Attachment 1.

Date 30/3/81

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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Shahjahan Kabir Trainee Investigator (if any) _____

Application No. 81-016 Supporting Agency (if Non-ICDDR,B) _____

Title of Study Investigations on the mechanism of adhesion of Vibrio cholerae

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: NA
   (a) Ill subjects Yes No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve: NA
   (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: NA
   (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: NA
   (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 summar
   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 sensitiv 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.

Shahjahan Kabir
Principal Investigator

Trainee

81-016
rec'd 1/4/81

SECTION I - RESEARCH PROTOCOL

1. Title: Investigations on the mechanism of adhesion of Vibrio cholerae

2. Principal Investigator: Shahjahan Kabir

3. Starting Date: April, 1981

4. Completion Date: March, 1983

5. Total Direct Cost: $64,988

6. Scientific Programme Head:

This protocol has been approved by the Working Group.

Signature of the Scientific Programme Head W.B.C.

Date 30/3/81

7. Abstract:

The study plans to elucidate the mechanism of adhesion of Vibrio cholerae from several strains belonging to both the biotypes (classical and El Tor) and the serotypes (Ogawa and Inaba). Vibrio cholerae will be grown under various cultural conditions. The nature of forces involved in adhesion will be studied by performing binding studies on model compounds. Hydrophobic gels will be used to study whether hydrophobic interactions are involved in the adhesive process.

Similarly ion-exchange mattrices will be used to study the role of ionizable groups in the process of adhesion. Binding studies with erythrocytes from different species will indicate whether there are carbohydrate mediated receptors to which Vibrios may adhere. The cellular specificity in adhesion will be studies by performing adhesion of Vibrios with intestinal epithelial and lymphoid cells and comparing the results with those isolated from non-intestinal organs. Adhesion of Vibrios to eukaryotic cells will be quantitated either by enumerating bacteria under a microscope or growing Vibrios in the presence of a radiolabelled nutrient and counting the adherent cells in a scintillation counter. To study the effect of different cell surface components in the process of adhesion, rabbits will be immunised with different cell surface components (flagella, lipopolysaccharides and outer membrane proteins). Adhesion of Vibrios will be studied in the intestines of immunised rabbits and results will be compared with those obtained from unimmunised rabbits. Experiments will be performed to correlate adherence and immune response by orally immunising mice with different strains of Vibrio cholerae based on their adherence properties and quantitating immunoglobulin production in the intestine at different time intervals. Prophylactic potential of model compounds such as hydrophobic gels and ion-exchange resins against experimentally induced cholera will be studied in rabbits.

A. INTRODUCTION

1. Objective: The long term objective of these studies is to control diarrhoeal disease caused by the toxin-secreting non-invasive bacteria, such as Vibrio cholerae. Hence, research will be carried out to understand the mechanism by which Vibrio cholerae colonize the host environment. It is anticipated that information obtained from such studies will make significant contribution to the design of studies to prevent enteric bacterial infections in humans.

Background: It has been known for sometime that bacteria possess adhesive properties. Bacteria have been found to adhere to materials as diverse as the surfaces of clays (1), glass (2) the root hair of surface plants (3), the gut of nematodes (4), the surface of protozoans (5) etc. In animals the route of infections for most pathogens is through the mucous membranes lining body entrances. The mucosal surfaces are continuously bathed by fluids such as tears, saliva, intestinal juice, urine which tend to wash away bacteria. To exert pathogenesis bacteria must adhere to mucosal epithelial cells by resisting the cleansing action

of these fluids. Therefore, adhesion represents an initial event in the colonisation of a habitat by bacterial species. After initial attachment bacteria may multiply and secrete toxic products which produce symptoms or induce infection by invading the mucosa and underlying tissues.

Recent results from several laboratories indicate that a high degree of specificity exists in the process of adhesion of bacteria to the host cell surfaces. Gibbons and Houte (6) observed that strains of Streptococcus salivarius and S. sanguis which are present in significant proportions on oral epithelial surfaces possessed definite capacity to adhere to epithelial cells obtained from cheek scrapings of humans. In contrast, strains of S. mutans which were found only in minor proportions, if at all, on oral epithelial cells exhibited feeble or no adherence at all. Frost (7) has reported that common pathogens of bovine mastitis such as Staphylococcus aureus and S. agalactiae adhere to ductular epithelial cells than do bacteria which do not frequently cause mastitis. Group A Streptococci associated with rheumatic fever were

reported to attach in greater number to buccal cells of patients with rheumatic fever than to normal subjects (8). Recently, it has been demonstrated that E. coli isolated from patients with urinary tract infection adhere better than E. coli from patients with asymptomatic bacteriuria (9). Gould et al (10) studied the adherence of bacteria to heart valves in vitro and observed that organisms that most frequently cause bacterial endocarditis were found to adhere best to heart valves suggesting that the ability to adhere to valvular endothelium may be an important or essential characteristic of bacteria that cause endocarditis. These results suggest that a high degree of specificity exists in the process of adherence of bacteria to the host tissue surfaces.

The interaction of bacteria with animal cell surfaces: Although considerable amount of work has been done to study the nature of interaction between eukaryotic cell surfaces, there has been little work of a similiar nature on prokaryotic-eukaryotic cell interactions. It can be anticipated that the following forces might play an important role in the interaction of bacteria with animal cell surfaces.

1. Cell surface potential:

The surface of prokaryotic and eukaryotic cells have a negative potential that results from the ionisation of various chemical groups of the cell. The surface potential of the human erythrocyte, for example, results mainly from the ionisation of the sialic acid carboxyl groups. The chemical entities responsible for the surface potentials of bacterial cells vary with species, strains and growth conditions. As example, the teichoic acids of the cell walls of Staphylococcus aureus (11), the glucuronic acid capsules of Klebsiella aerogenes (12), the fimbriae of E. coli (13), and the hyaluronic acid of S. pyogenes capsules (14) markedly influence surface potential. Surface charges have important effects on the adhesive properties of bacteria. Heckels et al (15) studied the mode of adhesion of non-fimbriate (non-adhesive) and fimbriate (adhesive) gonococci and observed that neutralisation of the negative charge of the gonococcal surface resulted in an increase in the adhesion of fimbriate gonococci. Neutralisation of the positive charge of gonococcal surface, in contrast, resulted in almost total elimination of adhesiveness.

Total charge also vary from one bacterial species to another. Thus, Hall et al (16) have been able to fractionate S. typhimurium cells by ion-exchange chromatography into two populations and could differentiate S. typhimurium from E. coli on the basis of their surface charge properties.

2. Cell surface receptors:

Increasing evidence in the literature suggest that there might be receptors on the host cell membrane mediating the bacterial adhesion. Although the major chemical constitutent of the eukaryotic cell membrane are lipids, proteins and carbohydrates, it is the cell surface carbohydrates which have been implicated as possible receptors for the attachment of bacteria. The phenomenon of agglutination of erythrocytes of human and other animals by E. coli was reported as early as in 1980. The first indication that cell surface sugars may be involved in these interactions was the observation made in 1955 by Collier and DeMiranda (17). D-Mannose, alone of many sugars tested, strongly inhibited the hemagglutination reactions. Later Duguid and Gilles (18) observed that the hemagglutinating activity was associated with the presence of pilli on the organisms. They observed

that E. coli adhered to intestinal epithelial cells and the adherence was blocked by D-mannose. Later Ofek et al (19) demonstrated that the adherence of several strains of E. coli to human epithelial cells was mediated by mannose (or mannose like) receptors present on the surface of the latter. Thus, of a variety of sugars tested, only D-mannose and methyl-α-D-mannoside at low concentrations, inhibited the bacterial adherence to epithelial cells. Yeast mannan, a polymer of D-mannose, was also a strong inhibitor.

Sodium metaperiodate is a reagent which cleaves vicinal hydroxyl groups in sugar residues. Epithelial cells treated with sodium metaperiodate failed to bind E. coli. Besides treatment of epithelial cells with concanavalin A, a lectin which binds to D-mannose (or D-glucose) residues on cell surfaces, inhibited bacterial adherence. These results suggest that cell surface sugars act as determinants of recognition in bacteral adhesion.

3. Chemical bonds involved in cell-cell interaction:

Physicochemical properties of the enterobacterial cell surface such as hydrophobicity (i.e., interaction between non-polar groups) might play a role in the phenomenon of bacterial adherence. Thus, localised hydrophobic areas of the bacterial cell surface might interact with similar groupings on the host through displacement of water and form an adhesive bond. Smyth et al (20) have demonstrated in vitro adhesion of porcine enteropathogenic E. coli strains possessing pilus-like structures (K-88 antigen) to the hydrophobic gel mattrices. E. coli strains lacking K-88 antigen did not adhere indicating that hydrophobic groups on the pilli might be involved in the bacterial adherence. Peres et al (21) have observed that lipophilic strains of S. typhimurium and E. coli showed a greater association with intestinal mucosa than that exhibited by more hydrophilic strains.

Studies with Vibrio cholerae:

Very few studies have been performed to investigate the adhesive properties of Vibrio cholerae.
Earlier Freter indicated that Vibrio cholerae interacted with mucosal surfaces of the intestine (22).

Later on Jones et al (23) observed that Vibrios adhered to rabbit intestinal cells and agglutinated human O-erythrocytes. Bacteria grown in broth were adhesive while those grown on agar plates lacked these abilities. The phenomenon of adhesion of Vibrio cholerae have been found to depend on the motility of these strains. Thus nonmotile Vibrio mutants lacked the ability to adhere to rabbit intestinal brush border membranes. Also, nonmotile strains did not agglutinate human group O-erythrocytes. The agglutination of human group O-erythrocytes was specifically inhibited by L-fucose and various glycosides of L-fucose and to a lesser extent by D-mannose. However, significant differences were observed when these studies were performed with intact slices of intestinal tissues (24). Nonmotile vibrios adhered to mucosal surfaces. Adherence was inhibited by crude as well as degraded mucosal scrappings. No differences were observed in association with slices of intestinal mucosa whether grown on agar or in slices. Besides L-fucose did not inhibit assocation with intestinal mucosa Nelson et al (25) have studied the mode of adhesion of Vibrio cholerae to the intestinal mucosa by scanning electron microscopy. They observed that Vibrios adhered

via their surface coats directly to the tips of microvilli. Adherence appeared to occur through surface coats rather than through flagella.

Prelimiarly studies:

Preliminary studies have been performed to study in vitro adhesive properties of Vibrio cholerae with model compounds such as hydrophobic gels, ion-exchange mattrices, erythrocyte surfaces. It appears from such a study that the mechanism of adhesion of Vibrio cholerae involves a multifaced process. Thus, strains of Vibrio cholerae were found to adhere to both hydrophobic and ion-exchange mattrices. The phenomenon of adhesion was dependent on the strain of Vibrio cholerae as some strains adhered more avidly as compared to that by others. Also, Vibrio cholerae agglutinated chicken erythrocytes and this process was inhibited by D-mannose. L-Fucose did not inhibit the the agglutination of human group O erythrocytes by Vibrio cholerae. This finding is in contrast to what has been reported by Jones et al (23).

3. Rationale:

Although there have been numerous attempts to develop effective vaccines against cholera the currently available vaccine consisting of killed whole cells do not give rise to a longer protection. The major approach to the management of enteric infections remains the use of antimicrobials to achieve a sufficient antibacterial effect. However, one of the major problems is the induction of resistance to antibiotics. As a result alternative strategies have to be sought.

One of the strategies is to prevent the association of bacteria with the intestinal mucosa and subsequent pathogenesis. This involves an understanding of the factors which determine bacterial adhesion with the host. But our knoweldge regarding the adhesive properties of Vibrio cholerae is rather limited. Thus no detailed study has been performed to determine the mode of interaction of Vibrio cholerae with in vitro model systems such as hydrophobic and ion-exchange mattrices. Besides the specificity of adhesion of Vibrio cholerae at a cellular level has not been studied. Nor the nature of the cell surface component involved in the process of adhesion is known. Hence there are several reasons which justify an investigation on the adhesive mechanism of Vibrio cholerae. The advantage to adopt

such an approach lies in the fact that colonisation of bacteria in the gut could be prevented by compounds which (a) would adhere to bacteria (b) block the receptor on the host or (c) would displace the adherent bacteria from intestinal mucosa. None of these approaches would induce antibiotic resistance. Identification of the cell surface components of the bacteria involved in adhesion would help in developing strategies in preventing adhesion by immunological methods. Also, a study of adhesion will permit us to select strains possessing greater adhesive properties. Such a strain or strains can be utilised for oral immunisation against cholera.

B. Specific aims:

1. To study in vitro adhesion of several strains of Vibrio cholerae to (i) mattrices containing ionised groups (ion-exchange resins), (ii) surfaces containing hydrophobic groups and (iii) erythrocytes from different species.
2. To study in vitro the phenomenon of adhesion of Vibrio cholerae using various paramenters such as the effect of media, growth kinetics, growth conditions.
3. To study the specificity of adhesion of Vibrio cholerae at a cellular level by comparing the adhesive properties of Vibrio cholerae between the components of the small intestine of experimental animals such as mouse (mucus layer, epithelial cells, lymphocytes etc.) and other cell lines (chinese hamster ovary, mouse adrenal etc.).
4. To identify the components of the cell surface of Vibrio cholerae involved in the adhesive process and to prevent the adhesion of Vibrio cholerae by antisera raised against the isolated components.
5. To study the prevention of diarrhoea induced by Vibrio cholerae in experimental animal such as rabbits by hydrophobic gels, sugars or ion-exchange resins.

C. Experimental:

Bacterial strains:

Vibrio cholerae has two major serotypes: Ogawa and Inaba. Within each serotype are there two biotypes: Classical and El Tor. The study will include several strains from both these biotypes and serotypes isolated from fresh human diarrheal stool as well as those present in the ICDDR,B collection.

Growth media and conditions:

Vibrio cholerae will be grown on nutrient agar plates as well as on the liquid media. The following liquid media will be used: 1) peptone-water, 2) semisynthetic and 3) synthetic.

Vibrio cholerae will be grown aerobically as well as under environmental conditions which might be present in the human intestinal lumen, such as anaerobiosis, a temperature of 37^{o}C and the presence of bile salts.

Detection of adhesive properties:

The following techniques will be used to detect the adhesive properties of Vibrio cholerae:

i) Hemagglutination assay

ii) Hydrophobic Interaction Chromatography (HIC)

iii) Ion exchange chromatography

iv) Radioadherence assay

i) Hemagglutination assay: Two fold serial dilution of bacterial suspensions will be prepared in O. 15 M sodium chloride using plastic microtiter plates. Erythrocytes from different species will be used for detecting hemagglutinatint activities.

ii) Hydrophobic Interaction Chromatography (HIC):

Hydrophobic derivatives of Sepharose such as octyl and phenyl will will be used for such a study. These gels will be washed extensively with buffered 4M NaCl to remove fine particles and sodium azide. Gels will be allowed to equilibrate in columns comprising of short-ended glass pasteur pipetts plugged with a little glass wool and fitted with clamped Teflon tubing. Bacterial suspensions (100 µl, $1x10^8$) will be allowed to drain into the gel beds which will be washed with 5 ml of buffered 4M NaCl or 1M $(NH_4)_2SO_4$ flow rate (1 to 2 ml per min). Release of bacteria adsorbed to hydrophobic gels will be done by decreasing the ionic strength.

Thus desorption will be performed by washing the gel bed with 10 ml of 10 mM Na_2PO_4 buffer (pH 6.8). The turbidities will be compared spectrophotometrically at A_{600} with appropriately diluted portion of the original suspensions.

iii) Ion-exchange chromatography:

Bacterial suspensions (100 µl, 1 x 10^8) will be allowed to drain into a column containing an ion-exchange resins such as DEAE-cellulose or cation-exchange resins such as Carboxymethyl (CM)-cellulose equilibrated with phosphate buffer(0.05M, pH 6.0.). The adherent bacteria will be eluted by a linear gradient of sodium chloride in 0.05 M phosphate buffer at pH 6.0. The number of bacteria in the eluent will be measured spectrophotometrically.

iv) Radioadherence assay: Vibrio cholerae will be grown in broth cultures at 37°C in the presence of 25 μC_i (methyl-3_H)-thymidine for 16 h. The bacterial suspension will be centrifuged for 10 minutes at 12,000 x g and washed extensively in phosphate buffered saline 0.05 M, pH 7.4. Bacteria will be resuspended in PBS to the required concentrations of optical density, verified by total radioactivity of bacterial suspensions and quantitative pour plates.

To 1 ml of radiolabellelled bacteria (10^8 colony forming units) will be added 1 ml of eukaryotic cells (10^6) and the mixture will be incubated at 37°C. After incubation, the suspension will be filtered on membrane filters (12 µm, nucleopore) which will then be washed extensively with PBS. The residual radioactivity on the filters will be determined and converted to the numbers of bactera. Results will be expressed as the number of adherent bacteria per cell.

v) Microscopic technique of adherence: Vibrio cholerae cell suspensions will contain 10^8 colony forming units. One ml of the bacterial suspension will be added to 1 ml (10^6) of the cell (isolated epithelial cells, lymphocytes etc). The mixture will be incubated at 37°C for periods varying from 10 seconds to 180 minutes. After incubation the cell suspensions will be centrifuged for 5 minutes at approximately 150 x g and washing procedure will be repeated. A drop of the mixture will be placed on a microscopic slide and cover slip. Then the number of bacteria on each cell will be counted.

Isolation of intestinal cells:

Intestinal cells can be isolated from mice or rabbits according to the procedure described by Cebra et al (26). Briefly, the small intestine, devoid of Peyer's patches, will be cut into pieces 3 cm long. The segments will be everted on a Pasteur pipette and washed briefly in Ca^{2+}/Mg^{2+} free-Hanks balanced salt solution (CMF-HBSS). They will be incubated with stirring for 15 min at room temperature in CMF-HBSS containing 0.75M EDTA. The pH will be kept constant between 7.2 and 7.4 by the addition of 7.5 (w/v) $NaHCO_3$. Gut pieces will then be treated with collagenase (10 units ml^{-1}, 20 ml per gut piece) in RPMI-1640 at room temperature. The cell suspension will be filtered through a gauze to remove debris, placed over a layer of Ficoll-Hypaque and centrifuged at 400 x g for 40 minutes at 20°C. Intestinal lymphocytes will be obtained at the interface. Binding studies will be performed on both intestinal epithelial cells and lymphocytes.

Antibodies to bacterial cell surface components and attachment:

The following are the major cell surface components which might play a significant role in the adhesive process:

(a) flagella

(b) outer membrane proteins

(c) lipopolysaccharides

These components will be isolated according to the procedure described by the principal investigator previously(27,28).Adult rabbits will be immunised with each of these cell envelope components. Rabbits will receive 100 µg of fagella, outer membrane proteins or lipopolysaccharides administered subcutaneously by 14 days apart. Rabbits will be challenged 1 week after the second dose of vaccine.

In vivo assay of Vibrios in the lumen and those adherent to the intestine: Vibrios present in the lumen will be collected by washing the loop in PBS and will be enumerated after appropriate dilution. The washed loops will be homogenized and diluted to obtain viable counts of Vibrios adherent to the intestine. The total viable

count will be the sum of bacteria in the lumen and those adherent to intestine; the percentage adherence being

$$\frac{\text{number of adherent vibrios}}{\text{total number of vibrios}} \times 100$$

Adherence to the intestine in immunised rabbits:

Strains of Vibrio cholerae will be injected into intestinal loops of rabbits immunised with either of these following components: lipopolysaccharides, outer membrane proteins and flagella. Results will be compared with unimmunised rabbits serving as controls.

In vitro antibody mediated adhesion neutralisation:

Antisera to a particular antigen (lipopolysaccharide, outer membrane proteins or flagella) will be purified from an affinity mattrix containing that antigen covalently linked to an immobilised surface such as Sepharose 4B. The affinity purified antiserum will be added to the bacterial suspension (10^8) and incubated at 37°C for 30 minutes. The serum-bacteria mixture will then be added to the intestinal epithelial cells and adhesion will be determined as described in a previous section.

Vibrio adherence and intestinal immune response:

Mice will be fed with several strains of Vibrio cholerae based on their adherence properties. For oral immunization each mouse will be administered 0.5 ml of the bacterial suspension ($4x10^9$) in phosphate buffered saline (PBS), further buffered by the addition of 0.3% $NaHCO_3$. Doses will be delivered intragastrically by a syringe fitted with a blunt-ended 19 gauge needle. Intestinal fluid will be collected by passing 5 ml of PBS containing 0.5% bovine serum albumin through the small intestine and collecting this wash out. This material will be pooled for each group of mice and centrifuged at 10,000 x g for 20 min at 4^oC. The supernatant will be harvested and stored at -20^oC. Intestinal fluid will be collected at different time intervals after the oral administration of Vibrio cholerae and the kinetics of immune response in the intestinal fluid will be monitored by quantitating immunoglobulin levels with the help of Enzyme Linked Immunoadsorbent Assay (ELISA).

Isoelectric equilibrium analysis of Vibrio cholerae:

The surface properties of the ionizable groups present on Vibrio cholerae will be determined by an analysis of the isoelectric points of Vibrio cholerae. The role of the cell surface ionizable groups such as carboxyl and amino will be studied by modifying cells with ethylene amine and formaldehyde respectively as described by Sherbet and Lakshmi (29). Isoelectric focussing will be performed in columns fitted with platinum electrodes. Cells will be electrofucussed in a pH range of 3-10 using Ficoll or glycerol as the supporting gradient.

Electron Microscopy:

The morphology of Vibrio cholerae will be studied under a variety of cultural conditions such as kinetics of growth, variation of media. Bacteria will be fixed in 2% glutaraldehyde in 0.1M sodium cacodylate (pH 7.00) buffer containing 0.1M sucrose for one week. The specimens will be examined by an electron microscope.

Prophylactic control of diarrhoea by model compounds:

Prophylactic potential of model compounds containing hydrophobic and ion-exchange groups against induced diarrhoea will be performed in infant rabbits.

Forty rabbits will be used for this purpose. They will be separated from their mothers and starved for 6 hours. They will be divided into 4 groups and each infant will be kept in a separate cage. They will be fed by a polyethylene tubing 1-3 ml glucose (10%) in physiological saline every 6 hours during the whole experimental period (3-4 days). By this tubing each rabbit will receive 10^9 organisms (in 0.5 ml saline). Preswollen hydrophobic gel such as octyl-sepharose (0.25 g) in 3 ml glucose-saline solution will be given by an oral tube according to the following schedule:

(a) Group 1: 15 min before 10^9 Vibrio cholerae

(b) Group 2: 6 hours after 10^9 Vibrio cholerae

(c) Group 3: 12 hours after 10^9 Vibrio cholerae

(d) Group 4: Control receiving 10^9 Vibrio cholerae

The severity of diarrhoea in experimental and in control animals will be monitored by visual inspection. Morbidity and mortality of rabbits will be recorded at different time intervals.

D. SIGNIFICANCE:

Successful completion of the proposed protocol will considerably increase our knoweldge about the mechanism of adhesion of Vibrio cholerae. The specific information which might arise out of this protocol are as follows:

1) In vitro studies with model compounds will suggest whether Vibrios adhere by ionic, hydrophobic or receptor mediated mechanism.
2) Studies with eukaryotic cells will demonstrate whether a) there is a specificity in adhesion and b) there are specific adhesion sites of cell surfaces.
3) The study will illustrate the nature of the cell surface involved in adhesion and the possible role of antibody against that component in preventing adhesion.
4) It will provide evidence whether there is a correlation between bacterial adherence and intestinal immune response. Such a study might help in selecting Vibrio cholerae strains which might provide better local immune response.
5) If model compounds such as hydrophobic gels can control experimentally induced cholera strategies can be developed to use these compounds against bacterial diarrhoea in humans.

E. FACILITIES REQUIRED

1. Office Space: Not required
2. Laboratory space: Laboratory space for one research worker preffered
3. Hospital resources: none
4. Animal resources: as listed in the budget
5. Logistical supprt: none
6. Equipments:
   a) High speed centrifuge
   b) Ultracentrifuge
   c) Liquid scintillation counter
   d) Phase-contrast microscope
   e) Eqoelectric focussing columns and ampholines
   f) Electron microscope

F. COLLABORATIVE ARRANGEMENTS:

Since the ICDDR,B does not have an electron microscope the Principal Investigator has made arrangements with the electron microscopists at the Radiobiologie Institut, Freiburg, Federal Republic of Germany who have kindly agreed to provide assistance in this project. They will examine a few speciemns which will be delivered to them at Freiburg, FRG.

G. PLAN OF THE STUDY:

The study is expected to be competed over a period of two years. Items 1 and 2 as specified in the section "Specific aims" will be completed during the first year (April, 1981 - March, 1982). The remaining items will be investigated during the second year of the study.

Section III - Budget (April'81 - March'82)

A. Detailed Budget

1. PERSONNEL SERVICES

NAME	POSITION	% TIME USED	ANNUAL SALARY	TAKA	DOLLAR
Dr. S. Kabir	Scientist	25	$27,768		6,947
To be appointed	Sr. Research Officer	100	Tk36,000	36,000	
Secreatary		50	Tk24,000	12,000	
		Sub Total:		48,000	6,947

2. SUPPLIES AND MATERIALS:

REagents, chemicals	5,000

3. EQUIPMENT 5,000

4. PATIENT HOSPITALISATION

None

5. OUTPATIENT CARE

None

6. TRAVEL AND TRANSPORTATION OF PERSONS

International Travel

Transport	1 meeting	5,000
Perdiem	15 days	1,200
Visiting relevant laboratories	15 days	1,200
		7,400

7. TRANSPORTATION OF THINGS

Import of equipments	1,000

8. RENT, COMMUNICATIONS AND UTILITIES

POStage	500

		TAKA	DOLLAR
9.	PRINTING AND REPRODUCTION		
	Printing forms		200
	Publication costs		500
			700
10.	ANIMAL REQUIREMENTS:		
	Rabbits 50	6,000	
	Mice 2000	10,000	
	Rat 100	2,000	
		18,000	

BUDGET SUMMARY

	Category	Year 1 Taka	Year 1 Dollars	Year 2 Taka	Year 2 Dollars
1.	Personnel	48,000	6,947	52,800	7,641
2.	Supplies		5,000		5,550
3.	Equipment		5,000		5,550
4.	Hospitalisation		-		-
5.	Outpatient care		-		-
6.	Travel		7,400		8,100
7.	Transportation		1,000		1,000
8.	Rent		500		550
9.	Printing		700		770
10.	Animal	18,000		19,800	
	Total:	66,000	26,547	72,600	29,201
			4,400		4,840
		Total $	30,947		34,041

Total $ 64,988

Conversion rate $1.00 = Tk. 15.00

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