Attachment 1. 06

REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS Date [illegible] 6
CRL

Principal Investigator [illegible] Trainee investigator(if any) _____

Application No 77-029 Supporting Agency(if Non-CRL) _____

Title of study [illegible] 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 possibly 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
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 question 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 area.
3. An indication as to when the questionnaire will be presented to the Board for review.

We agree to obtain approval of the Review Board on Use of Human Volunteers for any changes involving the rights and welfare of subjects before making such change.

[illegible]
Principal Investigator

Trainee

Please return 2 copies of entire protocol to Chairman, Review Board on Use of Human Subjects.

[redacted] ADDENDUM TO PROTOCOL - GASTRIC ACID IN ENTERIC DISEASE

11-029

Q6

We have found that 9 of 30 adult cholera patients have low acid 7 days after hospitalization. We have also found only 1 of 15 shigella patients with low acid (< 5 mEq per hour). We would like now to include children age 2-8 in this protocol. This will mean studying 30 children with cholera, 30 children with darkfield negative watery stool and 30 children with shigella. Initially well-nourished children will be selected since we have found that over 40% of children with severe malnutrition do not stimulate with a 1mg/kg Histalog dose.

There will be only 2 differences from the adult protocol. Immediately after gastric analysis on the day after admission, children will receive 48 hours of tetracycline therapy. If there is a pH drop below 2.0 he will be retested on the 8th hospital day. Children will be brought for one day after one month for a follow up gastric acid test as presently performed. Routine therapy for other diseases will of course be given.

Children with shigella will likewise receive a 100mg/kg ampicillin for 5 days given in 4 divided doses, but this will start on the day of admission.

We have found that quantitative gastric cultures of gram negative organisms correlate well with gastric acid titration studies. We would therefore like to do quantitative cultures on gastric juice on MacConkey and in cholera cases on Gelatin agar. This will require a technician 1 hour per day.

CONSENT FORM

I understand that refusal to enter this study will not affect the usual type of treatment my child will receive at the Cholera Hospital.

I understand that my child is admitted for therapy of diarrhea and will receive routine treatment, except antibiotic treatment will be withheld for 24 hours in the case of cholera.

I also understand that he/she will in addition receive a test of his/her stomach acid and the ability of his/her stomach to kill germs.

This will be done by putting a tube into his/her stomach and sucking stomach juice for 3 hours. He/she will also receive an injection of medicine to stimulate his/her acid production. This medicine may cause him/her to get a slight headache and to become flushed. During the test he will have sugar given through a vein so that he/she will not need to eat during the test.

He/she may stay in hospital for 7 days. This is 4 days longer than he/she would normally stay in hospital. You can leave the study at any time and in no way will be penalized for doing so.

All information obtained will be handled confidently.

_____
Signature

_____
Date

সম্মতি পত্র

আমি অবগত হয়েছি যে এই গবেষণায় অংশ গ্রহন না করলেও কলেরা হাসপাতালের নিয়ম মাফিক স্বাভাবিক চিকিৎসা থেকে আমার সন্তান/ সন্তুনি বঞ্চিত হবে না। আমি অবগত আছি যে আমার সন্তানকে উদারাময় (ডাইরিয়া) রোগের সুচিকিৎসার জন্য হাসপাতালে ভর্তি করা হয়েছে। রোগীকে এন্টিবাইওটিক ঔষধ ছাড়া অন্যান্য প্রকারের ঔষধ যথাযথভাবে দেয়া হবে। কলেরার জন্য এন্টিবাইওটিক ঔষধ ভর্তির ২৪ ঘন্টা পর শুরু করা হবে।

রোগী / রোগীনির পাকস্থলীর পাকরস নিয়ে পরীক্ষা করা হবে এবং তার পাকরসের জীবানু ধ্বংশী ক্ষমতাও পরীক্ষা করে দেখা হবে। পাকস্থলীর ভিতর একটি নাল প্রবেশ দ্বারা ৩ ঘন্টা পর্যন্ত পাকরস গ্রহন করা হবে। রোগী / রোগীনিকে তার পাকরস ক্ষরনের জন্য একটি উত্তেজক ইনজেকশন দেয়া হবে এর জন্য সামান্য মাথা ব্যাথা বা চোখ মুখে গরম ভাব দেখা দিতে পারে। পরীক্ষা চলাকালীন রোগীকে রওশিরার মাধ্যমে শর্করা জাতীয় ঔষধ দেয়া হবে যার ফলে ঐ সময় তার কোন খাদ্য গ্রহনের দরকার হবে না।

রোগী / রোগীনিকে ৭ দিন হাসপাতালে থাকতে হবে। এই সময় স্বাভাবিক চিকিৎসার চাইতে ৪ দিন অতিরিক্ত হবে। ইচ্ছা করলে আপনি যে কোন সময় আপনার সন্তানকে গবেষণা থেকে সরিয়ে নিতে পারবেন। তার জন্য কোন প্রকার ক্ষতির সম্ভবনা থাকবে না।

সমস্ত তাওশরী ফলাফল গোপন ভাবে রাখা হবে।

নাম

তারিখ :----------

Received 11/10/77-029

Attachment 1a

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The Board will not consider any application which does not include an abstract summary. The abstract should summarize the purpose of the study, the methods and procedures to be used, by addressing each of the following items. If an item is not applicable, please note accordingly:

1. Describe the requirements for a subject population and explain the rationale for using in this population special groups such as children, or groups whose ability to give voluntary informed consent may be in question.

2. Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

3. Describe procedures for protecting against or minimizing potential risks and an assessment of their likely effectiveness.

4. Include a description of the methods for safeguarding confidentiality or protecting anonymity.

5. When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.

   (a) If signed consent will not be obtained, explain why this requirement should be waived and provide an alternative procedure.

   (b) If information is to be withheld from a subject, justify this course of action.

6. If study involves an interview, describe where and in what context the interview will take place. State approximate length of time required for the interview.

7. Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the planned work. Indicate how the benefits outweigh the risks.

8. State if the activity requires the use of records (hospital, medical, birth, death or other), organs, tissues, body fluids, the fetus or the abortus.

The statement to the subject should include information specified in items 2,3,4 and 7, as well as indicating the approximate time required for participation in the activit

SECTION I - RESEARCH PROTOCOL

1. Title: Gastric Acid in Enteric Disease

2. Principal Investigator: Robert H. Gilman, M.D.

3. Starting Date: September, 1977

4. Completion Date: May, 1978

5. Total Direct Cost: $16,094

6. Abstract Summary:

A case control method will be used to evaluate gastric acid in patients with enteric disease. Adult patients with cholera, amebic dysentery and shigellosis (30 in each group) will be compared in their ability to produce gastric acid after a 50mg betazole stimulus. They will be studied the morning after admission, 7 days, 3 months and in a few cases 6 months after hospital admission. Gastrin levels will also be determined.

7. Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: To define gastric acid production in enteric disease.

2. Background: Gastric acid is hypothesized to be a barrier to enteric infection. Enteropathogenic bacteria die at low ph's, and volunteers challenged with cholera, E.coli and shigellosis all have reductions in the infective dose after bicarbonate buffering of the stomach.[1] Patients with partial gastrectomy have an increased risk of developing clinical cholera and salmonella.[4] Those who partake of lime (calcium carbonate) with their betel are also at a higher risk of developing cholera.

However, still uncertain is whether cholera is associated with lowered gastrin acid secretion in most cases, and if there is an association, whether cholera causes a decrease in gastric acid or whether low gastric acid predisposes patients to having cholera. Indeed, whether cholera patients have increased achlorhydria (ph> 3.5) is still extremely controversial. In addition, how frequent this is histamine fast is also unknown.

In Calcutta, achlorhydria and a blunted response to 50mg betazole injected dose was found.[6] In Dacca using a histamine infusion technique neither achlorhydria nor a diminished threshhold was found in an uncontrolled study.[7] In another study from Dacca, both achlorhydria and an inability to produce acid after a rice gruel meal were found.[8] In comparing these studies methodological differences make it difficult to arrive at a firm conclusion since the stimulus in each study was different. In addition, the studies were uncontrolled for the normal population and the effects of diarrheal disease on gastric acid. The Calcutta study although studying patients at various

times after disease did not study the same patients but rather used different patients at various periods after a bout of cholera.

Using age-matched controls with different enteric diseases, it should be possible to isolate whether inhibition of acid secretion is more common in one disease than in the others. Studies of gastric secretions in adult patients with shigellosis and enterotoxigenic E.coli have not been performed. Gastric acid would not be expected to protect patients from attacks of amebic dysentery since these patients ingest cysts which are not harmed by low ph. Patients with amebic dysentery would therefore not be expected to have a lowered acid secretion.

3. Rationale: The rationale of this study is the use of the case-control method to compare gastric acid secretions. As an attempt will be made to age and sex match patients, they should be comparable. Similarly, patients infected with E.coli would be expected not to have gastric acid changes different from those of shigella unless the toxin itself has some effect on gastric acid production.

B. SPECIFIC AIMS

1. Using the case control method we hope to show whether cholera cases have impaired acid production.
2. In cholera cases relate this impaired production to the duration extent of diarrhea and duration of cholera vibrio positivity.
3. Define the normal histalog stimulation test in Bengali adult males.
4. To compare gastric acid output in diarrheal diseases. It will be especially interesting to compare cholera and E.coli patients.

C. METHODS OF PROCEDURE

Adult male patients admitted to the ward with ulcerative and non-ulcerative amebic dysentery, shigella, or cholera will initially be studied. An attempt will be made to age-match these adult patients within 10 year groups. Thirty patients in each of these four groups will be studied. In addition, 15-30 normal controls will be tested.

Patients admitted within the previous 24 hours will have no oral fluids after 12 midnight. Purging will be replaced using intravenous therapy. All patients will have an I.V. with 5% Dextrose, and electrolytes if purging, 4 hours prior to testing to prevent mild hypoglycemia.

All patients will receive routine ward care. An initial history and physical will be performed and blood for Hct, CBC, and BUN, electrolytes and serum specific gravity will be determined. History will specifically note what medicines the patient took prior to admission, in cases of cholera, how many hours of diarrhea preceded admission and whether he was pulseless on admission. A chest X-ray to determine free air and a high right diaphragm will be taken as routine for patients with amebic dysentery. As a part of research, all cholera and shigella patients will also have chest X-rays. Routine urinalysis and stool microscopic examination will be performed. All amebic dysentery and shigella patients will have proctoscopy performed to define the severity of the disease. Stool culture will be performed as routine except in patients suspected of having E.coli diarrhea (watery diarrhea and a negative darkfield exam.). In these patients 2 individual lactose positive colonies will be picked and tested for enterotoxin production by cho and infant mouse assay.

Patients with cholera will be treated with I.V. hydration only. Tetracycline therapy will be withheld till discharge. This will be done so that the volume

and duration of diarrhea and the duration of vibrio carriage can be compared in patients with differing gastric acid. Metronidazole will be withheld till after gastric analysis has been performed. Patients with non-ulcerative amebic dysentery have low rates of complications and a mean duration of disease prior to admission to the hospital of 3 months. A 16 hour delay in therapy of these patients is felt to be low in risk. Patients with ulcerative amebiasis will be treated as usual with metronidazole given in divided doses except that the morning dose will be deferred till after the gastric acid study is completed. Similarly, in shigella cases the ampicillin dose (given initially) will be withheld after 12P.M. at night till after the gastric acid test is performed.

Patients will be intubated with a mercury weighted plastic tube with multiple holes cut at the end. The tube will be inserted into the stomach to the 50cm mark. The patients will be placed on his left side. Gastric juice will be collected for the next 30 minutes and discarded. Subsequently, using intermittent hand suction, 15 minute samples of gastric juice will be collected for one hour. An injection of histalogue 50mg will be given I.M. and 15 minute samples collected for the next 2 hours. All samples will have their volumes measured and then be sent to chemistry for free and total acid determinations. This determination will be made by titration on a pH meter to pH 3.5 to 7.0 with 0.01NNaOH. Titration will be performed on the day of the test. Patients will have gastric aspirates tested for the presence of bile in the stomach. All patients will have their gastric acid tests repeated at 7 days. Patients will be asked to return for follow up 3 and 6 months after hospitalization for repeat gastric acid tests.

A cohort of normal subjects (volunteers) will be asked to have gastric acid tests; they will be reimbursed for their time lost for work. This group will be drawn from attendants of patients asked at the outpatient department.

In addition, any patient found to have hyposecretion on the 7th day will be retested using pentagastrin (6ug/kg) as a stimulus. Achlorhydria resistant to histamine but sensitive to gastrin has been described.

Statistics - Assuming 90% acidity (ph 3.5) in one group and 60% acidity in the other, 30 per group will be adequate to obtain statistical differences. In addition, T tests will be performed on mean basal and stimulated values of MEQ/L per hour obtained in each of the 5 groups.

The time needed for this study will be 6 months.

D. SIGNIFICANCE

It is hoped by using a case control method that we can answer the question of reduced gastric acid in cholera with some degree of finality. This will say nothing however, about risk rates of the population at large. It should at the same time describe the status of gastric acid in shigellosis and amebic dysentery.

E. FACILITIES REQUIRED

Office space - Investigator and Study doctors as already provided.

1) Laboratory space - chemistry - routine - 6 cubic feet.
2) Storage space. For duration of study. ¼ of Revco for serum specimens.
3) Hospital beds - 10 beds per day per week for 6 months.
4) Animal resources - Infant mice - 200 for Infant Mice ST assay.
5) Vehicle - 2 months - for 3 hours daily for follow up visits and 3 Matlab visits.
6) No specialized requirements.

F. COLLABORATIVE ARRANGEMENTS

Arrangements depend on where c'strin and assays will be performed.

BIBLIOGRAPHY

4. DuPont, H.L. & Hornick, R.B. Clinical Approach to Infectious Diarrheas Medicine 52:265-269, 1973

5. Giannella, R.A. et al. Influence of Gastric Acidity on Bacterial and Parasitic Enteric Infections. Annals of Internal Medicine 78:271-276, 1973

5. Johns Hopkins University - International Center for Medical Research Annual Report 1974-1975. Nalin P 65. Cited in Lancet Editorial Lancet II 1283, 1976

6. Sack, G.H. et al. Gastric acidity in cholera and noncholera diarrhoea. Bull. Wld Hlth Org. 47:31-36, 1972.

7. Cash, R.A. et al. Acid in Cholera. Lancet 2:1192, 1970

8. Nalin, D.R & Levine, R.J. Cholera is Primarily Waterborne in Bangladesh. The Lancet II:1305, 1976

[illegible] & McGuigan, J.E. Relations between Serum Gastrin Levels and Rates of Gastric Hydrochloric Acid Secretion. The New England Journal of Medicine 284:408-412, 1971

[illegible] the histological and secretory changes in the stomach in patients with auto-immunity to gastric parietel cells. Lancet I:401, 1964

CONSENT FORM

I understand that I have been admitted for treatment of diarrhea or dysentery. I also understand that I will have a tube put down my stomach and the digesting and acid power of my stomach tested both on admission and 7 days later. This is 7 days longer than I would normally be hospitalized. The tube may produce slight gagging but otherwise will not be uncomfortable. I will be given an injection which may produce slight flushing and headache.

Blood from my vein in small amounts 5cc's will be taken during this test and at 2 day intervals. My stool will be collected at the same time to check the loss of protein. If I am an amebic dysentery case my medicine may be delayed for 18 hours after admission. This will be inconvenient but this short delay should not be harmful.

Refusal to participate in this study will not alter the therapy I would routinely get at the Cholera Hospital. Also, I am free to withdraw from this study at any time and will in no way be penalized for doing so.

I also understand that if I have cholera that my treatment will be only fluids and no antibiotics. This may increase the number of days I will be in hospital with diarrhea. However, cholera although shortened does not require antibiotic therapy. I will receive an antibiotic at the end of 7 days.

_____
Signature

_____
Date

The role of gastric acid in naturally acquired enteric disease is still controversial. Resolution of this problem with minimal risk and slight discomfort would appear to be reasonable.

ABSTRACT SUMMARY

Adult patients will be studied. All patients will be rehydrated. Tetracycline treatment will be withheld in the treatment of cholera patients. Similarly, in patients with non-ulcerative amebic dysentery therapy will be withheld till the initial gastric acid test. This will mean a delay of less than 24 hours. In shigellosis, patients with fever and in patients with ulcerative amebiasis there will be no delay in therapy.

Patients will be hospitalized 7 days after admission; this is 4 days longer than is necessary for cholera and 2-3 days longer than is necessary for non-ulcerative amebiasis and shigella.

The only invasive procedure which is associated with research will be passing a stomach tube. This is virtually without risk. Betazole (Histalogue) in the dose given may rarely produce slight symptoms of flushing and headache. Blood 5cc's will be taken at the time of the study for gastrin levels. Two cc's of blood will be taken every other day for fecal protein studies.

Tetracycline therapy in cholera is not essential to the treatment. Its absence however will increase the duration of diarrhea and stool volume and also increase the hospital stay of the patient. Withholding treatment is necessary to determin duration and volume of diarrhea in cholera patients with differing gastric acid. Non-ulcerative amebic dysentery patients have a mean history of 3 months prior to hospitalization and rarely have complications. Similarly, in shigellosis in adults, the effect of antibiotics is still controversial.

The risk in therapy delay is therefore minimal. In ulcerative amebic dysentery, a group of patients with a high risk of complications, therapy will not be withheld.

PROCEDURES FOR [illegible] CONFIDENTIALITY

Patients admitted to the study will be given a study number; records will be kept according to study number and all data will be kept in a locked file in the investigator's locked office. Following completion of the study, all identifying information will be cut off from the data sheet and the clinical information only will be kept at the Cholera Research Laboratory in a locked data storage office. Results of the study will be published in a medical journal and no identifying information will be included in the report of this study.

SCHEDULE

	Shigella	Amebiasis	E.coli	Cholera
Admission Day				
Procto (Non-research)	+	+		
Serum Pcc (Research)	+	+	+	+
UA - Lytes	+	+	+	+
X-ray - Chest	+ Research	+	+ Research	+ Research
U/A	+	+	+	+
C.C. & S.S.	+	+	+	+
Therapy	+	+ Delay - Research	-	Delay Research
4 hour prior	+	+		
25% Glucose or 5% D5W	D5W	D5W	25%	25%
Gastrin 8:30	+	+	+	+
Tube	+	+	+	+
Histalog - 50mg	+	+	+	+
Gastrin - 30 min.				
Post Histalog	+	+	+	+
Tube out - 2hr post stim.	+	+	+	+
Repeat at 7 days	+	+	+	+
Repeat at 5-6 months	+	+	+	+

These patients will also be used for protein-loss studies. This will be written as a separate protocol.

[illegible]

[illegible]

1. PERSONNEL SERVICES

	Name	Position	% of effort	Annual Salary	Project Requirements TAKA	Project Requirements DOLLARS
1.	Dr R. Gilman	Chief Investigator	10%	$ 33,000		3,300
2.	Dr Rabbani	Study doctor	60%	Tk27,084	16,250	
3.	Dr Asma	Study doctor	20%	Tk27,084	5,417	
4.	Ms Ghosh	[illegible], Staff Nurse	5%	Tk14,880	744	
5.	Dr Seaton		5%	$ 18,907		945
6.	Mr Akbar Ali	Study Tech.	10%	Tk32,076	3,208	
7.		Chemistry Tech.	40%	Tk 8,668	3,467	
8.		Sera organ.	10%	Tk15,927	1,593	
9.	Mr J[illegible]e Gomez	Serologist	70%	Tk15927	11,145	
10.		Ward clerk	50%	Tk 6,312	3,156	
11.		Field Assistant	50%	Tk 6,000	3,000	
12.		2 extra sweepers	25%	Tk 4,000	2,000	
13.		1 assistant nurse	25%	Tk 8,000	2,000	
					51,980	4,245

2. SUPPLIES & MATERIALS

Mercury, tubes, needles, syringes & mis. 4,000
If gastrin or ferritin studies are to be performed here * cost not added

3. EQUIPMENT

Use of one calculator

4. PATIENT HOSPITALIZATION

Each patient is hospitalized for 7 days x 120 = 840 days

840 days x 135 113,400

5. OPERATIONAL CAR

Sweeper to be used for transporting watery stool to lab. from the outpatient ward. An assistant nurse and senior sweeper to be used to assist gastric acid studies.

In addition, outpatient care will be needed to get good 3 month follow up. We expect only about ¼ of the patients to return and will offer 15 Taka per study at that time. 450

6. TRANSPORT

Car - 1,000 miles - Dacca - Matlab 1,400

7. OVERSEAS TRAVEL -

8. OUTSIDE TRANSPORT -

9. RENT, COMMUNICATION AND UTILITIES 1,000

10. PRINTING AND REPRODUCTION 4,000

11. CONTRACTUAL SERVICE

15 Taka per normal patient 1,500

12. CONSTRUCTION -

	Category	Year 1		Year 2
		Taka	Dollars	
1.	Personnel	51,900	4,245	-
2.	Supplies	4,000	-	-
3.	Equipment	-	-	-
4.	Hospitalization	113,400	-	-
5.	Outpatient Care	450	-	-
6.	CRL Transport	1,400	-	-
7.	Overseas Travel	-	-	-
8.	Transportation of things	-	-	-
9.	Rent/Communication	1,000	-	-
10.	Printing and Reproduction	4,000	-	-
11.	Contractual Service	1,500	-	-
12.	Construction	-	-	-
		177,730	4,245	

Total Direct Cost = $[illegible],094

Conversion rate $U.S.1 = Tk. 15