Attachment 1.
(FACE SHEET)

Date February 11,1993

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr.P.K.Bardhan & Dr.D.Mahalanabis

Application No. 93-009

Title of Study Evaluation of anti-diarrhoeal effects of bismuth sub-salicylate in paediatric population.

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

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

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects Yes (No)
   (c) Minors or persons under guardianship (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects Yes (No)
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects Yes (No)
   (d) Discomfort to subjects (Yes) No
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) (Yes) No
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids (Yes) No
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
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes (No)
5. Will signed consent form be required:
   (a) From subjects Yes (No)
   (b) From parent or guardian (if subjects are minors) (Yes) No
6. Will precautions be taken to protect anonymity of subjects (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ✓ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

Pradip Bardhan
Principal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

Title	:	EVALUATION OF ANTI-DIARRHOEAL EFFECTS OF BISMUTH SUB-SALICYLATE IN PAEDIATRIC POPULATION
Principal Investigator	:	Dr. P. K. Bardhan Dr. D. Mahalanabis
Co-Investigators	:	Dr. S. Samiul Huq Dr. S. A. Sarkar Dr. J. Albert Mr. M. Mujibur Rahman
Consultant	:	Prof. R. B. Sack Prof. K. Gyr
Starting Date	:	As soon as possible
Completion Date	:	3 years after starting
Total Direct Cost	:	US$ 265,800.00

Associate Director
Clinical Sciences Division.

Date : February 11, 1993

Summary : Although no specific medications are recommended in acute watery (non-cholera) diarrhoea or persistent diarrhoea, a number of non-specific anti-diarrhoeal are currently available, most of which are of limited effectiveness or have undesirable side-effects. There is a substantial amount of evidence that bismuth sub-salicylate is an effective anti-diarrhoeal agent in travellers' diarrhoea, acute non-cholera watery diarrhoea and chronic non-specific diarrhoea. This study proposes to assess the antidiarrhoeal effect of bismuth sub-salicylate in children with acute non-cholera watery diarrhoea and persistent diarrhoea. A total of 270 children, aged 6 months to 3 years,suffering from acute watery diarrhoea, and 210 children, 6 months to 2 yrs of age, suffering from persistent diarrhoea will be studied. The patients will be divided into three groups and a randomised placebo-controlled double-blind clinical trial will be done where each child will recieve bismuth sub-salicylate, colloidal bismuth sub-citrate or placebo, and their subsequent clinical course will be monitored and compared. The patients will also be followed up after discharge at home to evaluate any potential preventive effect of the bismuth compounds against recurrence of diarrhoea. It is expected that this study will provide valuable information regarding the anti-diarrhoeal properties of bismuth sub-salicylate.

SECTION II - RESEARCH PLAN

OBJECTIVES

1. GENERAL :

To evaluate the anti-diarrhoeal efffect of Bismuth Sub-salicylate (BSS) in acute watery and persistent diarrhoeas in children.

2. SPECIFIC :

Primary :

a) To examine if BSS reduces stool output and duration of diarrhoea in children suffering from acute watery and persistent diarrhoeas.

b) To observe if BSS causes clearance of diarrhoeagenic pathogens and reduces net electrolyte secretion in children with acute watery and persistent diarrhoeas.

c) To evaluate if anti-diarrhoeal effects of BSS (if any) are due to the Bismuth moiety of BSS.

Secondary :

To see if treatment with BSS and Colloidal Bismuth Sub-citrate (CBS) reduces the number of diarrhoeal episodes in the post-treatment folllow-up period.

BACKGROUND

Acute diarrhoeal illness are a major cause of morbidity and mortality through out the world. Estimates of the number of deaths worldwide from diarrhoea range from 5 to 10 millions[1]. Acute diarrhoea has its greatest impact on the paediatric population, especially in the developing countries. A 1980 WHO study estimated that 0.7 to 1 billion episodes of diarrhoea occur annually in children younger than 5 years of age, with a mortality of 4.6 million per year[1].

Persistent diarrhoea in children is identifiied as one of the major health problems in the developing world and is of high research priority. As much as 50% of diarrhoeal deaths in children under 5 years of age may be attributed to persistent diarrhoea.

Effective rehydration is the treatment of choice in acute childhood diarrhoea irrespective of aetiology, and its use has resulted in dramatic decreases in the morbidity and mortality in developing countries. The WHO has taken the position that 90% of children with watery diarrhoea can be treated safety with oral rehydration therapy. However, oral rehydration therapy does not reduce the amount of stool or duration of diarrhoea[2]. In

persistent diarrhoea, management mostly consistes of dietary manipulation, and maintenance of hydration. Although a number of non-specific anti-diarrhoeal agents are available, they have limited effectiveness or undesirable side effects. In many instances specific antimicrobial therapy is initially unavailable and the acuteness of the disease demands immediate therapy before the results of diagnostic studies become available. Antibiotics are the agents most commonly used under these circumstances, often in empiric fashion because they are available "over the counter" in many countries. Their indiscriminate use may result in undesirable side effects and their chronic use results in the development of bacterial resistance. However, if a chemical compound that could lessen the diarrhoeal illness or threat of illness without selecting for the occurrence of resistance were available, this would have important implication for public health. Therefore an important need for safe and effective adjunctive therapy exists.

Bismuth compounds are old drugs that have enjoyed great popularity for at least three countries. BSS was first used in 1901 in the treatment of "cholera infantum" a common diarrhoeal disease in children. It's subsequent commercial success has been such that it is now estimated by the manufacturer to be a fixture in the medicine cabinet in 60% of American households. Despite this venerable history systematic investigation of the mechanism of action and clinical efficacy of bismuth compounds has only begun in recent years. There is a substantial amount of clinical evidence that bismuth subsalicylate (BSS) is effective in the treatment of diarrhoeal disorders, including studies that have demonstrated a beneficial effect both in the prevention and therapy in traveler's diarrhoea [3]. Dupont et al. [4] demonstrated that BSS reduces the frequency of unformed stools, improves stool consistency, and decreases the accompanying symptoms of nausea and abdominal cramps. Steinhoff et al[5] inoculated 59 volunteers with the Norwalk agent, an illness characterized by vomiting and diarrhoea; subsequently diarrhoea developed in 34 subjects, who were randomly assigned to a course of BSS or placebo in double-blind fashion. There was a significant reduction in the severity and duration of abdominal cramps and in the median duration of gastro intestinal symptoms in the group treated with BSS.

Gryboski et al[6] reported that BSS was effective in the treatment of chronic diarrhoea of diverse aetiologies in infants and children. In this study, 29 children aged 2-7 months, were treated in double-blind fashion with BSS or placebo. The BSS treated group had significantly fewer stools with less water content and had a significant improvement in clinical status compared with placebo treated group.

BSS has shown promising result in acute diarrhoea, although the studies are rather limited. Soriano-Brucher et al[7], studying 123 infants and children with acute diarrhoea in Chile, administered BSS (100 mg/kg/day) for 5 days in a placebo-controlled clinical trial. The BSS group had significant improvements over the placebo group in the following parameters : number of stools/day, requirement of I/V fluids, stool weight and consistency and length of hospital stay. D. Figueroa et al[8] has shown in a well-designed placebo-controlled trial with two doses of BSS (100 mg/kg/day, 150 mg/kg/day) over 275 male children, aged 3-59 months with mild to moderate dehydrating watery diarrhoea in Peru that diarrhoea remained prolonged (>5 days after admission) significantly in placebo group, which was three times more than BSS group. Children treated with BSS (at either dose) had clinically important and statistically significant reduction in (i) volume of ORS consumed (20%), (ii)

volume of diarrhoeal stools (30%), (iii) duration of diarrhoea (25%), and (iv) duration of hospital stay (about 1 day). It was concluded that BSS may be a clinically useful, safe, and inexpensive adjunct to oral rehydration therapy in children with acute watery diarrhoea.

The mechanism(s) of action of BSS is not completely understood. The possible mechanisms include a direct anti-microbial effect and anti-secretory and anti-toxin effects.

There is considerable evidence to indicate that BSS has direct antimicrobial activity against diarrhoeal pathogens. Several bacterial pathogens including enterotoxigenic E.coli, Salmonellae, Campylobacter jejuni and Clostridium difficile are inhibited *in vitro* by BSS or its metabolic product at concentration that can achieved in the intestinal tract[9,10,11]. The isolation of bacterial pathogens from stools of patients with infectious diarrhoea is markedly reduced in BSS-treated patients compared to placebo treated controls. The exact anti-microbial mechanism is not known, though it is believed that bismuth binds to the bacteria, disrupting the cell wall and transmembrane transport. Although bismuth compounds inhibit bacterial pathogens, as well as many of the aerobic and anaerobic bacteria when tested *in vitro*[12], no change were noted in the overall composition of the normal flora of the gut or in faeces[13] during BSS administration in therapeutic doses.

BSS was found to significantly reduce fluid accumulation in ligated intestinal loops (rabbit and pig) and unligated intestinal segments (rat) from both E. coli and cholera toxins as well as arachidonic acid[14,15]. This anti-secretory effect may be attributable to the salicylate component of BSS, as this was not found by bismuth subcarbonate. It should be noted that salicylates have been found to be effective anti-secretory agents. Thus it is possible that both the Bismuth and salicylate components of BSS, either alone or together, are responsible for the reported anti-diarrhoeal effects of BSS.

Previously, many compounds of Bismuth were used clinically for symtomatic treatment of diarrhoea, though most of these compounds are no more available now for clinical use. The other Bismuth-containing drug currently avilable for clinical use besides BSS is Colloidal Bismuth sub-citrate (CBS). CBS has been widely used in the treatment of duodenal and gastric ulcer disease, essential non-ulcer dyspepsia, duodenitis, NSAID-induced disease and *Helicobacter pylori* associated gastro-duodenal disease with high clinical efficacy. Currently, the commonest use of CBS, alone or with combination with antibiotics, is in treatment of *H. pylori* associated gastro-duodenal disease, and long-term eradiacation of *H. pylori* in upto 95% patients have been reported[16]. CBS appears to act via several mechanisms including inhibition of peptic activity, acceleration of ulcer healing, and accumulation of epidermal growth factor in the diseased mucosa[17]. However, the most prominent mechanism may be its bactericidal effect against *H. pylori*.

The effect of CBS in diarrhoeal disorders has not been investigated. Like other bismuth compounds, the bismuth part of CBS may have similar actions. That CBS has potent bactericidal action is shown by its effect on *H. pylori*. It has also been shown to be capable of reducing fermentation by colonic bacteria[12]. A recent report has shown the protective effect of CBS against distal colitis produced experimentally in rats[18]. The safety record of CBS is excellent[19], and it has been used in children for eradicating *H. pylori*

without any untoward effect[20]. Thus, the potential anti-diarrhoeal effect of CBS is worth a trial.

An addtional benefit of using bismuth compounds for the treatment of diarrhoeal diseases is the potential preventive effect. Hypochlorhydria is known to be a predisposing factor to repeated gastrointestinal infections[21]. *H. pylori* is a major cause of gastritis, and a striking association has been noted between the presence of *H. pylori* and gastric hypochlorhydria[22]. In fact, *H. pylori* infection has been shown to be a significant risk-factor for persistent diarrhoea[23], and may well be a risk factor for acute diarrhoea. Prelimenary results show that *H. pylori* is quite common in the paediatric population in Bangladesh. It is known that children recovering from one diarrhoeal episode are more prone to develop another diarrhoeal episode when compared to healthy children, and thus suppression of *H. pylori* in children suffering from diarrhoea may have some beneficial protective effect upon subsequent developement of diarrhoeal episodes.

Safety of BSS and CBS : BSS has been found quite safe and well-tolerated, as evident by the fact that despite being around for a long time, it is still an over-the-counter medicine in most of the countries including USA and UK. CBS has also been found to be a very safe drug, and there is no report of any significant side-effect .Bismuth toxicity is rare, and has only been reported after long-term treatment[24]. Bismuth levels in plasma observed after treatment of children with BSS or CBS has been found to be well below toxic levels[7,8,20,25]. Though the salicylate component of BSS is almost completely absorbed[26], no signs of salicylate toxicity have been noted in the previous reports, and serum salicylate levels remain well below toxic levels[7,8]. No association has been found between Reye's syndrome and the use of BSS or other non-acetylsalicylic acid salicylates[27,28].

RATIONALE :

Existing treatment for dehydrating acute watery diarrhoeas in children includes replacement of fluid loss, mainly through oral rehydration therapy (ORT), and appropriate feeding to minimise nutritional consequences. However, they reduce neither the severity nor the duration of diarrhoea. Other than in proven cholera patients, antibiotics are generally not recommended in acute watery diarrhoeas. The current treatment of persistent diarrhoea consists mostly of dietary manipulation and maintenance of hydration; antibiotics are generally not indicated and there is no specific intervention. Thus, it is important to evaluate non-antibiotic antidiarrhoeals. BSS is widely used as an anti-diarrhoeal for decades, and and has proven its effect in travellers' diarrhoea. Recent reports have also shown BSS as an effective adjunct in acute and chronic diarrhoeas in children, where it was found to reduce both the severity as well as the duration of diarrhoea. It is a very safe, non-allergenic, over-the-counter drug, with minimal side-effects, and does not change the normal gut flora. If found useful, this may prove to be a valuable adjunctive therapy to the current management of dehydrating diarrhoeas in children. CBS may have some potential anti-diarrhoeal effects, and comparing such effects with those of BSS will also help to find out whether the bismuth or the subsalicylate moiety of BSS is the major contributor to its anti-diarrhoeal effect. Again, the anti-*H. pylori* effects of BSS and CBS may be important as protecting against this important risk-factor. A negative outcome

of this study will also be helpful, as it will discourage unjustified use of BSS.

MATERIALS AND METHODS

The study will be conducted in the Clinical Research Centre of ICDDR,B. Every year about 70,000 patients are treated in this centre, 60% of whom are below the age of 2 years. The study will be conducted for a period of 36 months from the date of starting the study.

1. ***PATIENT RECRUITMENT***:

Children presenting at the CRC, ICDDR,B with a history of watery diarrhoea will be evaluated and if they fulfill the remaining inclusion and exclusion criteria will be admitted to the study.

(A) INCLUSION CRITERIA:

Acute Diarrhoea

1. Age : 6 months to 3 years
2. Sex : Males only
3. History of acute watery diarrhoea of <72 hours duration and had 4 or more liquid stools over 24 hours prior to admission.
4. Presence of some signs of dehydration.
5. Nutritional status: Wt. for height > 60 of N.C.H.S. median.

Persistent Diarrhoea

1. Age : 6 months to 2 years
2. Sex : Males only
3. History of watery diarrhoea of >14 days duration and had 4 or more liquid stools over 24 hours prior to admission.
4. Presence of some signs of dehydration.
5. Nutritional status: Wt. for height > 60 of N.C.H.S. median.

(B) EXCLUSION CRITERIA:

1. Systemic infection requiring prompt antibiotic treatment e.g. pneumonia, meningitis, septicaemia.
2. History of bloody diarrhoea (gross blood in stool).
3. Patients suffering from cholera.
4. Any rehydration therapy (intravenous), antibiotics or antidiarrhoeal drugs given during the week before admission.

(C) INFORMED CONSENT:

If the patient is found suitable for inclusion into the study an informed consent will be obtained (English and Bangla consent form attached). The consent form will be administered by one of the investigators and then will be witnessed by another staff member.

2. ***CASE MANAGEMENT***:

Patients will receive routine care which includes -

a) replacement of fluid and electrolytes mainly with ORT supported by I.V. treatment for those who need it;
b) unrestricted breast feeding of those still breastfed during rehydration and maintenance therapy;
c) formula milk for non-breastfed children starting after initial rehydration period; and
d) Feeding : According to the standard feeding regimen followed in the ICDDR,B hospital for children with diarrhoea.
Acute Diarrhoea : Semisolid and solid food appropriate for age during maintenance fluid therapy.
Persistent Diarrhoea : A rice-based diet will be used.

e) Medicine : A double-dummy tecnique will be used in providing BSS/CBS/placebo to the patients in a randomised fashion. Oral BSS will be administered to 90 patients at a dose of 150 mg/kg/day in 5 divided doses daily and oral CBS to another 90 patients at a dose of 480 mg/1.73 m^2 of body surface area/day in 5 divided doses daily (6 a.m., 10 a.m., 2 p.m., 6 p.m. and 10 p.m.). In the control group placebos will be given identical in appearance to BSS and CBS to 90 patients in the same dosage schedule. The treatment will continue for 5 days in patients with acute diarrhoea and for 10 days in patients with persistent diarrhoea. The individual doses will be worked out on admission (after rehydration) and will be followed for rest of the study.

CRITERIA FOR INTRAVENOUS FLUID:

1. Severe dehydration
2. In patients whose hydration status cannot be maintained adequately by ORS or patients with intractable vomiting.
3. Patients with gross electrolyte imbalance requiring correction by appropriate intravenous fluid.
4. Patients developing paralytic ileus.

TREATMENT FAILURE :

Patients still having diarrhoea after full course of treatment, i.e. 5 days in acute diarrhoea and 10 days in persistent diarrhoea, will be considered as treatment failures. Patients on whom dehydration reappears requiring i.v. hydration or those who develop complications during the course of treatment will constitute as patients with deviated clinical course. Such patients (treatment failures and patients with deviated clinical course) will however recieve appropriate management according to the standard ICDDR,B clinical practice.

Summary of Procedures

BASELINE EXAMINATION:

A standard history and a thorough physical examination will be carried out after admitting the patient into the study by one of the investigators. Patient will be weighed and the following investigations will be done.

a) Blood for T.C., D.C., Hct., serum glucose (random).
b) Blood for electrolytes (Na+, K+, Cl-, TCO_2), serum Sp. Gr., protein (2ml of blood will be required for the tests mentioned).
c) Fresh stool for microscopy.
d) Fresh stool for culture for Shigella, Salmonella, Vibrios, E. coli (ETEC, EPEC, EAEC), Aeromonas, Plesiomonas and Campylobacters.
e) Stool for rotavirus (ELISA)
f) Intake and output measurements will be instituted using urine bags and cholera cots.
g) C^{13}-Urea breath test for detection of *H. pylori*.
h) Faecal α-1-antitrpsin for assessing intestinal protein loss.

DURING THE COURSE OF ILLNESS :

i) Patients will be evaluated every 8 hours and intake/output measurements will be summarised from tally sheets.
ii) Patients will be weighed and physical examination findings will be recorded and time of cessation of diarrhoea noted.
iii) Stool samples for electrolyte analyses from every output period will be sent.
iv) A second blood sample will be taken for serum levels of Bismuth and salicylate on day-6 from patients with acute diarrhoea and on day-11 from patients with persistent diarrhoea, 2-3 hrs after the last dose of medicine.
v) Repeat stool examination (Culture + ELISA for RV) on day-6 from patients with acute diarrhoea and on day-11 from patients with persistent diarrhoea.
vi) Repeat faecal α-1-AT on day-6 and day-11 in acute diarrhoea and persistent diarrhoea respectively.

AFTER DISCHARGE (During follow-up) :

The patients will be followed up for 6 months after discharge every 2 weeks, the first follow-up being on the 15th day after admission. The Urea breath test will be repeated at 3 and 6 months to determine the status of *H. pylori*. On each visit, anthropometric measurements will be taken, and a general information of the health of the child during the preceding 2 weeks will be sought. Any recurrence of diarrhoea will be noted and the course will be followed up, if necessary at the hospital.

3. *SAMPLE SIZE CALCULATION:*

A. If we assume that there will be a 25% reduction of total stool output (ml/kg) in the BSS groups compared to control group, then taking power as 80% and significance level as 0.05 (Stool output 208 ml/kg; $S.D._{pooled}$ = 113.5).

$$n = \frac{2 \times (113.5)^2}{(52.03)^2} \times 7.9 = 75$$

Assuming a 10% dropout, the total sample size should be 83 in each group.

B. Assuming a 20% reduction in ORS intake in the groups recieving BSS, with 80% power and significance at 0.05% (ORS intake 371 ml/kg, $S.D._{pooled}$ 161.3), the sample size becomes -

$$n = \frac{2 \times (161.3)^2}{(74.2)^2} \times 7.9 = 75$$

With a 10% dropout, the sample size is 83 per group.

C. We assume that there will be a 20% reduction of duration of diarrhoea in the BSS groups compared to control group. Taking power as 80% and significance level as 0.05 (Mean duration = 92 hours, S.D. = 39).

$$n = \frac{2 \times (39)^2}{(18.4)^2} \times 7.9$$

or 72 in each group.

Assuming a 10% dropout, the total sample size should be 80 in each group.

D. Expecting a 20% reduction in the duration of diarrhoea in the persistent diarrhoea group, and with 80% power and 0.05% significance (Mean duration 6.5 days, S.D. 2.6 days),

$$n = \frac{2 \times (2.6)^2}{(1.3)^2} \times 7.9 = 63$$

With a 10% dropout, the sample size is 65 per group.

Thus the sample size is taken as 90 (rounded up) in each of the 3 groups with acute diarrhoea, i.e. a total of 270 patients for acute diarrhoea, and 70 (rounded up) in each group with persistent diarrhoea, or a total of 210 children with persistent diarrhoea.

4. *RANDOMIZATION:*

The patients in the study groups will receive rehydration and maintenance fluid along with BSS or CBS , while those in the control will receive rehydration and maintenance fluid with placebos. A double-placebo technique will be used i.e., BSS or its identical placebo and CBS or its identical placebo. Thus the three groups of patients will recieve either BSS and CBS-placebo, CBS and BSS-placebo, or BSS-placebo and CBS-placebo. The medicines and their respective placebos will be identical in appearance, packaged in identical containers, and will be dispensed in the same amount. The containers will be arranged in a sequence of drugs and placebos that corresponds to the randomization code then numbered sequentially. Containers of medicine with serial number will contain drugs or placebo syrup according to master randomization chart.

	Patient group		Recieves	Net result
I	Acute diarrhoea persistent diarrhoea	- 90 pts - 70 pts	BSS + (CBS placebo)	BSS
II	Acute diarrhoea Persistent diarrhoea	- 90 pts - 70 pts	(BSS placebo) + CBS	CBS
III	Acute diarrhoea Persistent diarrhoea	- 90 pts - 70 pts	(BSS placebo) + (CBS placebo)	Placebo

5. *OUTCOME VARIABLES:*

* Stool output
* Fluid Intake - ORS & Plain water
* Duration of diarrhoea after adm.
* No. of patients with treatment failure/deviated clinical course.
* No. of patients with persisting infection after Day 5/Day 10.
* Faecal electrolyte loss.
* Faecal α-1-antitrypsin excretion.
* Time of first post-discharge diarrhoea, number and duration of diarrhoeal attacks during the 6 months follow-up period

6. *DATA ANALYSIS:*

The study groups will be compared for all the variables prior to intervention. Major outcome variables will be compared after evaluating their descriptive statistics for distribution etc. The quantitative outcome measures will be compared using ANOVA or Students 't' test on primary data or after appropriate transformation when indicated and also by an equivalent non parametric test e.g. Kruskall-Wallis test or Mann Whitney U test. Dichotomus outcome measures will be compared by X^2 test or Fisher's exact test as appropriate. Time for the first post-discharge diarrhoeal attack will be noted and compared between the three group with logrank test. Data from the patients declared as treatment failures or with deviated clinical course will be analysed seperately.

7. *IMPORTANT DEFINITIONS:*

1. Duration of diarrhoea after admission: The time in hours from initiation of study treatment until passage of the last liquid or semi-liquid stool prior to two formed stools or prior to 12 hours during which no stool is passed.

2. Stool output: The weight of stool in g/kg of admission body weight expressed per time period (from admission to 24 hours or for entire duration of diarrhoea).

3. ORS and plain water intake: The volume of ORS or plain water taken in ml/kg of admission body weight expressed per time.

8. COLLABORATIVE ARRANGEMENTS :

An inter-institutional collaboration will be arranged with Prof. K. Gyr, University of Basel, Switzerland.

^{13}C-Urea Breath Test

This test is based upon the principle that in the presence of the enzyme urease in stomach, orally adminstered urea will be hydrolysed into CO_2 and ammitcing gastric pathogen, and therefore a positive urea breath test can gen. The urea breath test has proven to be very robust, attaining sensitivity and specificity greater than 90%. This non-radioactive, non-invasive test has been successfully utilised as a diagnostic tool in *H. pylori* infection, including in children.

After obtaining a baseline breath sample at a fasting state (2 hours fast), a test dose alongwith a liquid meal (to delay gastric emptying), an dthen breath samples will be collected every 10 minutes for 1 hour through a two-way non-rebreathing paediatric mask into vacutainer tubes in duplicate. $^{13}CO_2$ will be measured in these sam ratio in the breath samples after the testdose will indicate a positive test. Increase in the $^{13}CO_2/^{12}CO_2$

PERSONNEL		1st Year	2nd Year	3rd Year	
Dr. P. Bardhan	20%	2,424	2,670	2,935	
Dr. Samiul Huq	20%	1,705	1,875	2,065	
Dr. S. A. Sarkar	10%	1,215	1,340	1,470	
Medical Officer (Trainee)	100%	2,210	2,320	2,555	
Health Assistant (1)	100%	2,900	3,190	3,510	
Trainee Health Asstt (2)	100%	2,850	2,990	3,290	
Field Worker (2)	100%	3,160	3,160	3,160	
Field Asstt. (2)	100%	1,900	1,900	1,900	
	Total :	18,365	19,445	20,885	58,695
LAB TESTS					
Blood CBC,HCT		580	580	105	
Electrolytes		1,980	1,980	360	
Creat., Sp.Gravity					
Bismuth		1,280	1,280	235	
Salicylate		1,210	1,210	220	
Stool M/E		365	365	70	
C/S (Salm, Shig, Cholera, Campy, ETEC, EPEC, EAEC Rotavirus, Non-CV)		14,960	14,960	2,720	
Faecal α-1-AT		8,800	8,800	1,600	
Breath Analysis		4,000	4,000	725	
	Total :	33,175	33,175	6,035	72,385
PATIENT HOSPITALISATION		43,625	43,625	11,000	98,250
SUPPLIES AND MATERIALS					
Drugs		6,000	-	-	
Urine collection bags		500	500	200	
Non-Stock items		250	250	250	
Stationaries		750	750	250	
Syringes, Containers, Vials, etc.		500	500	200	
C^{13}-Urea		8,000	-	-	
	Total :	16,000	2,000	900	18,900
MAIL, FAX, TELEX, etc.		500	500	500	1,500

PRINTING AND PUBLICATION	250	250	1,000	1,500
ICDDR,B TRANSPORT	500	500	500	1,500
INTERNATIONAL TRANSPORT	400	400	200	1,000
LOCAL TRANSPORT	1,500	2,000	1,000	4,500
DATA ENTRY AND ANLYSIS	500	500	1,500	2,500
INTERNATIONAL TRAVEL	-	-	3,000	3,000
CAPITAL EXPENDITURE				
HPLC Column for Salicylate	1,200			
File Cabinet (1)	150			
Total :	1,350	-	-	1,350
GRAND TOTAL	**116,165**	**102,395**	**46,240**	**265,800**

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ICDDR-B Protocol Reviewer: Smlzore 23 Nov. 1992

Evaluation of Bismuth Sub-Salicylate's anti-diarrhoeal effects in paediatric patients (acute and persistent diarrhoeas)

Summary: follow-up aspect needs more attention; good rationale
2-level trial proposed (please see last page 4)

Minor comments.

p 1	last line	in the developing world
p 2	line 4	in dramatic increeses
p 2		improved ORS does reduce stool volume + diarrhoea duration
p 2	line 9	In persistent diarrhoea
p 2	line 11	Although a number ... is available
p 2	line 20	lessen the diarrhoeal illness or threat of illness
p 2	line -2	Its subsequent commercial success ...
p 3	line 7	in Traveller's diarrhoea
p 4	line 1	What has Figueroa shown? (has reported a well-designed)
p 4	line 4	Diarrhoea remained prolonged ( ) three times more frequently in placebo than BSS groups.
p 5	line 8	salicylates have been found ...
p 5	line -9	Currently, the commonest use ... is in treatment of ...
p 5	line -6	accelerates
p 6	last line	no signs of ... have been noted
p 7	line 2	No association has been noted (delete 'been found')
p 7	line 12	consists mostly of dietary manipulation.

Detailed comments

p 8 (B) Persistent diarrhoea

p 9 (C) Exclusion criteria

p 9 (4) Why exclude such patients — stratify, if you must.

p 9 Case-management

c) is formula milk half strength for 1st 24 hours?

d) standardized regime of feeding?

p 10 Dose: since dose depends on kg/or m^2, will dose be adjusted daily (esp persistent diarrhoea) or worked out on day 1 and same dose applied daily thereafter.

p10 Design — Timing of 5 ~~daily~~ doses per day (24 does not divide by 5!)?

Yes: see p13 (should be stated earlier!!)

Why 83 — round up to 90 per treatment group

Will double-dummy technique be used? OR will all children be randomized to a) trial (BSS or CBS)

b) treatment within trial

Why 5 days for acute; 7 days for persistent

PLEASE IGNORE SINCE DOUBLE DUMMY PLANNED

360 ELIGIBLE PATIENTS

NB randomization also needs to be stratified for acute/persistent

a) RANDOMISE

180 Eligible patients in BSS trial

180 eligible patient in CBS trial

b) RANDOMISE

b) RANDOMISE

(i) 90 to BSS

(ii) 90 to BSS-placebo

(iii) 90 to CBS

(iv) 90 to CBS placebo

double-blind

double-blind

single-blind

single-blind

single-blind

(i) versus (ii) double-blind

(iii) versus (iv) double-blind

(i)/(ii) versus (iii)/(iv) ~~single~~ 1½ blind!

I don't know of a terminology for this comparison

(i) versus (iv)

different treatments but investigator does not know which of (i) & (iv) are active!

Definition of treatment failure. Why include such definitions
How will 'treatment failure' be handled at analysis
Because definitions of the type 'in any 24 hour period'
8 hours – measurements will be in blocks of ?6 or ?8 hours
(see p or that in practice any consecutive ?4 or ?3
recording periods. Over 5 days, there are ?19 or
?14 possible blocks!
Should failure type 3 read 7 days for
persistent diarrhoea cases?

p 11. During course of illness When will parents be weighed?
What physical exam findings will be recorded,
how frequently in course of illness?

p12 Follow-up Methodology of follow-up is poorly described
by whom?
What will be asked every 2 weeks and how recorded?
NB Will follow-up be every 2 weeks after randomization
or after discharge (some children will take longer to
recover: better to schedule from randomization, else
biassed timing dependent upon recovery from initial
episode).

p12 Sample size Justify mean and sd.
p13 Stratification Only now do we learn that randomization shall be
stratified with $3 \times 83 = 249$ (round up to 270)
cases of acute diarrhoea
and $3 \times 65 = 195$ (round up to 210)
cases of persistent diarrhoea 480

b Data analysis Logrank test for time to first diarrhoeal episode post discharge
admission; for time to H pylori found/disappeared post discharge;
distribution of # subsequent diarrhoeal episodes (? treated episodes: define!)

p13 Study size.

No sample size calculations for follow-up

What is the impact of H. pylori on new episodes of diarrhoea (attributable risk)? What is the plausible eradication effect of BSS or CBS & hence reduction in attributable risk? ~~How~~ many children need to be followed up?

a) to assess eradication effect

b) to assess reduction in diarrhoeal episodes.

NOTE: if the above calculations gave answers of 1500, not 500 randomized, then you could randomize eligible patients to levels of study as follows

2000 eligible patients — RANDOMIZE —

LEVEL 1

Randomize to inhospital drug administration + only record breath tests at 3 months + 6 months post-discharge 1500 children (500 M + 1000 F)

LEVEL 2

Randomize to inhosp drug admin, monitoring + ~~follow~~ follow-up for 2 weeks for diarrhoeal episodes + 3, 6 month breath tests 500 males

i.e. All females + half males (random half) assigned to LEVEL 1
half males (other half) assigned to LEVEL 2 (the protocol)

i.e. Need detailed study within large, simple trial

** Even use capture-recapture via your 4% sample to track readmissions of LEVEL 1 randomized children **

Need extra

Title: Evaluation of anti-diarrhoeal effects of Bismuth sub-salisyclate in Paediatric population.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project		✓	
Adequacy of Project Design		✓	
Suitability of Methodology		✓	
Feasibility within time period		✓	
Appropriateness of budget	-	-	-
Potential value of field of knowledge		✓	

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification

- on technical grounds ☒
- on level of financial support ☐

Detailed comments

The objectives are to replicate the results of the two previous studies on BSS effects in acute diarrhea from Chile and Peru and to extend the observations to persistent diarrhea. The scientifically most interesting part of this project is to evaluate whether Bismuth or salicylate is the major contributor to any effect seen. The study design is adequate to perceive differences larger than 25% between groups.

Several questions need to be addressed as follows:

1. Why is the age group restricted to 2 year olds. In fact there is a great lack of data in 2-12 year olds. Although this age group is less susceptible to diarrhea it is still an important pediatric group from the point of view of anti diarrheal drug use.

2. Males-only designs are under some attack. It would be desirable to include females in future protocols and use a stool collection device in future studies. I do not expect this study to do this.

3. Why are cholera patients excluded they could be analyzed as a separate group and the study numbers increased. To date there are no data on the effect of BSS on cholera. This would be an important opportunity missed. If data were collected, an increase in the study size and budget would be necessary.

4. There will be a need to decide how to analyze effects by etiology. If there is a serious wish to assess effect by etiology then the study size must be increased substantially. Otherwise comprehensive bacteriology and virology will not be needed except to describe the populations in each group.

5. For persistent diarrhea a 7 day course is probably not adequate and 10 days to 2 weeks should be considered. Follow-up for relapse would be desirable but may not be feasible in this study.

6. Some measurement of recovery of the intestine other than the end of diarrhea would be very desirable. This could be alpha-1-antitrypsin measurements of protein loss or stool lactoferrin or some test for absorption of fat, protein or carbohydrate. This would be of special importance in persistent diarrhea.

7. I assume rice ORS will be used in this study. To date there have been no studies on BSS except with glucose ORS. Since rice-ORS decreases severity and length of illness, it may be difficult to show an effect of BSS. Glucose-ORT should be used to avoid confounding variables.

This protocol is really testing a series of hypotheses which need better articulation. I would understand then implicitly as follows:

Hypotheses

a) Does bismuth or bismuth and salicylate speed recovery from acute or persistent diarrhoea patients treated with nice-based ORT. To test this hypothesis one would need a glucose-ORS/RSS vs rice-ORS/BSS composition. This has even been done - no data available. Unless addressed specifically, nice-ORT will be a confounding issue.

b) Is bismuth the active agent or does salicylate contribute to the beneficial effects of BSS on diarrhoea? This hypothesis really should be tested in patients treated with glucose-ORS, best in order to relate findings to previous studies.

c) Does either BSS or CBS alter the course of persistent diarrhoea?

d) Does either bismuth or salicylate accumulate to excessive levels in patients treated with BSS or CBS?

An important neglected hypothesis is whether signs of intestinal disorder that could impact on nutrition are altered by BSS or CBS. Clearly the protein loss/intoleration/malabsorption engendered is of great consequence to the health of the child. In a hospitalized setting, such as ICDDR,B, some parameters should be accessible to comment on this.

WBG:sc:mh/REVIEW

RESPONSE TO REVIEWERS' COMMENTS

Reviewer 1.

1. Appropriate corrections have been made.

2. Inclusion criteria for persistent diarrhoea and the exclusion criteria have been detailed. Patients to be excluded from the study are those who have complications or are otherwise likely to recieve antibiotics or other specific medicines.

3. Feeding of the children will be according to the standard regime followed in the ICDDR,B.

4. The dose schedule will be worked out on Day 1 after rehydration and will be adhered to for the duration of the study.

5. Patients with diarrhoea after full treatment, i.e. 5 days for acute diarrhoea and 10 days for persistent diarrhoea will be considered as treatment failures. Patients who develop dehydration during treatment requiring i.v. fluids or developing complications will constitute as deviated course. Data from patients with treatment failure or deviated course will be analysed seperately.

6. Measurements of inputs, outputs, vital signs, etc. are done 8 hourly as a standard practice. The patients are weighed before and after rehydration, and then daily till discharge from the hospital. Physical examinations are done as frequently as necessary, but at least twice daily.

7. Follow-up methodology is now provided in greater detail. The data will be recorded in a pre-designed form. Follow-up will be done every 2 weeks after randomization.

8. Sample sizes are rounded up as suggested.

9. Data analysis will be performed as suggested.

10. Examination of any preventive effect of bismuth compunds on diarrhoea will need a properly designed seperate study. There is still not enough evidence to treat anybody with H. pylori and any symptom other than dyspepsia\peptic ulcer disease. Assessment of any potential preventive effect of BSS/CBS on diarrhoea is a secondary objective in the present study.

Reviewer 2.

1. Children suffering from persistent diarrhoea are mostly less than 1 year of age. Children suffering from acute diarrhoea are also mostly less than 3 years of age. The age group for acute diarrhoea has therefore been extended upto 3 years.

2. Because of inadequacy of the stool collection device now available, males-only design has been retained.

3. The present study aims at examining the effects of BSS on those type of childhood diarrhoeas for which no specific medicines are recommended. Appropriate antibiotics are generally recommended in cholera, and assessing the effect of BSS on cholera may need a seperate study.

4. The microbiological laboratory examinations required in the present study are mostly for descriptive purpose, and the repeat examinatios are to examine the clearance of the responsible organism, if any.

5. The course of medicine in persistent diarrhoea has been extended upto 10 days.

6. Faecal alpha-1-antitrypsin will be assayed as suggested.

7. Rice-ORS is currently used in the ICDDR,B as the standard in ORT, and also will be used in the study.