
6. Measles			
7. Skin infection			
8. Cough			
9. Mumps			
10. Chichen pox			
11. Ear infection			
12. Oral thrus			
13. Oral sore			
14. Other			