ttachment 1.

REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS Date_____
CRL

80

rincipal Investigator Dr. Ayesha Molla Trainee investigator(if any) —

pplication No 79-003 Supporting Agency(if Non-CRL) —

tle of study Digestive enzyme activ- Project status:
ies during diarrheal diseases ( ) New Study
in the children of Bangladesh ( ) Continuation with change
( ) No change (do not fill out rest of form)

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rcle the appropriate answer to each of the following(If Not Applicable write NA):

Source of Population:
a) Ill subjects (Yes) No
b) Non-ill subjects Yes (No)
c) Minors or persons under guardianship (Yes) No

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 possibly damaging to subject or others Yes (No)

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

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 Board for review.

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agree to obtain approval of the Review Board on Use of Human Volunteers for any
anges involving the rights and welfare of subjects before

1. Adult male and female upto 40 yrs of age suffering from cholera and E.coli will be selected for studying digestive enzymes. Children of both sexes upto 5 yrs of age suffering from diarrheal diseases will also be included in the study. The rationale for including this group of population is that in our country children suffers mostly in diarrhea both bacterial and viral and this has tremendous impact on the nutritional status of the children. Moreover some viral infection called rotavirus diarrhea is quite common in our children population and absent in adult people. Therefore children with rotavirus diarrhea are the most appropriate subjects for studying the impact of rotavirus diarrhea on activity of digestive enzymes.

2. There is no potential risks - physical, psychological, social or legal involved in the study except there will be some discomfort of the patient during nosogastric introduction of a polyvinyl tubing of diameter 1.7 to 2.1 mm.

3. Does not apply.

4. There will be no personal identification of the patient.

5. Consent will be obtained from the patients. Informed consent will be obtained from the adult and for children informed consent will be obtained from parents or legal guardians at the hospital premises.

   (a) does not apply.

   (b) no information will be withheld from the subject.

6. Except asking clinical history, there will be no question in relation to private affiars of the subject.

7. Diarrhea is one of the common cause of death in children not only because of loss of fluid but also because of association of diarrhea with malnutrition and malabsorption. For better understanding of the pathophysiology of the diarrheal diseases this protocol is devised, which ultimately may lead us in better management and in prevention of the disease.

8. This study requires the use of body fluids: duodenal juice for pancreatic enzyme estimations. Blood will be taken for routine patient care not for research purposes.

79-003
Rec'd 5/1/79

SECTION I - RESEARCH PROTOCOL

1) Title: Digestive enzyme activities during diarrhoeal diseases

2) Principal Investigators: Dr. Ayesha Molla
Dr. A.M. Molla

Co-Investigators: Mr. M.A. Wahed
Dr. M. Mujibur Rahaman
Dr. W.B. Greenough III

3) Starting Date: January 1979

4) Completion Date: December 1979

5) Total Direct Cost:

6) Abstract Summary:

For efficient utilization of nutrients the presence of digestive enzymes e.g. proteolytic, lipolytic and carbohydrate splitting enzymes are essential in the gut lumen. Enterokinase, trypsin, chymotrypsin, lipase and amylase are the main digestive enzymes secreted into the duodenum. In different diarrheal episodes these digestive enzymes may be varyingly affected and thus may have important impact on the severity of these diseases. So far very little information is available about the digestive enzymes in cholera, rotavirus, and E.coli diarrhea. Knowledge on this aspect of these enzyme activities may help to delineate the mechanism of malabsorption in acute diarrheal disease in Bangladesh.

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

a) To have knowledge about the levels of digestive enzyme activities during and after the acute diarrheal diseases like cholera, E.coli and rotavirus.

b) To have information regarding the impact of nutritional status on the enzymatic activity and its role on the etiology (if any) and the severity of these diarrheal disease.

c) To know if the above mentioned proteolytic, lipolytic or carbohydrate splitting enzyme supplementation in case of severely deficient patients would reduce the severity or would help in quick recovery of the disease.

d) Establishment of the laboratory procedures for these enzymatic estimation which could be applied for further studies relating to malabsorption.

2. Background:

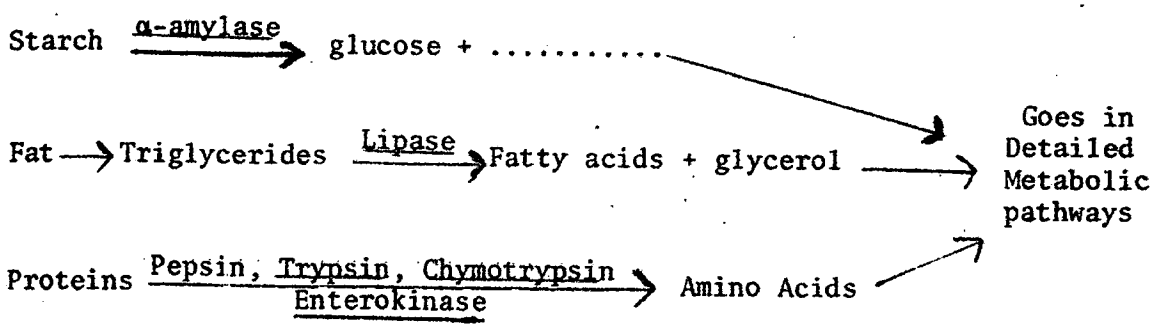
Diarrhea is a major problem of this country and widespread malnutrition of this society is probably one of the main causes in the development of intestinal infection and subsequent diarrhea. Malabsorption could occur due to bacterial overgrowth, toxin elaboration with subsequent affection of gut epithelium and villus abnormalities due to direct invasion of the mucosal layer.

It is well known that due to interaction with cholera toxin mucosal concentration of C-AMP increases attributing to the accumulation of fluid in the gut lumen. Diarrhea produced due to E.coli infection also occurs approximately in a similar fashion. The mechanism of rotavirus diarrhea production in still unclear. Due to different pathophysiology of the diseases alternations in pancreatic enzyme activities relating fat, carbohydrate and protein metabolism are expected to occur. Moreover in protein calorie malnutrition studies on pancreatic function indicate that pancreatic output after stimulation with secretin and pancreozymin is markedly reduced. Lipase, trypsin, chymotrypsin, and amylase activity was found to be lower than the normal level (1). This suggests that in case of diarrhea associated with protein caloric malnutrition the deficiency of these enzymes might have important nutritional impact on these patients.

So far little work has been done to evaluate the impact of diarrhea on the pancreatic enzyme activities in relation to absorption of fat, protein and carbohydrate. There are some work done on mucosal enzymes in acute and convalescent phases of cholera. For example it was found that the disaccharidase activity in duodenal biopsy is temporarily impaired during the acute phase of the disease and increases quite rapidly to a normal level after recovery (2). Recently Sherr et. al. (3) studied the effect of cholera toxin on rabbit jejunal carbohydrate metabolizing enzymes and found that fructose diphosphatase activity, an enzyme unique to gluconeogenesis increases and pyruvate kinase, an enzyme unique to glycolysis reciprocally decreases after incubation of the rabbit jejunal mucosa with

cholera enterotoxin. Moreover the key enzymes responsible for metabolism of fat, protein and carbohydrate are under the control of C-AMP, increasing concentration of which might enchance or decrease the enzyme levels affecting the normal metabolism.

There is another study reported by Gyr et. al. ( ) relating cholera in protein depleted patas monkey's with trypsin activity of their pancreatic secretion. Monkeys with severe protein caloric malnutrition have very low duodenal tryptic activity and when they are challenged with cholera the severity was found to be higher in the protein depleted group than the trypsin supplemented group. He concluded that trypsin might play a role in the local immunity of the gut of patas monkeys. Following is a sketch of the carbohydrate fat and protein digestion by amylase, lipase, enterokinase, trypsin and chymotrypsin. The underlined enzymes will be assayed in this study. Considering the above background this study aimed at an analysis of the duodenal juice during and after attack of the diarrheal episodes to investigate the effect of cholera, E.coli and rotavirus infection on the secretion of pancreatic enzymes.



Simplified sketch of fat, protein, and carbohydrate metabolism

3. Rationale:

Acute diarrheas like cholera, rotavirus and E.coli are common in our population. The mechanism of disease production are not same in the different etiology of diarrhea and their effect on the digestive enzymes are also likely to be different. Since little information are available on the level of enzymatic activities during cholera, E.coli and rotavirus diarrhea, it will be of immense value to have first hand information about these enzymes and their role on the etiology and severity of these diseases. Establishment of these methodology will also be useful for further studies on malabsorption.

B. SPECIFIC AIMS:

1. To establish the laboratory methods for estimations of enterokinase, trypsin, chymotrypsin, lipase and amylase in duodenal juices during and after diarrheal attacks.

2. To know the mechanism of malabsorption in relation to the enzymetic activities associated with bacterial and viral diarrhea.

3. To find out the enzymatic activity during basal condition and after stimulation by a standard liquid meal.

4. To establish the relation between acute diarrheal disease (cholera, E.coli and rotavirus) and the individual enzyme functions of the gut.

C. METHODS OF PROCEDURE:

The detailed methods of the enzyme activity measurements are described in Appendix I.

Composition of the Test Meal: The test meal of 500 ml will be a balanced composition used by Borgstrom et. al. (5). It will consist of a homogeneous mixture of fat, protein and carbohydrate, the distribution of calories being 40, 15 and 45 respectively. The meal will contain an unabsorbable marker polyethelene glycol 4000 (PEG). The composition of the test meal is given below.

Composition of Test Meal (TM)

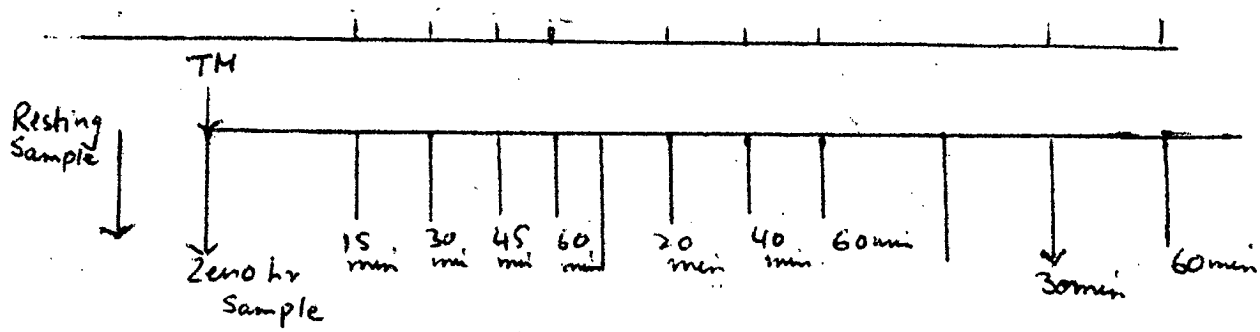
	g	Fat	Carbohydrate	Protein
Corn or any vegetable oil	74	74	-	-
Skim vitaminized milk powder	126	n	y	2
Glucose	138	-	138	-
PEG	5	-	-	-
Water	1000 ml			

Intubation method: This technique of intubation was originally devised by Borgstrom et. al. (5). Later on Ziene et. al. (6) used it for estimating the secretion of pancreatic enzymes.

Our plan of study will be as follows: Adult patients will be selected after rehydration and proper electrolyte balance. In case of children a sedative pentobarnitone 2 mg/kg or diazepum of 0.5 mg/kg will be given intramascularly prior to intubation. Either second or third day will be chosen for the study. The patients should be kept fasting

at least 8-10 hours before the start of the study. A small polyvinyl tube of internal diameter of 1.7 to 2.1 mm will be introduced through the nose and allowed to move into the duodenum till 70 to 125 cm has passed from nose. The pH of the juice will be checked by a pH paper and a resting sample of duodenal fluid will be aspirated. The test meal of 500 ml fluid will be allowed to drink by the patients. Immediately after ingestion a zero hour sample of 5 ml volume will be aspirated and collected over ice. In the first hour of study a series of samples at 15, 30, 45 and 60 min will be aspirated. In the second hr every 20 min sample and in the third hr every 30 min sample will be aspirated. This can be shown diagramatically in Figure 1. The test subjects will be sitting in a chair during the collection period. The tube position will be determined by fluroscopy.

FIGURE 1



In the convalescent phase of the disease i.e. after 2 to 3 weeks of the discharge of the disease the same intubation will be done and the samples will be collected for enzyme estimations. Each individual will be its own control.

Subjects: Adult male and female upto 40 yrs of age having cholera, E.coli and rotavirus will be selected for the study. patients with moderate to severe dehydration with/~~any~~ out complication like pneumonia, high fever and tuberculosis will be included in the study. In each group of infection minimum 20 subjects will be studied for this protocol.

ANALYSIS OF DATA

Calculation of the total enzymatic activity:

Calculation will be done according to zieve et. al. (6). All estimations will be corrected for dilution by estimating the concentration of PEG in the duodenal fluid.

Suppose M_t = Total amount of PEG added in the TM.

M_s = The amount of PEG left over in the stomach.

$M_d = M_t - M_s$ = Amount entered the duodenum.

M_r = Amount of PEG recovered in the duodenum.

$\frac{M_t}{M_r} \times 100$ = Percentage recovered in the duodenum.

$\frac{M_d}{M_r}$ = Factor by which volume of aspirate or enzyme content of aspirate must be multiplied to get total volume or total activity.

This calculation assumes that there is thorough mixing in the duodenum prior to aspirate.

The mean enzyme activities at first, second and third hour during and

The enzyme activities at successive collections of duodenal juice will also be shown in a tabulated form to find out the peak response after ingestion of TM.

The difference of the mean activities of control and diseased subjects at different time interval after ingestion of TM will be tested by student's t test.

D. SIGNIFICANCE:

There has been no systematic studies performed relating the secretion of pancreatic enzymes in duodenal juice with cholera, E.coli and rotavirus infection. Thus due to lack of information in this area, the pathophysiology of the intestine during bacterial infection was not clearly understood. Therefore research in this field would be a valuable contribution in science.

E. FACILITIES REQUIRED:

1. Office space for the principal investigators and filing cabinets are required for storing reprints and text books.

2. A bench space will be required for performing the assays. A technician's help will be needed to assist in the assay producers.

3. Two hospital beds and an attendant nurse will be required to assist the clinical investigator.

4. An enzyme analyzer (already ordered by Dr. B. Seaton) and an autotitrator will be required to perform titrimetric assays.

5. Transport will be required for the patients returning home. For control studies transport facilities will be needed.

6. One Field Assistant will be required to follow the patients up.

REFERENCES

1. Barbezat, G.O., and Hansen, J.O.L.: The exocrine pancreas and protein calorie malnutrition. Pediatrics 42: 77, 1968.

2. Hirschhorn N, Molla A, Molla A.M.: Reversible jejunal disaccharidase deficiency in cholera and other acute diarrhoeal diseases. Johns Hopkins Med. J. 125(6): 291-300, December 1969.

3. Howard P. Sherr, M.D., Fred B., Stifel Ph.D., and Robert H. Herman, M.D.: Effect of cholera toxin on rabbit jejunal carbohydrate-metabolizing enzymes. Gastroenterology 75: 711-716; 1968.

4. Gyr, K. M.D.,M.P.H. & T.M., O. Felsenfeld, M.D. M.Sc. and M. Zimmershining, M.D. Gastroenterology 74: 54-513, 1978.

5. B. Borgstrom, A. Dahlqvist, G. Lundh, and J. Sjovall,: Studies of intestinal digestion and absorption in the human J. Clin. Invest. 36: 1521-1536, 1957.

6. Leslie Zieve, M.D. Beatrice Mulford, M.D., and Ann Mohale, B.A. Secretion of Pancreatic Enzymes. II Comparative response following test meal or injection of secretion and pancreozymin, Am. J. Dig Dis. II (9), 685-694, 1966.

APPENDIX I

ESTIMATION OF PANCREATIC LIPASE ACTIVITY IN DUODENAL CONTENTS

Ref: Erlanson and Borgstrom. Scand. J. Gastro, (1970), 5, 293

PRINCIPLE

The neutralisation with standard (.01N NaOH) alkali of the free fatty acids liberated from the substrate triglycerides by the enzyme pancreatic lipase.

REAGENTS

1. Glycerol tributyrate
2. 0.002 m Tris-HCl buffer pH 8.0
3. Sodium hydroxide 0.01 M

TITRATION

- A Burette containing sodium hydroxide adds to the mixing container (see the operating instruction of the multidosigraph)
- Titration goes towards up the pH scale (i.e. from acid side towards pH 8.0)
- End point pH 8.0
- Titration performed at room temperature. The volume of alkali needed to maintain the end point constant at pH 8.0 over a period of 2 minutes would measure equivalent amount of acid liberation by the pancreatic enzyme.

PROCEDURE

1. Add 1 ml of tributyrate to 10 ml TRIS-buffer in titration container.
2. Add 20 µl of duodenal juice (undiluted).
3. Place titration container over the magnetic stirrer of the titro meter and immediately mix at maximum speed. Stirring at high speed is important to obtain good emulsion of fat, so that lipase can act on its substrate.
4. Bring pH to 8.0 with titrator (will hear valve open and shut).
5. Start stop watch and wait for 30 seconds to allow for overshoot or equilibration.
6. After 30 seconds note the volume of NaOH needed to bring the end point to pH 8.0.
7. Then note the volume delivered in 2 one minute periods; stop further titration after 2 minutes.
8. Check linearity between first and second minutes.
9. Run duplicate and take the mean reading over 2 minutes.
10. Express results as mEq fatty acid liberated per mlof juice per minute.

CALCULATION

.01 M i.e. 10 mM/1

i.e. 10 µM/ml NaOH used for titration

For example, if 3.13 ml 10 µM NaOH is used in one minute titration time by 20 µl of duodenal juice, the equivalent amount of fatty acid liberation by 1 ml of juice would be:

$$\frac{3.13 \times 10 \times 1000}{20} = \frac{\text{m/ml/minute}}{1000} = \text{meq/ml/min}$$

$$= 1.51 \text{ meq/ml/minute}$$

ESTIMATION OF PANCREATIC CHYMOTRYPSIN ACTIVITY IN DUODENAL CONTENTS

Ref: Haverback, B.J.; Gastroenterology. 44, 588-597, 1960.....

PRINCIPLE

Chymotrypsin hydrolyses the peptide bond at tyrosine or phenylalanine. The amino acid released by the action of chymotrypsin is neutralised by standard alkali (0.01 N NaOH).

REAGENTS

1. Prepare 0.036MATEE (acetyl-L-tysosone-ethylester) in 50% methanol and 50% 0.01 tris buffer.
2. Standard sodium hydroxide 0.01N

TITRATION

- A motorised burette containing 0.01N NaOH adds automatically to the mixing container.
- Titration goes up the pH scale.
- End point pH 8.0.
- Titration performed at room temperature.
- Speed of titration should be kept minimum.

PROCEDURE

1. Add 100 µl of undiluted juice to 10 ml of .036 ATEE solution in a titration vessel.

2. Place titration vessel over magnetic stirrer, mix with maximum speed and slowly bring pH to 8.0 by adding NaOH automatically (will hear valve open and shut).

3. Start stop watch and wait for 30 seconds to allow overshoot or equilibration. After 30 seconds, note the volume of NaOH needed to maintain pH at 8.0.

4. Note volume after 2 x 1 minutes and stop titration.

5. Run a duplicate sample and take the mean of 2 minutes reading.

6. Express results as meq acid liberated per min per ml of juice.

CALCULATION

If 2.0 ml NaOH of 0.01 N conc. is needed to maintain the pH at 8.0, an equivalent amount of acid liberated per ml of juice per minute will be

$$\frac{2 \times 10 \times 1000}{100 \times 1000} = 0.2 \text{ meq/ml/min.}$$

ESTIMATION OF TRYPTIC ACTIVITY IN DUODENAL CONTENTS

Ref: Wiggins, H.S. (1967). Simple method for estimating trypsin. Gut 8, 415-416

PRINCIPLE

Trypsin hydrolyzes the peptide bond between the carboxyl group of arginine of lysine with the amino group of amino acid. The acids released by the action of trypsin is neutralised by standard alkali (.o1N NaOH).

REAGENTS

1. Prepare 5 mM (.788 gm/1) TRIS-HC1 buffer, pH 8.0 containing 40 mM NaCl (2.338 gm/1) and 30 mM C a Cl_2 (4.4 gm/1).

2. Prepare 8 mM (0.758 gm/250 ml) P-Tosyl-1-Argenine methyl ester (TAME) in TRIS buffer and adjust the pH at 7.8.

3. Sodium hydroxide 0.01N.

TITRATION

- A Burette containing .01N NaOH adds to the mixing container (see operating instruction of the multidosigraph).

- Titration goes towards up the pH scale.

- End point pH 8.0.

- Titration performed at room temperature.

- Speed of titration should be kept below zero.

PROCEDURE

1. Add 100 ul of undiluted juice to 10 ml 8 mM TAME solution in a titration vessel.

2. Place titration vessel over the magnetic stirrer and immediately mix it at maximum speed.

3. Slowly bring the pH to 8.0 with the titrator (will hear valve oper and shut).

4. Start stop watch and wait for 30 seconds to allow overshoot or equilibration. After 30 seconds, note the volume of NaOH needed to maintain the pH at 8.0.

5. Note volume delivered after this in 2 x 1 minutes and stop titration after 2 minutes.

6. Run a duplicate and take the mean of 2 minutes reading.

7. Express results as meq acid liberated per minute per ml of juice.

CALCULATION

If 2.0 ml NaOH is needed to maintain the pH at 8.0, an equivalent amount of acid liberated per ml juice per minute will be:-

$$\frac{2 \times 10 \times 1000}{100 \times 1000} = 0.200 \text{ meq/ml/minute}$$

ESTIMATION OF POLYETHYLENE GLYCOL (PEG)

Ref: Stig Hyden - Kungl Lantboukshogskol. Ann/r. Vol. 22 139-145, 1956.

REAGENTS

Barium Chloride 10%

Barium Hydroxide 0.3N

Zinc Sulphate $7H_2O$, 5%

300 gms Trichloroacetic adic + 50 gms barium chloride per one litre of H_2O.

STOCK STANDARDS

600 mg% PEG/4000

METHOD

Mix all the reagents in the following order:-

- water 2 mls
- test solution 50 µl of standard 100 ml
- Ba Cl_2 solution to remove any SO_4, 250 µl
- Ba $(OH)_2$ solution 500 µl
- Zn SO_4 $7H_2O$ 500 µl

This gives dilution of 1/33 for the standard and 1/66 for test.

Run a series of standard PEG solutions (0.5, 1.0, 1.5, 3.0, 4.5, 6.0 mg/ml) in parallel to the test and leave overnight at $4^{o}C$. Filter next morning using Whatman 41 (5.5 cm) ashless filterpaper. A blank is also put

through using water in place of test solution. To 1 ml of supernatant add 1 ml of TCA reagent. Mix and read after 5 minutes standing at room temperature (turbidity reaches at peak and after 5 minutes goes off). Read at 500 nm on Gilford Spectrophotometer. Plot concentration of PEG standards against optical density and find the unknowns from the curve.

STANDARD CURVE

Stock solution of PEG (6 mg/ml) is diluted with water as follows to give concentrations of 0.5, 1.5, 3.0, 4.5, and 6 mg/ml.

Stock solution of PEG in ml.	Add water in ml	Final concentrations mg/ml
0.5	5.5	0.5
1.0	5.0	1.0
1.0	3.0	1.5
2.0	2.0	3.0
3.0	1.0	4.5
4.0	0.0	6.0

ENTEROKINASE ACTIVITY OF DUODENAL CONTENT

Ref: Hadorn, B., Tarlow, M.J., June, L.K., and Wolff, O.H., Lancet 1, 812-13, 1969.

REAGENTS

(1) Trypsinogen

(2) 0.1 M Tris-maleate buffer pH 6.0

(3) TAME

(4) .005 M Hcl

PROCEDURE

- Take 0.1 ml of trypsinogen in dilute acid (1 ng. bovine trypsinogen signa per ml of 0.005 M Hcl).
- Mix with 0.1 ml of 0.1 M tris maleate buffer, pH 6.0
- Add 0.1 ml of 1/50 diluted duodenal juice and 1.2 ml distilled water to the above mixture.
- Measure the tryptic activity in this mixture by titration method as is measured for trypsin mentioned previously.

Under this condition the increase of tryptic activity in the incubation mixture is proportional to the amount of enterokinase concentration. One enterokinase unit is expressed in micrograms of trypsin formed from 1 mg of trypsinogen per minute, per ml of duodenal juice.

Abstract Summary

Title: Digestive enzyme activities during diarrhoeal diseases

For efficient utilization of nutrients the presence of digestive enzymes e.g. proteolytic, lipolytic and carbohydrate splitting enzymes are essential in the gut lumen. Enterokinase, trypsin, chymotrypsin, lipase and amylase are the main digestive enzymes secreted into the duodenum. In different diarrheal episodes these digestive enzymes may be varyingly affected and thus may have important impact on the severity of these diseases. So far very little information is available about the digestive enzymes in cholera, rotavirus, and E.coli diarrhea. Knowledge on this aspect of these enzyme activities may help to delineate the mechanism of malabsorption in acute diarrheal disease in Bangladesh.

SECTION III - BUDGET
A. DETAILED BUDGET

PERSONNEL SERVICES

Name	Position	% or # of days	Annual Salary	Project Requirem. Taka	U.S.
Dr. Ayesha Molla	Investigator	50%	76,190	38095.0	-
Dr. Abdul, Majid Molla	Investigator	20%	94,610	18922.0	-
Mr. M.A. Wahed	Acting Head, Biochemistry Br.	10%	31,580	3158	-
Dr. W.B. Greenough III	Stf. Director	5%	$ 52,700		$ 2630
Dr. M.M. Rahaman	Deputy Director	5%	116,740	5837	-
Mr. Rahim	Research Asstt.	30%	23,660	7098	-
A Nurse		40%	16,040	6416	-
A field assistant		20%	12,000	2400	-
Microbiology Technician		20%	23,660	4,732.00	-

2. SUPPLIES & MATERIALS	Taka	U.S. $
Items		
Enzyme substrate		150.00
Reagents		300.00
Plastic beaker & container etc.		100.00
Stopwatch 2		50.00
Trypsin reagents		120.00
For ELISA test @ 2.00 Tk./patient	240.00	
	240.00	720.00
EQUIPMENT		
Auto titrator (SHANDON)		1000.00
Total		1000.00
PATIENT HOSPITALISATION		
300 patient days	45,000.00	
Tltal:	45,000.00	

(some patient days will be shared with food absorption study)

CRL TRANSPORT	
200 miles	680.00
TRAVEL & TRANSPORTATION OF PERSONS	
Nil	
Transportation of supplies	
Reagents & materials	180.00
Equipments	250.00
Total:	430.00

RENT, COMMUNICATION & UTILITIES

N i l

PRINTING & REPRODUCTION Tk. 2000.00

OTHER CONTRUCTUAL SERVICES

N i l

CONSTRUCTION, RENOVATION & ALTERATIONS

N i l

BUDGET SUMMARY

	CATEGORY	YEAR - 1 TAKA	YEAR - 1 DOLLAR
1.	Personnel Services	86,658.00	2,630.00
2.	Supplies & materials	240.00	720.00
3.	Equipment	-	1000.00
4.	Patient hospitalization	45,000.00	-
5.	CRL Transport	680.00	-
6.	Travel	-	-
7.	Rent etc.	-	-
8.	Printing & reproduction	2,000.00	-
9.	Other contractual	-	-
10.	Construction & Renovation	-	-
	Total	134,578.00	4,780.00

Total cost in U.S. $ 13,752.00

Conversion Rate U.S. $ = Taka 15

International Centre for Diarrhoeal Disease Research, Bangladesh

Short Title: Enzyme Activity during Diarrhoea.

CONSENT FORM

International Centre for Diarrhoeal Disease Research has undertaken a research programme to examine the digestive enzymatic activity in the duodenal juice. We would like your child to take part in this programme.

The impact of diarrhoeal diseases on the nutritional status of the patient will be evaluated by this study and thereby the result of this study will be guidance for necesssry advise, treatment and prevention of the patients suffering from diarrhoeal disease.

If you or your child wishes to take part in this study the following procedures will be undertaken.

1) Patient will be kept on NPO from 10 p.m. to 9:30 a.m. (next) only for one day when the study will start.

2) A polyvinyl tubing of diameter 1-7 to 2.1 mm will be introduced through the nose of the patient. This will not hurt the patient but will cause some discomfort.

3) Then the patient will be given a standard Test Meal consisting of vitaminized skim milk powder, vegetable oil, and glucose to drink.

4) After ingestion of the meal duodenal fluid will be aspirated from time to time and this study will continue for three hours. Then the tube will be withdrawn.

5) The patient will be brought back to hospital after 2 to 3 weeks of discharge and the above mentioned intubation will be performed again.

6) At this stage the patient will receive treatment for all kinds of gastrointestinal diseases and vitamin will be supplied.

7) At any time during this study the patient has right to withdraw consent,in that case usual treatment will be given.

If you agree to participate in this research activity, please sign here.

Signature _____

Relation with the patient _____

Date _____

সম্মতি পত্র

ডায়রিয়া চলাকালীন রোগীর খাবার হজম হওয়ার জন্যে যে সমস্ত এনজাইম অন্ত্রনালীতে কাজ করে, তাদের কার্যকারিতা পরীক্ষা করার নিমিত্তে এই গবেষনাগার একটি গবেষনা চালাচ্ছে। আমরা চাই যে, আপনি এবং আপনার বাচ্চা এতে অংশ গ্রহন করুন।

ডায়রিয়ার সঙ্গে পুষ্টিহানিজনিত যে ক্ষতি হয় এই গবেষনার ফলে তার মান নির্নয় করা যাবে এবং প্রয়োজনেমত ঔষধ এবং উপদেশ দিয়ে এই রোগ নিবারনের জন্যে রোগীকে সাহায্য করা হবে।

আপনার আপত্তি না থাকলে আপনি বা আপনার বাচ্চা এই গবেষনায় সংস্থা নিলে নিম্নলিখিত ব্যবস্থা গ্রহন করা হবে।

(১) রোগীকে রাত্রি দশটার পর কোন খাবার দেয়া হবে। সকাল ৯-৩০ মিনিটে রোগীর গবেষনা শুরুর পূর্ব পর্যন্ত রোগীকে কোন খাবার খেতে দেয়া হবেনা।

(২) একটা পাতলা প্লাস্টিকের নল নাক দিয়ে অন্ত্রনালীতে ঢুকানো হবে। এতে রোগীর সামান্য অসুবিধা হবে কিন্তু কোন কষ্ট হবেনা।

(৩) তারপর রোগীকে ভিটামিনযুক্ত দুধ, চিনি এবং তেল মেশানো একটা খাবার পরিমান মত দেয়া হবে।

(৪) এই খাবার দেওয়ার পর তিনঘন্টা সময় কিছুক্ষন পর পর অন্ত্রনালীর জুস সংগ্রহ করা হবে। এতে রোগীর কোন কষ্ট হবেনা। তারপর প্লাস্টিক নল ফেলে দেয়া হবে।

(৫) রোগী বাসায় চলে যাবার পর ২ থেকে ৩ সপ্তাহ পর আবার উপরোল্লিখিত গবেষনা করার জন্যে রোগীকে হাসপাতালে আসার উপদেশ দেয়া হবে।

(৬) এই সময়ে রোগীকে সকল প্রকার পেটের পীড়ার জন্যে চিকিৎসা এবং প্রয়োজনেমত ভিটামিন বা ঔষধ দেয়া হবে।

(৭) যে কোন সময় রোগী এই গবেষনা থেকে নিজেকে প্রত্যাহার করে নিতে পারে এবং এতে স্বাভাবিক চিকিৎসার কোন ত্রুটি হবেনা।

এই গবেষনার কাজে রাজী থাকলে স্বাক্ষর করুন।

স্বাক্ষর _____

রোগীর সঙ্গে সম্পর্ক _____

তারিখ _____