Library copy

Library

ETHICAL REVIEW COMMITTEE, ICDDR,B

Date 20.12.83

27,12,83

ICDDR,B Library

Room—18

Principal Investigator P. Speelman

Application No. 83-049 (P)

Title of Study Role of endogenous prostaglandins in secretory diarrhoeas

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)

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.

Principal Investigator

27 DEC 1983

Trainee

SECTION 1 - RESEARCH PROTOCOL

1.	TITLE:	THE ROLE OF ENDOGENOUS PROSTAGLANDINS IN SECRETORY DIARRHEAS
2.	PRINCIPAL INVESTIGATORS:	Dr. P. Speelman (Dhaka) Dr. G.H. Rabbani (Dhaka) Dr. J. Rask-Madsen (Denmark) Dr. K. Bukhave (Denmark)
3.	STARTING DATE:	1 April 1984
4.	COMPLETION DATE:	1 September 1984
5.	TOTAL INCREMENTAL COST:	US $ 1260
6.	SCIENTIFIC PROGRAMME:	This protocol has been approved by the Pathogenesis & Therapy Working Group.

PROGRAMME HEAD

Date: 21/12/83

7. ABSTRACT SUMMARY:

Preliminary studies on patients with acute cholera have shown that PGE_2 levels in jejunal fluids were significantly raised and were negatively correlated to the time following onset of diarrhea. PGE_2 levels were normal in convalescence. Jejunal flow rates of PGE_2 were significantly raised and correlated positively to stool output.

In this project we propose to study 16 patients with acute cholera during "slow marker" and "steady-state" perfusion techniques; we will measure levels of all endogenous PG's (PGE_2, PGE_1, PGF_2 alfa, 6 keto PGF_1 and tromboxane) as well as 5 hydroxytrypramine.

We will study the effect of a bolus injection of indomethacin (1.0 mg/kg) on these parameters. The patients studied will be requested to return to the hospital one week after discharge for intubation and control perfusion studies without administration of drug.

8. REVIEWES:

(a) Research involving human subject: _____

(b) Research Review Committee: _____

(c) Director: _____

N.B. This research protocol is a part of a collaborative research project with Dr. J. Rask-Madsen from Denmark. Funds for this study will be requested from the WHO.

A. INTRODUCTION:

1. Objectives:

a. To study the effect of indomethacin, a PG-synthesis inhibitor, on endogenous prostaglandin production and secretion of fluids and electrolytes.

b. To measure not only PGE_2 but all endogenous PG's as well as 5-HT in jejunal fluid of patients with cholera during the acute phase and in convalescence.

2. Background:

The role of the AA-PG system in the regulation of intestinal ion transport has not yet been fully established, but there is now ample evidence to suggest that this system makes up a significant regulatory mechanism (1,2).
PGs are synthetized throughout the gastrointestinal tract where different regions are characterized by different profiles of AA-metabolizing enzymes (3). Both hormones, paracrine mediators, and neurotransmitters may interact with a specific surface receptor which in turn initiates the enzymatic release of AA from the phospholipid pool (4). The released AA can then be oxygenated via the cyclooxygenase pathway to the unstable endoperoxides which, dependent on the specific cell, are further converted to PGE_2 and $PGF_{2\alpha}$ - the major products in the intestinal mucosal cell - as well as prostacyclin(PGI_2) and thromboxanes(TX). Alternatively, AA may be oxygenated via the lipoxygenase pathway which leads to the formation of leukotrienes(LT) - substances which have recently been discovered in leukocytes and appear to play an important role in chemotaxis and immune response (5) and may be involved in diarrhoea caused by invasive organisms that is accompanied by inflammation as well as in chronic inflammatory bowel disease.
The cyclooxygenase pathway is blocked by non-steroid-anti-inflammatory compounds like indomethacin and aspirin. The lipoxygenase pathway is not affected by cyclooxygenase inhibitors, but the transformation of LTA_4 to LTB_4 appears to be inhibited by 5-amino-salicylic acid(5-ASA), the active moiety of sulphasalazine. In contrast, glucocorticoids act by reducing the supply of substrate for PG/LT biosynthesis due to activation of peptide phospholipase inhibitors, provisionally named macrocortin or lipomodulin (6).
PGs are rapidly metabolized, even within the same tissue, with no evidence of storage, and their release is considered to reflect *de novo* synthesis. Thus PGs appear to have paracrine actions and may be regarded as local regulators, rather than circulating hormones - more than 90% being inactivated during a single passage through the lungs (7).
In the gastrointestinal tract PGs are considered to play a role in the control of motor activity and have been implicated as determinants of secretion (8,9). Thus PGs have been known to cause diarrhoea in humans since it was incidentally observed in 1970 that women receiving PG infusions for termination of pregnancy, frequently developed diarrhoea as an adverse effect (10). Initially this effect was attributed to changes in motility, but later it was shown that the copious watery diarrhoea following parenteral, oral, and jejunal administration of PGs had the characteristics of secretory diarrhoea (4,8,11).
Previous *in vitro* studies have been handicapped by the inability of isolated intestinal mucosa to respond to PG concentrations which may be considered physiological. However, recent studies by Bukhave and Rask-Madsen (11) have demonstrated that secretory responses to low (physiological) doses (10^{-11} - 10^{-7}M) of PGE_2 can be obtained by the Ussing chamber preparation of human jejunal mucosa, provided that the production of endogenous PGs is blocked by indomethacin. These studies convincingly illustrated that the inability of untreated tissues to respond to so-called "physiological" concentrations of exogenous PGE_2 is caused by preformed PGE_2, since the *in vitro* formation of endogenous PGE_2 by untreated tissues equalled the threshold concentration for effect of exogenous PGE_2 in the same tissue. Furthermore, pretreatment of the tissue with indomethacin practically abolished PGE_2 formation *in vitro*, at the same time increasing its sensitivity to exogenous PGE_2.
PGs may have pathophysiological (9,12), in addition to pharmacological (8,13) and physiological (11), effects on intestinal ion transport. However, the primary obstacle for establishing the pathophysiological role of PGs in secretion is the artificial *in vitro* production of PGs by aggregating platelets, which occur spontaneously with blood sampling, or by tissue specimen as a result of mechanical damage by the biopsy forceps. These events cannot be controlled by the addition of PG synthesis inhibitors or anticoagulants to the test tube (14,15). On the other hand determination of PG-metabolites in plasma or urine would at best reflect the total body production (15).

Considering the named methodological problems data on the amount of AA metabolites released into the gastrointestinal fluids appear presently to provide the most reliable index of the balance between gastrointestinal PG synthesis and degradion *in vivo* (12, 16-22). This "atraumatic" approach is also attractive because it permits estimation of parent PGs and their metabolites in parallel, as well as specific stimulation by luminal and neurohumoral secretagogues, in addition to nonspecific stimulation due to hypoxia and chemical or physical damage *in vivo*.

Using the above mentioned "atraumatic" approach Rask-Madsen et al. have shown abnormally high concentrations of PGs in the intestinal lumen in:

1. The irradiation syndrome following physical damage to the epithelial membranes (4).
2. Collagenous colitis, maybe due to hypoxia caused by a diffusional barrier associated with subepithelial deposits of collagen (19).
3. Fluid-discharging villous adenoma of the rectum, maybe as a result of the neoplasia *per se* (12), or maybe hypoxia due to low vascularization of the tumour epithelium.
4. Malignant carcinoid syndrome (21), probably in response to high circulating levels of 5-HT (4).
5. "Nervous diarrhoea", as observed in certain patients classified as irritable bowel syndrome, maybe due to increased parasympathetic influence (17) or possibly specific food intolerance (22).
6. Coeliac disease as a consequence of crypt hyperplasia (4) with increased local release of 5-HT (23), because PGE_2 levels are significantly raised even in the absence of active inflammation (20).
7. Inflammatory bowel disease primarily due to release of PGs from infiltrating leukocytes (24).

Analysis of the data obtained in the above mentioned clinical conditions shows that a positive correlation exists between luminal PGE_2 and stool volume in patients with secretory diarrhoea sensitive to indomethacin treatment (25). On the other hand, PGE_2 levels appear to be independent of the diarrhoea *per se*, since normal PGE_2 levels were found in patients not responding to indomethacin, *e.g.* Verner Morrison's syndrome (cAMP being the second messenger) and disaccharidase intolerance (osmotic diarrhoea). In contrast, the elevated luminal PGE_2 levels found in patients with inflammatory bowel disease correlate primarily with the severity of mucosal inflammation, in agreement with the observation that treatment with potent cyclooxygenase inhibitors decreases absorption and increases the relapse rate (26) - probably by a diversion of AA metabolism via the lipoxygenase pathway.

The effects caused by PGEs are in many respects similar to those caused by cholera toxin, and since both PGE in pharmacological doses and cholera toxin increase intestinal cAMP by stimulating the intestinal adenylate cyclase activity (27,28), it has been speculated that PGs might be mediators of the secretory effects of cholera toxin (29). The use of PG synthesis inhibitors has been reported to decrease or inhibit the secretory effects of cholera toxin *in vitro* (30,31), but it has been generally accepted that the mechanism by which PGs elicit secretion depends on cAMP and that the role of PGs is secondary, rather than primary (32-33).
However, the above mentioned studies on stripped human jejunal mucosa showed evidence of secretory effects that could be obtained with PG concentrations 100-1000 times lower than those required to affect the adenylate cyclase activity, provided that the *in vitro* formation was suppressed by indomethacin (11). Furthermore, recent observations suggest that intramural nervous reflexes play a role in secretion induced by cholera toxin and dihydroxy bile acids, since these secretagogues in experimental animals - besides activating the adenylate cyclase activity - trigger the release of 5-HT (34), a substance that is postulated to activate phospholipases in the cell membrane, hydrolyze phospholipids, and lead to the formation of AA and its metabolites (4,25).

Since intestinal secretion is induced by 5-HT and cholinergic agonists (35), both being neurotransmitters which stimulate PG synthesis and raise intracellular Ca without affecting cAMP, a revised view for the cause of secretion would be that PGs act by increasing the gating of Ca across the serosal cell membrane and cAMP by releasing intracellular reservoir Ca (25). Consequently, both PGs and cAMP may be considered "true" second messengers for the stimulus-secretion coupling *via* intracellular free Ca, as illustrated in the model of postulated intracellular control mechanisms adapted from Powell and Field (35), but modified by indicating that PG formation occurs in response to secretagogoues like 5-HT (25).

This hypothesis on the mechanisms involved in secretory diarrhoea has been further substantiated by recent studies on the mechanism of diarrhoea in a patient with carcinoid syndrome (21) and following withdrawal of morphine from morphine tolerant rats (36). In the carcinoid syndrome PGE_2 levels in the jejunal fluids were markedly increased, but both indomethacin and ketanserin reduced the diarrhoeal volume and the local intestinal PGE_2 concentrations. In morphine tolerant rats naloxone-induced morphine withdrawal reversed fluid absorption to secretion without changing mucosal cAMP levels, but markedly enhanced PGE_2 and 5-HT release. Indomethacin prevented withdrawal-induced fluid secretion and the increase in PGE_2 release. In contrast, ketanserin prevented secretion without influencing the release of 5-HT. Also the α_2-receptor agonist, clonidine, promoted absorption during withdrawal, whereas atropin failed to influence fluid transport (36).

Previous work:

Preliminary studies have demonstrated that PGE_2 levels in jejunal fluids of patients with cholera were significantly raised and were negatively correlated to the time following onset of diarrhea. Jejunal flow rates of PGE_2 were also significantly raised and positively correlated to the stool output during the acute phase of the disease. These results suggest an important role of PG's, in addition to cAMP, in human cholera. (Manuscript available for interested persons).

3. Rationale:

The data as summarized under "previous work" form the rationale for further studies. Both PGE and cholera-toxin(CT) increase intestinal c-AMP. It has therefore been speculated that PG's might be mediators of the secretory effect of CT. So far it has been accepted that PG's elicit secretion via the c-AMP pathway and that the role of PG's is secondary. However, it has recently been shown that PG's can elicit secretion in concentrations 100-1000 times lower than required to affect the adenylate cyclase activity.

Another argument to reconsider the role of PG's is the fact that CT has been shown to trigger enterochromaffin cells to release 5-HT. 5-HT is a neurotransmitter which stimulates PG synthesis, raises intracellular Ca. without affecting c-AMP and induces intestinal secretion. These 2 arguments combined with the exciting results of the pilot-study form the rationale for this study.

The study of the effect of indomethacin on intestinal release of endogenous PG release and the transport of fluids and electrolytes, combined with measurements of 5-HT is therefore a logic next step in our research.

B. SPECIFIC AIMS: See objectives

Patient selection:

Adult patients, male and female, presenting to ICDDR,B Treatment Centre with a history of acute watery diarrhoea (duration less than 24 hours) are eligible for the study. Patients should be, at least moderately dehydrated. Only those patients with initially a purging rate of 200 ml/hour and more will be eligible for this study. No prior medication is allowed. Fresh fecal specimen will be examined by darkfield microscopy for the presence of V. cholerae and a specimen will be sent for culture. The study will be explained to the patients by a local Bangladeshi doctor and the patient will be invited to participate in the study. As soon as informed written consent has been obtained the patient will be transferred to the study ward. A complete physical examination will be done and rehydration will be performed with intravenous fluid. No oral rehydration solution will be used during the study period.

Perfusion studies:

Patients will undergo jejunal intubation by an oral or nasogastric triple-lumen tube. Preferentially, the intubation will be carried out in the morning, the patient being in a fasting state. The position of the tube (distal aspiration port 20 cm distal to the ligament of Treitz) will be checked under fluoroscopy. Ten ml of jejunal fluid will be aspirated for determination of fasting concentrations of PGs and 5-HT. Hereafter a "slow

marker" (42) or a "steady state" perfusion (43) of the jejunum segment will be performed, using BSP as non-absorable marker.

In the "slow marker" perfusion technique the test segment will be prefused with a rate of 0.5 ml/min for approximately 45 minutes for equilibration. Then 10 ml of jejunal fluid will be sampled for determination of fasting intestinal flowrates, PG's and 5-HT. Hereafter the response to a bolus injection of indomethacin (1.0 mg/kg) will be studied, collecting samples at 30, 60 and 90 minutes.

The equilibration period in the "study-state" perfusion technique will also be 45 minutes with an infusion rate of ± 10 ml/min. After the equilibration period 4 sequential 15 min. collections of 10 ml will be used for determination of transport rates of fluid, Na, Cl, K, PG's and 5-HT. Hereafter the response to a bolus injection of indomethacin (1.0 mg/kg) will be studied collecting samples at 30, 60 and 90 minutes.

Indomethacin I.V. in this dose has been used before by Thornell et al.(45). He treated 20 patients with confirmed gallbladder disease with intravenous indomethacin during 24 separate attacks of biliary pain. Pain was relieved within 30 min. of each of all 24 treatments. Apart from some vertigo and slight nausea in 7 treatments in males and 5 in females there were no side effects.

In summary, 16 patients will be investigated during acute cholera and convalescence. In 8 patients the effect of indomethacin will be studied during slow marker perfusion; 8 other patients will be studied during steady-state perfusion.

All patients studied will be requested to return to the hospital one week after discharge for jejunal intubation, sampling of jejunal fluid, and control perfusion studies without administration of drugs.

Laboratory analyses:

PG measurements:

Radioimmunological measurements (RIA) will be performed for determination of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TXB_2 in jejunal fluids aspirated during "slow marker" perfusion and in fluids collected during "steady-state" perfusions of the small intestine. PGF_2 is considered to be the PG responsible for ion secretion, whereas $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ are used as indices of basal PG production in epithelial and endothelial cells, respectively. TXB_2, which originates primarily in platelets, is used as marker of vessel injury with bleeding. Determination of the named PGs, which include purification by extraction and column chromatography before the quantifications are performed by RIA (17), are currently carried out in the Danish laboratory. The RIAs for PGE_2 and $PGF_{2\alpha}$ were recently checked by quantitative gas chromatography-mass spectrometry (48). The addition of the relevant internal standards to the biological samples will be performed at the biochemical laboratory at ICDDR,B prior to the preliminary extraction in order to correct for losses of unstable PGs during storage and transport of samples.

5-HT measurements:

Determination of 5-HT will be performed by HPLC according to Sperk (50). The samples will be mixed immediately with cold perchloric acid (final concentration 0.2 M) and ascorbic acid (final concentration 0.01 mM). Degradation during storage and transport from Dhaka may provide problems. However, any storage problem can be solved by adding radiolabelled 5-HT to the samples immediately following their collection in ICDDR,B - thus providing an internal standard for correction of decay.

Data analysis:

The data will be analysed using relevant standard parametrical statistical methods, such as the Student's t-test for paired and impaired variates and the analysis of variance or non-parametrical statistical analyses - preferentially Wilcoxon's test for paired variates and Mann Withney's U-test. Subjects will serve as their own controls whenever possible.

D. SIGNIFICANCE:

Definition of the role of PG's in intestinal secretion in patients with diarrhoea may provide a rationale for the clinical use of potential anti-diarrhoeal drugs that inhibit PG metabolism or interfere with the action of secretagogues on arachidonic acid metabolism.

E. FACILITIES REQUIRED:

No new facilities required.

F. COLLABORATIVE ARRANGEMENT:

This protocol is a part of a collaborative research project between ICDDR,B and Dr. J. Rask-Madsen in Denmark.

REFERENCES

1. Binder HJ. New modes for regulating intestinal ion transport. Gastroenterology 1980; 75: 642-7.

2. Rask-Madsen J, Bukhave K. Inhibitors and gastrointestinal function. In PW Ramwell (ed). Prostaglandin synthetase inhibitors. AR Liss Inc, New York 1980; 374-96.

3. LeDuc E, Needleman P. Regional localization of prostacyclin and thromboxane synthesis in dog stomach and intestinal tract. J Pharmacol Exp Therap 1979; 211: 181-8.

4. Rask-Madsen J, Bukhave K. The role of prostaglandins in diarrhoea. Clin Res Rev 1981; 1/Suppl. 1: 33-48.

5. Stenson WF, Lobos E. Sulphasalazine inhibits the synthesis of chemotactic lipids by neutrophils. J Clin Invest 1982; 69: 494-7.

6. Blackwell GJ et al. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. Nature 1980; 287: 147-9.

7. Bukhave K, Hansen HS. Elimination of low steady-state concentrations of (5,6-$^3H_2$) prostaglandin E_1 in the pulmonary and the systemic circulations of anaesthetized rats. Biochem Biophys Acta 1977; 489: 403-14.

8. Matuchansky C, Bernier JJ. Effect of prostaglandin E_1 on glucose, water, and electrolyte absorption in human jejunum. Gastroenterology 1973; 64: 1111-8.

9. Rask-Madsen J, Bukhave K. Prostaglandins and chronic diarrhoea: clinical aspects. Scand J Gastroenterol 1979; 14/Suppl. 53: 73-8.

10. Karim SMM, Filshie GM. Therapeutic abortion using $PGF_{2\alpha}$. Lancet 1970; I: 157-9.

11. Bukhave K, Rask-Madsen J. Saturation kinetics applied to *in vitro* effects of low prostaglandin E_2 and $F_{2\alpha}$ concentrations on ion transport across human jejunal mucosa. Gastroenterology 1980; 78: 32-42.

12. Steven K, Lange P, Bukhave K, Rask-Madsen J. Prostaglandin E_2 mediated secretory diarrhea in villous adenoma of rectum: Effect of treatment with indomethacin. Gastroenterology 1981; 80: 1562-6.

13. Bennett A, Fleshler B. Prostaglandins and the gastrointestinal tract. Gastroenterology 1970; 59: 790-800.

14. Johansson C, Bergström S. Prostaglandins and protection of the gastroduodenal mucosa. Scand J Gastroenterol 1982; 17/Suppl. 77: 21-46.

15. Bukhave K, Rask-Madsen J. Prostaglandins and chronic diarrhoea: Methodological problems. Scand J Gastroenterol 1979; 14/Suppl. 53: 67-72.

16. Rask-Madsen J, Bukhave K, Madsen PER, Bekker C. Effect of carbenoxolone on gastric prostaglandin E_2 levels in patients with peptic ulcer disease following vagal and pentagastrin stimulation. Eur J Clin Invest 1983; 13: 351-6.

17. Bukhave K, Rask-Madsen J. Prostaglandin E_2 in jejunal fluids and its potential diagnostic value for selecting patients with indomethacin-sensitive diarrhoea. Eur J Clin Invest 1981; 11: 191-7.

18. Peskar BM, Günter B, Peskar BA. Prostaglandins and prostaglandin metabolites in human gastric juice. Prostaglandins 1980; 20: 419-27.

19. Rask-Madsen J et al. Colonic transport of water and electrolytes in a patient with secretory diarrhea due to collagenous colitis. Dig Dis Sci 1983. In press.

20. Bukhave K, Rask-Madsen J. An approach to evaluation of local intestinal PG production and clinical assessment of its inhibition by indomethacin in chronic diarrhea. In B Samuelsson, PW Ramwell, R Paoletti (eds). Advances in Prostaglandin and Thromboxane Research, vol 8. Raven Press, New York 1980; 1627-31.

21. Antonsen S, Hansen MGJ, Bukhave K, Rask-Madsen J. The influence of a new selective 5-RT_2 receptor antagonist (ketanserin) on jejunal PGE_2 release and ion secretion due to malignant carcinoid syndrome (abstr). Gut 1982; 23: A887.

22. Alun-Jones V et al. Food intolerance: A major factor in the pathogenesis of irritable bowel syndrome. Lancet 1982; II: 1115-7.

23. Challacombe DN, Dawkins PD, Baker P. Increased tissue concentrations of 5-hydroxytryptamine in the duodenal mucosa of patients with coeliac disease. Gut 1977; 18: 882-6.

24. Lauritsen K, Hansen MGJ, Bukhave K, Rask-Madsen J. 1983. Unpublished observations.

25. Rask-Madsen J, Bukhave K. Prostaglandins and intestinal secretion. In LA Turnberg (ed). Intestinal Secretion, Proceedings of the third British Society of Gastroenterology-SK&F International Workshop 1982. Welwyn Garden City 1983; 76-83.

26. Rampton DS, McNeil NI, Sarner M. Analgesic ingestion and other factors preceding relapse in ulcerative colitis. Gut 1983; 24: 187-9.

27. Kimberg DV, Field M, Johnson J. Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. J Clin Invest 1971; 50: 1218-30.

28. Kimberg DV. Cyclic nucleotides and their role in gastrointestinal secretion. Gastroenterology 1974; 67: 1023-64.

29. Bennett A. Cholera and prostaglandins. Nature 1971; 231: 536.

30. Finck AD, Katz RL. Prevention of cholera-induced secretion in the cat by aspirin. Nature (New Biol) 1972; 238: 273-4.

31. Gots RE, Formal SB, Giannella RA. Indomethacin inhibition of salmonella typhimurium, shigella flexneri, and cholera-mediated rabbit ileal secretion. J Infect Dis 1974; 130: 280-4.

32. Kimberg DV, Field M, Gershon E, Henderson A. Effects of prostaglandins and cholera enterotoxin on intestinal mucosal cyclic AMP accummulation. Evidence against an essential role for prostaglandins in the action of the toxin. J Clin Invest 1974; 53: 941-949.

33. Hudson NS, Hindi SE, Wilson DE, Poppe L. Prostaglandin E in cholera toxin-induced intestinal secretion: Lack of an intermediary role. Am J Dig Dis 1975; 20: 1035-9.

34. Cassuto J, Jodal M, Sjövall H, Lundgren O. Nervous control of intestinal secretion. Clin Res Rev 1981; 1/Suppl. 1: 11-9.

34a. Karlström L, Cassuto J, Jodal M, Lundgren O. The effect of hexamethonium on the secretion induced by sodium deoxycholate in the rat jejunum. Experientia 1981; 37: 991-2.

35. Powell DW, Field M. Pharmacological approaches to treatment of secretory diarrhea. In: M Field, JS Fordtran, SG Schultz (eds). Secretory Diarrhea. American Physiological Society, Bethesda 1980; 187-209.

36. Beubler E, Bukhave K, Rask-Madsen J. Involvement of prostaglandin E_2 and 5-hydroxytryptamine in morphine withdrawal diarrhea in the rat. Gastroenterology 1983. Submitted for publication.

37. Speelman P, Rabbanni GH, Bukhave K, Rask-Madsen J. 1983. Unpublished observations.

38. Rask-Madsen J, Bukhave K. The difficulties to establish the pathophysiological role of prostaglandins in secretion. Proceedings of the 36th Falk-symposium. 1983. In press.

39. Hamberg M. Inhibition of prostaglandin synthesis in man. Biochem Biophys Res Commun 1972; 49: 720-6.

40. Oddsson E, Rask-Madsen J, Krag E. A secretory epithelium of the small intestine with increased sensitivity to bile acids in irritable bowel syndrome associated with diarrhoea. Scand J Gastroenterol 1978; 13: 409-16.

41. Rask-Madsen J, Bukhave K. The irritable bowel syndrome: The role of intestinal secretion. In NW Read (ed). Proceedings of Symposium on the Irritable Bowel Syndrome. Academic Press. London 1983. In press.

42. Banwell JG et al. Intestinal fluid and electrolyte transport in human cholera. J Clin Invest 1970; 49: 183-95.

43. Cooper H, Levitan R, Fordtran JS, Ingelfinger FJ. A method of studying absorption of water and solute from the human small intestine. Gastroenterology 1966; 1: 1-7.

44. Wrong O et al. In vivo dialysis of faeces as a method of stool analysis. I. Technique and results in normal subjects. Clin Sci 1965; 28: 357-75.

45. Thornell E, Kral G, Jansson R, Svanvik J. Inhibition of prostaglandin synthesis as a treatment for biliary pain. Lancet 1979; I: 584.

46. Janssen Pharmaceutics, R. 41468: The first pure and selective 5-HT_2 receptor blocking agent. (Investigational new Drug procedure, fifth Edition, january 1981).

47. Davis GR, Camp CR, Raskin P, Krejs GJ. Effects of somatostatin infusion on jejunal water and electrolyte transport in a patient with secretory diarrhea due to malignant carcinoid syndrome. Gastroenterology 1980; 78: 346-9.

48. Bukhave K, Gréen K, Rask-Madsen J. Comparison of radioimmunological determinations with gas chromatography mass spectrometry dosage. A study of PGE_2 and $PGF_{2\alpha}$ in gastrointestinal fluids. Biomed Mass Spectrom 1983; 10: 265-8.

49. Sharon P, Stenson W. Production of leukotrienes by colonic mucosa from patients with inflammatory bowel disease (IBD). Gastroenterology 1983; 84: 1306 (abstract).

50. Sperk G. Simultaneous determination of serotonin, 5-hydroxyindoleacetic acid and homovanillic acid by high performance liquid chromatography with electrochemical detection. J Neurochem 1982; 38: 840-3.

51. Uvnäs-Wallensten K et al. Effect of intraluminal pH on the release of somatostatin and gastrin into antral, bulbar and ileal pouches of conscious dogs. Acta Physiol Scand 1980; 110: 391-400.

52. Page EI, McCubbin JW. Artarial pressure response to infused serotonin in normotensive dogs, cats, hypertensive dogs, and man. Am J Physiol 1956; 184: 265-70.

53. Warner RRP, Janowitz HD, Dreiling DA. The effects of serotonin on human gastric and pancreatic secretion and bile flow. Clin Res 1959; 7: 32.

54. Misiewicz JJ, Waller SL, Eisner M. Motor responses of human gastrointestinal tract to 5-hydroxytryptamine *in vivo* and *in vitro*. Gut 1966; 7: 208-16.

ABSTRACT SUMMARY:

1. Adult male patients with diarrhea caused by cholera form the subject population.
2. Introduction of a double or triple lumen tube till beyond the ligament of Treitz does not bear any substantial risk. No problems or complications are known. No complications were encountered in the previous study. Perfusion of the small intestine for a few hours with a non absorbable marker as sodium sulfobromphtalein (BSP) is without any risk. Injection of indomethacin (1.0 mg/kg bodyweight is safe and has been used for treatment of acute cholecystitis (Lancet 1979:I:584). Indomethacin, which has already been used for years, has well-known side-effect and will not be used in patients with a history of ulcer disease.
3. The procedures are carried out by qualified and experienced doctors; it is highly unlikely that any complication will occur.
4. Data collection sheets will be kept in a locked place. If published, data will show no reference to the identity of the patient.
5. Informed consent (signed or thumb printed) will be obtained from the patients at the time of admission into the study.
6. does not apply
7. Direct benefit to the patient will be the cost free treatment of the diarrheal episode. Society in general may benefit in the future of the development of new antisecretory drugs which act through inhibition of prostaglandin synthesis.
8. In this study we will use the normal hospital charts and we will collect fluid from the jejunum through aspiration.

SECTION III - BUDGET

				Project requirment	
1.	Personnel Services:				
	Name	Designation	%Time	Taka	Dollars
	Dr. Speelman	Pr. Invest.	20	-	3,200
	Dr. Rabbani	Co. Invest.	20	6,000	-
	3 Senior staff nureses		25	18,750	-
	3 Cleaners		25	7,500	
2.	Supplies and materials:				
	Stool-cultures 32x25			800	-
	Other supplies, I.V. needles			-	160
3.	Equipment - Nil				
4.	Patient hospitalization: 16x7x150			16,800	-
5.	Outpatient: Nil				
6.	Transport:				100
7.	Transportation of samples:			-	200
8.	Printing, publication, reproduction:			-	100
			Total :	49,850	3,760

Total US $: 5,760

Personnel ± $: 4,500

Other ± $: 1,260 (Conversion rate US$1=Taka 25)

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

CONSENT FORM : PG-STUDY II

You have been attacked with cholera which provokes a very important loss of water from your body and requires replacement of the lost water by I.V. fluids. We want to study your intestinal fluid. Therefore we want to introduce a small tube through your mouth or nose to the intestines to collect this fluid. This procedure will take about half a day. Hereafter the tube will be removed. This procedure is completely safe but may cause some discomfort in nose or throat.

We will request you to come back to the hospital 2 weeks after discharge. If you come back, we will reimburse your travel expenses and a daily income.

If you do not want to be included in this study, you will not be penalized in any way but you will receive the same proper treatment in the hospital. You may also decide to withdraw from the study at any time.

If you accept to join the study, please sign the consent form here below.

_____
Signature of patient
or thumb impression

_____
Signature of the Investigator

Date: _____