

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Ayesha Molla

Trainee Investigator (if any) \_\_\_\_\_

Application No. 81-027

Supporting Agency (if Non-ICDDR,B) \_\_\_\_\_

Title of Study Hydrogen Breath Test for Estimation of Lactase Malabsorption in healthy volunteers and children with diarrhoea

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
- Are subjects clearly informed about:
  - (a) Nature and purposes of study  Yes No
  - (b) Procedures to be followed including alternatives used  Yes No
  - (c) Physical risks Yes No
  - (d) Sensitive questions Yes No
  - (e) Benefits to be derived  Yes No
  - (f) Right to refuse to participate or to withdraw from study  Yes No
  - (g) Confidential handling of data  Yes No
  - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure  Yes No

- 5. Will signed consent form be required:
  - (a) From subjects  Yes No
  - (b) From parent or guardian (if subjects are minors)  Yes No
- 6. Will precautions be taken to protect anonymity of subjects  Yes No
- 7. Check documents being submitted herewith to Committee:
  - Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
  - Protocol (Required)
  - Abstract Summary (Required)
  - Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
  - Informed consent form for subjects
  - Informed consent form for parent or guardian
  - Procedure for maintaining confidentiality
  - Questionnaire or interview schedule \*

\* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

Ayesha Molla  
Principal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

- 1. TITLE: Hydrogen Breath Test for Estimation of Lactose Malabsorption in Healthy Volunteers and Children with Diarrhoea.
- 2. Principal Investigator : Dr. Ayesha Molla
- Collaborating Investigator : Dr. Joel Mason (4th year Medical Student), Chicago, U. S. A.
- Co-Investigator : Dr. A. M. Molla
- : Dr. Shafique Sarker
- 3. Starting Date : May 1, 1981
- 4. Completion Date : December 31, 1981 (will be extended if required number of patients were not available by this time limit).
- 5. Total Direct Cost : Tk.61,450.00
- 6. Scientific Programme Head : Dr. M. Mujibur Rahaman

This Protocol has been approved by the Nutrition Working Group.

\*Signature of Scientific Programme Head: M. Mujibur Rahaman  
 Date: 23/6/1981

7. Abstract Summary:

The aim of this study is to standardise and establish a simple non invasive test like Hydrogen breath test using Quintron Gas Chromatograph to diagnose Lactase deficiency among the Diarrhoea patients in ICDDR,B. The breath test will also be validated against usual standard Lactose Tolerance Test. Once established, this test may be routinely used to diagnose Malabsorption and a suitable dietary regimen can be formulated for the affected children.

8. Review:

- (a) Ethical Review Committee : \_\_\_\_\_
- (b) Research Review Committee : \_\_\_\_\_
- (c) Director : \_\_\_\_\_
- (d) BMRC : \_\_\_\_\_

ABSTRACT SUMMARY FOR ERC

1. (a) Twenty healthy volunteers of both sexes of age between 20-40 years for standardization of hydrogen breath test technique.
- (b) Fifty children of 6 months to 10 years age group of either sex with a history of diarrhoea will be selected because lactose malabsorption or lactose intolerance appears to be most prevalent among the children in rural Bangladesh and also mortality and morbidity is relatively high.
2. The study will collect blood and breathing air sample from the volunteers. From children only breathing air sample will be collected by an anaesthesia mask. There will be no potential risk related to physical, psychological, social and legal, to the children or volunteers.
3. Not applicable.
4. Confidentiality of the records of the subjects will be maintained.
5. (a) There will be no such potential risk to the patients. Informed consent will be considered from the legal guardian or parents of the patients. Small quantity of blood samples will be collected from the consenting volunteers only.
- (b) Not applicable.
- (c) Not applicable.
6. No involvement of interview except to obtain the history of illness which is usually done routinely for clinical purpose.
7. The direct benefit to the subject will be, general medical care during the Course of diarrhoea, as well as the diagnosis of lactase deficiency in them. Above all, results obtained from this study will help us in treating and formulating an appropriate dietary regimen for the lactase deficient children during and after the diarrhoeal episode.

## SECTION II - RESEARCH PLAN

### 1. INTRODUCTION

#### 1. Objective

The main objective of the proposed study is to validate the hydrogen breath test technique in volunteers and then to utilise this technique for the investigation of secondary lactase deficiency in children with rotavirus associated diarrhoea.

#### 2. Background

Lactose malabsorption associated with the deficiency of intestinal enzyme lactase has only been established during the past decade (1-3). Normally, dietary lactose is hydrolysed by intestinal brush border lactase into glucose and galactose prior to absorption (4). Primary lactase deficiency occurs in either children or adults (5,6) and is presumably genetically determined. Secondary lactase deficiency, which is a transient phenomenon, may occur as a result of acute, infectious diarrhoea, protein - calorie malnutrition and other inflammatory disorders of the gut (7). Rosenberg et al (8) have provided an excellent review and discussion of malabsorption associated with acute diarrhoeal disease. Recently, Brown et al studied the prevalence of lactose malabsorption in Bangladeshi village children in relation to age, history of recent diarrhoea, nutritional status and breast feeding (9). Results showed that over 80% of diarrhoea-free children of more than 36 months of age were lactose malabsorbers but no (diarrhoea-free) children under 7 months age demonstrated malabsorption. Furthermore, a history of recent diarrhoea was associated with a significantly increased prevalence of lactose malabsorption in this study.

Numerous studies during the past decade have shown that rotavirus is an exceedingly common agent associated with childhood diarrhoea (10, 11,12). The viral infection is especially prevalent in the ages from 6-24 months. Kapikian et al found that 42% of 143 infants and children admitted to the hospital for acute gastroenterites had rotavirus in their stool (10). Furthermore, between 60-80% of the

infants aged 6-24 months had a rotavirus associated diarrhoea as defined by electron microscopic exam of the stool and/or a four-fold greater increase in serum anti-rotavirus titer. A recent study has emphasized the importance of rotavirus - associated diarrhoea among infants in Bangladesh (13).

Several studies have demonstrated carbohydrate malabsorption associated with acute diarrhoea in infants (5,6). One study performed in this Centre, stratified diarrhoeal patients according to aetiology and demonstrated transient carbohydrate malabsorption in patients with acute "non-specific gastroenteritis (14). However, no study to our knowledge has been investigated the cause of lactose malabsorption in infants with laboratory defined rotavirus associated diarrhoea.

Lactose malabsorption can be estimated by the conventional "lactose tolerance test" (LTT) in which failure of blood glucose to rise greater than 20 mgs% over the baseline level after ingestion of a loading dose of 2 gms/kg body wt. (not exceeding 50 gms of lactose). Lactose intolerance is indicated by the presence of borborygmy, intestinal cramps, flatulence, and diarrhoea. Recently the lactose breath hydrogen test (L-BHT) has been shown to be practical and effective non-invasive means of determining small intestinal lactase deficiency (15-18). The method obviates the necessity of repeated blood sampling. Newcomer et al (16) found that this method was the most sensitive and specific means of detecting intestinal hypolactasia when compared to three other commonly used indirect methods.

Recently, two groups of investigators have questioned the effectiveness of diagnosing lactose malabsorption with the L-BHT during acute infectious diarrhoea (19,20). These groups believe that either the overgrowth of the normal intestinal flora by pathogenic bacteria or the changes in peristaltic patterns during the acute phase of diarrhoea reduce production and absorption of intestinal hydrogen gas with a subsequent increase of "false negative" with the L-BHT.

This criticism of the L-BHT is based on experience with a very small number of patients and the conclusions drawn are quite tentative. However, in a study such as we are proposing this criticism should be kept in mind. Number of reasons lead us to believe that the L-BHT will prove to be an effective tool in the acute phase of rotavirus - associated diarrhoea. 1) The changes in intestinal flora during rotavirus infection are not well-defined but there is thought to be considerably less change than in the bacterial enterities and 2) rotavirus enterities is primarily an infection of the small bowel whereas the hydrogen producing anaerobic flora are located in the colon (21, 22).

The purpose of this present study is threefold. First the sensitivity and specificity of the L-BHT, as used in this Centre will be defined by comparing test results with those obtained with a concurrently run LTT in healthy volunteers. Secondly, the study aims to define the prevalence of lactose malabsorption in rotavirus - associated diarrhoea and to follow the time course of this malabsorption. Finally, the results obtained from this study will hopefully enable physicians to better formulate the appropriate dietary regimen during and after a bout of rotavirus - associated diarrhoea.

3. Rationale

Previous studies at this Centre have shown the presence of high prevalence rate of lactose malabsorption in the Bangladeshi village children. Hydrogen breath test is simple, non-invasive and effective means for diagnosis of lactose malabsorption in children. Setting up hydrogen breath test as a routine test in ICDDR,B might help diagnosing many children with lactase deficiency and advise proper dietary regimen.

B. SPECIFIC AIMS:

The specific aims of the proposed study are:-

- (1) To correlate the L-BHT with the LTT using healthy volunteers.
- (2) To demonstrate and describe the secondary lactase deficiency resulting from rotavirus - associated diarrhoea.

### C. PATIENTS AND METHODS

The study will have two parts. In the first part 20 healthy, consenting Volunteers will be selected and in the second part 50 diarrhoea patients aged 6-24 months will be studied.

#### PART I - VOLUNTEER STUDY

Twenty volunteers aged 20-40 years and without any history of diarrhoea and no history of antibiotic use during the past month will be selected for the study. The procedure will be explained in detail and verbal consent will be obtained. The subjects will be asked to fast from the previous midnight or for at least 6 hours prior to testing. A base line blood sugar and breath sample will be taken from each subject and then a single dose of lactose, 2 gms/kg (maximum: 50 gms), made into a 10% solution in water will be fed. Breath samples will be collected at 30 mins. 1 hour, 2 hrs., 3 hrs., 5 hrs., and 6 hrs. after ingestion of lactose as described by Solomons et al (15). Each time 1 cc blood samples will be collected at 15, 30, 60 and 90 mins. as described by McGill et al ( 23 ).

When ingested carbohydrate escapes intestinal absorption and is exposed to certain colonic bacteria, the resultant fermentation leads to the intraluminal evolution and subsequent absorption, of H<sub>2</sub> gas. Part of this H<sub>2</sub> is exhaled in expired air (21,22) and an increase in H<sub>2</sub> excretion after an oral carbohydrate load as small as 2 mgs. can be detected by H<sub>2</sub> breath analysis tests (15).

#### PART - II'

##### H<sub>2</sub> Breath Test on Patients

50 infants, aged 6-24 months, with an onset of acute diarrhoea within the past week will be selected randomly from the patient population presenting in the treatment Centre of the ICDDR.,B. Patients will be excluded for any of the following reasons: (1) antibiotic use during

the past 4 weeks. A list of commonly used antibiotic preparation will be recited to the guardian to prevent the inclusion of patients with occult antibiotic use (2) a severe degree of malnutrition i.e. less than 40% of expected weight for age according to local standards (3) any patient with obvious complications other than dehydration and (4) any patients whose clinical status dictates the use of antibiotics immediately. Informed consent will be obtained from the legal guardians of the child.

Stool and urine analysis, 1 cc of blood will be withdrawn for routine estimation of TBC, HCT and Spgr. Rectal swab and stool will be cultured for salmonella, shigella, E. coli and rotavirus. E. coli isolates will be tested for heat labile and heat stable enterotoxin. Management of the diarrhoea will be carried out as decided by the physician of the ward. Within three days of admission a L-BHT will be performed as described above except that breath samples will be collected by placing an anaesthesia mask connected to the collection apparatus over the mouth and nose of the infant and collecting the ggs from multiple respirations. About 30% of a mixed gas sample, which is collected with the anaesthesia mask, consists of gas from the anatomical dead space. Thus the analysis of the mixed gas samples will have a H<sub>2</sub> level of 70% of the corresponding collection of alveolar air, which is from the adult volunteers with an end-expiratory sample. Therefore, a correction factor of 1.4 - (100/70) will be made for all samples collected via the mask i.e. results obtained for mask samples will be multiplied by 1.4. Preliminary results obtained so far from the Volunteers, showed that most of the subject's breath-hydrogen peak at 2 hrs. after ingestion of lactose. Therefore breath samples will be collected from the children for only upto 3 hrs. after ingestion. Children those who will have confirmed diagnosis of rotavirus, will be followed up after 4 and 8 weeks of discharge. Each time BHT will be repeated to demonstrate the presence of lactase deficiency. Stool samples will also be tested each time for the presence/absence of rotavirus.



### STATISTICAL ANALYSIS

Part I The results obtained from the two tests (LFT and BHT) will be plotted and correlation co-efficient will be determined to test the validity of BHT.

Part II 50 children studied will be stratified into two groups i.e. pure rotavirus and mixed infection. BHT results during acute period will be compared between the two groups. Only pure rotavirus diagnosed patients will be brought back after 4 and 8 weeks of discharge. Results obtained at 4 and 8 weeks will be compared with the results obtained at acute stage.

### SIGNIFICANCE

Available information suggests that lactose malabsorption and lactose intolerance is highly prevalent among the children in rural Bangladesh. The proposed plan of research will aid in the early diagnosis of the above symptoms and help in prescribing a proper dietary regimen for the affected children.

### FACILITIES REQUIRED:

- (1) Office Space - Already existing space will be utilised.
- (2) Laboratory Space - ICDDR,B existing Laboratory will be utilised.
- (3) Hospital Ward - The present study ward will be utilised.

### COLLABORATIVE ARRANGEMENTS:

Collaborative arrangements for standardization of the BHT procedure:

Mr. Joel Mason, last year Medical Student from University of Chicago worked in ICDDR,B for 6 weeks on Quintron Gas Chromatograph.

REFERENCE

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3. Bayless TM, Rosenweig NS: A racial difference in incidence of lactase deficiency: A survey of milk intolerance and lactase deficiency in healthy adult males. *JAMA* 197: 968-972, 1966.
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10. Kapikian AZ, Kim Hw, Wyatt RG et al: Human reovirus - like agent as the major pathogen associated with "winter gastroenteritis in hospitalised infants and young children. *NEJM.* 294: 965-972, 1976.
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14. Lindenbaum J: Malabsorption during and after recovery from acute intestinal infection. *BMJ*. 2: 329, 1965.
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18. Levit MD. Donaldson RM: Use of respiratory hydrogen (H<sub>2</sub>) excretion to detect carbohydrate malabsorption. *J. Lab. Clin. Med.* 75: 937, 1970.
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20. Brown KH, Black RE, Parry L: The effect of diarrhoea on incidence of lactose malabsorption among Bangladeshi Children. *Am. J. Clin. Nutr.* 33: 2226 - 27, 1980.
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22. Levit MD: Production and excretion of hydrogen gas in man. *NEJM* 281: 122, 1969.
23. McGill et al. Comparison of venous and capillary blood in LFT. *Gastro* 53: 371, 1967.

SECTION III BUDGETA. DETAILED BUDGET1. Personnel Service

<u>Name</u>	<u>% Effort</u> <u>No. of Days</u>	<u>Annual</u> <u>Salary</u>	<u>Project Requirement</u>	
			<u>Taka</u>	<u>Dollars.</u>
Dr. A. M. Molla	10% 6 month	\$ 40,000	-	2000.00
Dr. Ayesha Molla	25% 6 month	Tk.140,00	17500	-
Dr. Joel Mason	100% 4 weeks	-	-	-
Research Assistant	100% 6 month	Tk.32,000	15000	-
Dr. Shafique	25% 6 month	Tk.32,400	4050	-
Nurse	30% 6 month	Tk.24,000	3600	-
		Sub total =	Tk.40150	\$2000.00

2. Supplies and Materials

Stationery goods	1000	-
Xeroxing and Mimeographing	5000	-
	Sub total = Tk. 6000	-

3. Equipment

Standard H <sub>2</sub> Cylinder (two)	-	\$ 250.00
Carrier Gas Cylinder (two)	-	\$ 580.00
Space for Quintron Gas Chromatograph	<u>Tk.15000</u>	<u>\$1000.00</u>
	Sub total=	Tk.15000 \$1830.00

4. Laboratory Expenses

Blood Test ( 50 Children) 100 samples (TBC: HCT etc.)	Tk. 400	-
Stool Test (50 Children) 100 Samples	Tk. 300	-

Laboratory ExpensesProject RequirementTaka                      DollarStool Culture 100 samples  
toxin testing

400.00

-

ELISA (rotavirus) 100 samples

700.00

-

Blood glucose determination  
100 samples

150.00

-

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1,950.00

-

5. TransportationFor follow up of patients  
2000 miles

700.00

-

6. Patients Hospitalisation

70 x 3 = 210 days x 150

31,500.00

-

Lunch for Volunteers

500.00

-

Grand Total              Tk. 98,500.00

3830.00

Incremental Cost        55,650.00

1830.00

B. BUDGET SUMMARY

<u>Category</u>	<u>Year 1</u>		<u>Year 2</u>		<u>Year 8</u>	
	<u>Taka</u>	<u>Dollars</u>	<u>Taka</u>	<u>Dollars</u>	<u>Taka</u>	<u>Dollars</u>
1. Personnel	40,150	2000.00				
2. Supplies	2,950	-				
3. Equipment	15,000	1830.00				
4. Hospitalisation	32,000	-				
5. Outputpatients	-	-				
6. CRL Transport	700	-				
7. Travel Persons	-	-				
8. Transportation Things	-	-				
9. Rent/Communication	-	-				
10. Printing/Reproduction	5,000	-				
11. Contractual Service	-	-				
12. Construction	-	-				
	<hr/>		<hr/>		<hr/>	
Total:	95,800	3830.00				
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Total \$	5,635 + 3830.00					
	= 9,465.00					

Conversion Rate \$ 1.00 = Tk. 17.00.

CONSENT FORM

Hydrogen Breath test in Children with Diarrhoea and in healthy volunteers.

International Centre for Diarrhoeal Disease Research, Bangladesh is actively carrying out research to improve and simplify different methods for easy diagnosis and treatment of diarrhoeal disease of different aetiology. During and after diarrhoeal disease some children as well as adults are not able to digest milk (which contains lactose). To diagnose this particular inability we are undertaking this study to evaluate a simple noninvasive method, which will analyse a breathing air sample of your child after feeding him/her a harmless sugar solution. By using this technique we will be able to administer treatment and advise proper dietary regimen to your child.

Only volunteers will be requested to give their blood samples before and after the ingestion of lactose solution.

Following tests will be done:-

1. To determine the amount of sugar content blood samples will be estimated before and after ingestion of sugar solution. This procedure will be applied for the volunteers only. Breathed air will be collected to carry out the test. This procedure will be applied to both volunteers and the patients.
2. We would like you to participate in this study. You will have complete freedom to drop your children from the study at any time you wish. In such cases there will be no change in usual treatment.

If you agree please sign bellow.

Name of Patient \_\_\_\_\_

Signature or thumb  
impression

Patient's Number \_\_\_\_\_

Relationship of  
Patients with Signator.

Signature of Physician \_\_\_\_\_





1. Die erste Aufgabe ist die  
Erstellung eines Zeitplans für  
die verschiedenen Aufgaben.  
Dieser Zeitplan sollte die  
Dauer der einzelnen Aufgaben  
sowie die Abfolge der  
Aufgaben berücksichtigen.  
Die Aufgabe besteht darin,  
den Zeitplan so zu erstellen,  
dass alle Aufgaben rechtzeitig  
erledigt werden können.

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