achment 1.

62 ETHICAL REVIEW COMMITTEE, ICDDR,B

Date 26.6.83

ncipal Investigator K.A. AlMahmud

lication No. 83-026

e of Study An animal model for e study of invasive colitis

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B)

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

le 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 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
(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

NA

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.

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

K A Mahmud.

Principal Investigator

Trainee

83-026
26/6/83

SECTION I - RESEARCH PROTOCOL

1. Title: An animal model for the study of invasive colitis

2. Principal Investigator: Dr. K.A. Al-Mahmud

   Co-Investigators: Dr. T. Butler
   Dr. I. Kabir
   Dr. A.S.M. Hamidur Rahman

3. Starting Date: July 1983

4. Completion Date: June 1984

5. Total Direct Cost: $ 8,225

6. Scientific Program Head: Dr. T. Butler

This protocol has been approved by the Host Defense Working Group

Signature of the Program Head: _____

Date: 21 6 83

7. Abstract Summary:

The normal colon absorbs water, sodium, and chloride and disease may impair this absorptive ability. A new model of colonic absorption will be tested by using a ligated loop of proximal colon in the rat. The ability of theligated loop to absorb solutions over time will be examined and the action of Shigella infection, endotoxin, and leukocytes to inhibit absorption will be tested.

8. Reviews:

   a. Research Involving Human Subjects: _____

   b. Research Review Committee: _____

   c. Director: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

   a. In the adult rat to measure the ability of a ligated colonic loop to absorb water, sodium, and chloride.

   b. To examine the effect of Shigella infection, and endotoxemia on the function of the loop.

2. Background

The normal human colon receives up to about 2000 ml of ileal effuent per day and absorbs all but about 100cc, which is the normal volume stool water passed each day. Diarrhea can result either from increased net secretion of water by the small intestine, as occurs in cholera, or by failure of the colon to absorb water. The normal colon has a capacity to absorb about 5,000 ml per day (1). During diseases that cause inflammation of the colon, as occurs in ulcerative colitis, water absorption and sodium absorption are impaired and more potassium is secreted (2). In the monkey model of shigellosis, the colon is converted from its normal absorptive function to being secretory (3).

In addition to inflammation in the intestine, diarrhea can result in humans from diseases outside the intestine, such as pneumonia or influenza. Physicians refer to this kind of diarrhea as "parenteral diarrhea." The mechanism of this diarrhea is unknown and animal models have not been used to study it.

Large numbers of fecal leukocytes are excreted in the stool of patients with invasive diarrhea. The leukocytes are used as diagnostic markers by physicians, but there is a possibility that fecal leukocytes play a role in producing diarrhea. The role of fecal leukocytes in an animal of diarrhea has not been tested.

The rat colon has been used by Donowitz and Binder to study colonic function (4). They used a closed loop of caecum and showed under normal conditions absorbed water, sodium, and chloride and secreted bicarbonate. After exposure to cholera toxin, the caecum secreted water, sodium, and chloride and secreted more bicarbonate. Shigella enterotoxin had no effect colonic function in this model.

The proposed work intends to modify the rat colon model of Donowitz and Binder by ligating the ascending colon, which is a region of the colon more relevant to human colitis than is the caecum. The action of Shigella infection in the loop will be tested and an effect of circulating endotoxin on colonic function will be assessed.

B. SPECIFIC AIMS:

1. To develop a new animal model of colonic function by isolating the ascending colon of the rat.
2. To measure the ability of the colon to absorb water, sodium, and chloride.
3. To test the effects of Shigella infection and endotoxin and leukocytes on the function of the colon.

C. METHODS:

1. Adult Long-Evans rats breeded in the Animal Facilities will be used. Rats with weights 250-300g will be chosen. They will be fasted for 48 hours before the experiments.
2. Rats will be anesthetized with pentobarbital 65mg/kg body weight IP. The ascending colon will be gently squeezed to remove contents into the distal colon. One ligature will be placed around the ascending colon at its exit from the caecum and another placed about 6 cm away to create an isolated loop.
3. About 3 ml of water and electrolyte solution containing polyethylene glycol (PEG) will be injected into the loop using a 26-guage needle. Solutions to be tested will include free water, ½ strength normal saline, and Na 140, K 5, HCO_3 40, and Cl 105 meq/L.
4. The loop will be returned to the abdominal cavity and the wound sutured closed. Rats will be sacrificed at intervals of 3hr, 6hr, 24hr and 48hr. The contents of the loop will be measured in a graduated cylinder, and the PEG and electrolyte concentrations measured.
5. In some experiments, loops will be injected with cultures of Shigella dysenteriae 1, S. flexneri, S. boydii, and S. sonnei. In some rats, endotoxin solutions will be injected intravenously after the loops have been created and injected with water-electrolyte solutions.
6. From some rats, specimens of the colonic loops will be placed into formalin for histologic examination to assess degrees of inflammation.
7. Leukocytes will be concentrated from heparinized rat or human blood by dextran sedimentation, washed, counted, and suspended in 10% serum

in normal saline. The leukocytes will be injected into colonic loops to test the action of leukocytes on colonic function. Stimulated leukocytes will be prepared by incubating leukocytes with Shigella bacteria or endotoxin for 2 hr at 37°C prior to injection of leukocytes into the loop. If any effect is observed, the action of cells will be separated from chemical mediators by testing supernatants of incubation mixtures after centrifugation.

8. Alternative model, if the rat colon does not respond to Shigella infection, the guinea pig colon will be tested as an alternative.

9. Data analysis. From the volume of fluid in the loop, electrolytes, and PEG concentration the net absorption and secretion of water and electrolytes will be calculated. The kinetics of absorption will be assessed and the effects of Shigella infection and endotoxemia measured.

D. SIGNIFICANCE

Shigellosis and other forms of colitis are serious diseases in Bangladesh and other developing countries. Convenient and inexpensive animal models are required to advance our knowledge about pathogenesis and treatment of colitis. The rat colon could prove to be a useful model to test virulence characteristics of infectious-agents and to test new therapies.

E. FACILITIES REQUIRED

The Animal Facilities, Biochemistry, and Microbiology Labs will be required.

F. COLLABORATIVE ARRANGEMENTS

No collaborating arrangement would be needed from oversease for this study.

References

1. Binder H.J.: Colonic secretion. Chapt. 39 in Physiology of the Gastrointestinal Tract, edited by L.R. Johnson, Raven Press, New York, 1981

2. Rask-Madsen, J: Simultaneous measurement of electrical polarization and electrolyte transport by the entire normal and inflammed human colon during in vivo perfusion. Scand. J. Gastroenterol 8:327-336,1973

3. Rout W.R., Formal S.B., Gianella R.A., and Dammin G.J.: Pathophysiology of Shigella diarrhea in the rhesus monkey: intestinal transport, morphological, and bacteriologic studies. Gastroenterol. 68:270-278,197

4. Donowitz, M. and Binder H.J.: Effect of enterotoxins of Vibrio cholerae, Escherichia coli, and Shigella dysenteriae Type 1 on fluid and electrolyte transport in the colon. J. Infect.Dis.134:135,197

Abstract Summary:

1. This study requires use of animals only
2. No risks are involved
3. Pain to animals will be minimized by use of anethesia.
4. NA
5. NA
6. NA
7. Benefits will be to learn more about mechanisms of diarrhea in the colon.
8. No use of records, organs, tissues, or body fluids from humans.

SECTION III - DETAILED BUDGET

1. Personnel Services:

Name	Designation	% Effort	Salary Taka	Salary Dollar
Dr. K.A. AlMahmud	Pr.Investigator	20	26,400	-
Dr. A.S.M. H. Rahman	Co-Investigator	10	6,500	-
Dr. T. Butler	Co-Investigator	5	-	3,500
Not named	Sr. Med.Officer	20	18,700	-
Not named	Technician	20	16,000	-

2. Supplies and materials:

Rats : 400 Tk.20 each	8,000	
Pentobarbital :	-	100
Surgical instruments :	-	100
Syringes, gloves :	-	200
Bacteriological media :	-	200
Histological specimens :100 spec.Tk.80 each	8,000	
Electrolytes : 300 Tk. 12 each	3,600	
PEG : 300 Tk. 12 each	3,600	
Dextran & media for leukocytes :	-	300

3. Equipment - None

4. Patient - None

5. Outpatient - None

6. ICDDR,B transport - None

7. Travel - None

8. Rent, utilities - None

9. Printing, reproduction : 6,000

10. Other Contractual services : Pathology Consultant 500x10 5,000

11. Construction : None - -

Total :	Tk.101,800	US$ 4,400
	$: 3825	

Total US$: 8,225

(Conversion rate US$1=Taka-1)

BUDGET SUMMARY

		US$
1.	Personnel services	6,316
2.	Supplies and materials	1,866
3.	Equipment	-
4.	Patient	-
5.	Outpatient	-
6.	ICDDR,B Transport	-
7.	Travel	-
8.	Rent, utilities	-
9.	Printing, reproduction	00,250
10.	Other Contractual services	00,209
11.	Construction	-
	Grand Total :	8.225