20

Date 7/4/85
9/4/85

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. F.P.L. Van Loon Trainee Investigator (if any) _____

Application No. 85-012 Supporting Agency (if Non-ICDDR,B) _____

Title of Study "Role of Endogenous Prostaglandins in E. coli Secretory Diarrhoeas"

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.

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

F P L Van Loon

Principal Investigator

Trainee

85-012
9/4/85

SECTION 1 - RESEARCH PROTOCOL

1. TITLE: ROLE OF ENDOGENOUS PROSTAGLANDINS IN E. COLI SECRETORY DIARRHOEAS.

2. PRINCIPAL INVESTIGATOR: Dr. F.P.L. Van Loon (Dhaka)

   CO-INVESTIGATORS: Dr. G.H. Rabbani (Dhaka); Dr. J. Rask-Madsen (Denmark); Dr. K. Bukhave (Denmark)

3. STARTING DATE: 15 April 1985

4. COMPLETION DATE: 15 September 1985

5. TOTAL DIRECT COST: US $ 3100

6. SCIENTIFIC PROGRAMME: This protocol has been approved by the Pathogenesis and Therapy Working Group.

Signature of Scientific Programme Head: [signature]

Date: 8/4/1985

7. ABSTRACT SUMMARY:

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

Since secretory diarrhoea can be induced by non cholera germs like E. coli as well , we propose to study 16 patients with acute E. coli diarrhoea 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 PGE_1 and tromoboxane) as well as 5 hydroxy-tryptamine). In order to account for the gap of clinical diagnosis and bacteriological confirmation of E. coli diarrhoea, we are keeping plan in the budget for 20 patients.

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

8. REVIEWS:

(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 in E. coli diarrhoea.

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

Background:

(a) Role of PG as a mediator of secretory diarrhoea:

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 non-specific 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 secretagogues 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).

(b) Increased PG levels in cholera patients (Speelman, Rabbani; GUT 1985).

(b)

Supraphysiologic doses of prostaglandins (PGs) mimic the effect of cholera toxin and cAMP in the small intestine, but not all observations are explicable in terms of the theory that links PGs to cAMP. Because no data exist on endogenous PGs in human cholera we measured PGE_2 concentrations in jejunal fluids and fasting intestinal flow rates of PGE_2 during slow marker perfusion of proximal jejunum in nine patients with high purging cholera. Nine patients in the recovery phase of cholera or other watery diarrhoeas served as controls. In acute cholera PGE_2 concentrations were significantly ($p<0{\cdot}001$) raised (172-1435 (n=9) *vs* 60-270 (n=9) pg/ml) and negatively correlated ($r=0{\cdot}71$; $p<0{\cdot}05$) to the time following onset of diarrhoea. Also fasting jejunal flow rates of PGE_2 were significantly ($p<0{\cdot}005$) increased (0·77-8·22 (n=7) *vs* 0·21-0·92 (n=6) ng/min), and positively correlated ($r=0{\cdot}84$; $p<0{\cdot}01$) to stool output (2·9-9·5 ml/min). By extrapolation, at normal stool output fasting jejunal flow rates of PGE_2 equalled those measured during convalescence. The results support the notion that PGs, in addition to cAMP, may play a pathophysiologic role in human cholera. As the ratio between the medians of the highest values measured during the acute phase of cholera and in late convalescence was at least 15, local intestinal PGE_2 formation in full blown cholera should result in mucosal PGE_2 concentrations above those required for a maximal secretory response. This observation might explain why conventional doses of aspirin and indomethacin had no significant antidiarrhoeal effect in clinical trials.

The data has been shown in the accompanying table and data illustrated by the Fig. 1

Table *PGE_2 concentrations in fasting jejunal fluids, fasting jejunal flow rates, and stool output in acute cholera and in convalescent phase of cholera and other watery diarrhoeas*

	PGE_2 (pg/ml)			*Flow rate (ml/min)*	*PGE_2 × flow rate (ng/min)*		*Stool output (ml/min)*
	Preperfusion	*Min**	*Max**		*Min*	*Max*	
Acute cholera							
Median	483	490	990	5·2	2·11	5·17	6·9
Range	175–1435	172–885	421–1435	3·5–6·9	0·77–4·60	2·00–8·22	2·9–9·5
No of patients	(9)	(9)	(9)	(7)	(7)	(7)	(9)
		$p<0{\cdot}01$			$p<0{\cdot}01$		
Convalescence							
Median	155	115	190	3·2	0·24	0·38	<0·2
Range	60–270	60–270	100–270	1·5–4·2	0·21–0·92	0·22–0·92	—
No of patients	(9)	(9)	(9)	(6)	(6)	(6)	—
p less than	0·002	0·001	0·0005	0·02	0·005	0·005	—

* Min and max denote median of lowest and highest values, respectively, measured before and during slow marker perfusion.

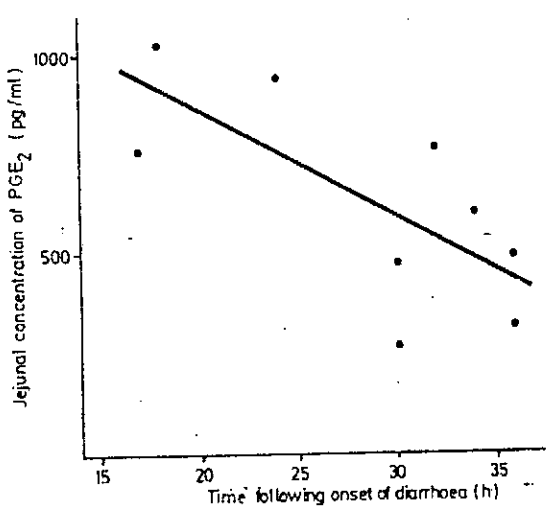

Fig. 1 *Correlation between PGE_2 concentrations in fasting jejunal fluids (means of all values observed in seven patients and preperfusion concentrations in two patients) and the time following onset of diarrhoea in patients with acute cholera ($r=0{\cdot}71$; $p<0{\cdot}05$).*

C. Results of the currently on-going study: in cholera patient at ICDDR,B:

So far, we have studied 7 patients in the acute phase and only 4 patients in the convalescent phase. A preliminary analysis of the data showed the following observation.

1. The PGE_2 concentration in the jejunal fluid is significantly increased during the acute stage of cholera and declined to normal level during the convalescent phase (see summary table). This finding is consistent with our previous studies done at ICDDR,B.

2. In the on-going study we are also examining the effect of indomethacin to inhibit the PG synthesis and therefore the jejunal secretion. The preliminary data indicate that after intravenous indomethacin (1 mg/kg/ body weight) there is a significant drop in the level of PGE_2 in the jujunal fluid. This effect will be further evaluated (see summary table).

3. The fasting intestinal flow rate (FIFR) of PGE_2 and water is also consistent with the previous data. The FIFR is significantly increased during acute cholera and declined during the convalescent stage.

All these finding strongly indicate that PG may have some role in the mechanism of fluid secretion due to cholera toxin. Also the PG inhibitory drugs may be useful to control diarrhoea in cholera patient. We look forward to complete this study by the next cholera season; and give you more interesting and detailed results.

Table: Prostaglandin E_2 concentration in jejunal fluid, fasting intestinal flow-rate (FIFR) and effect of indomethacin on jejunal PG in patients with severe cholera.

PGE_2 (Pg/ml) at different time period	Acute stage (N=7)	Convalescent stage (N=4)
	Mean ± SD	
Fasting stage	757 ± 251	398 ± 109
At 'O' time after fasting	533 ± 356	221 ± 78
At 30 min	395 ± 327	203 ± 133
At 60 min	303 146	162 ± 149
FIFR for water (ml/min) (Median, Range)	5.2 (0.8-35)	2.25 (0.7-17.9)
FIFR for PGE_2 (ml/min), Median, Range	2.15 (0.1-20.9)	0.15 (0.1-4.6)
PGE_2 before indomethacin (Mean ± SD)	508 242	-
PGE_2 after indomethacin (Mean±SD)	137 66	-

Rationale:

(1) The mechanism of action of E. coli enterotoxin (LT, ST) is not yet definitely known. There is conflicting evidence in the literature about the role of several mediators such PG, 5HT, CAMP and so on. According to existing literature the role of PG seems highly likely and the current study has been designed to examine if PG is an important factor for mediating the effect of E. coli toxin. This will substantially advance the knowledge of pathogenic mechanism of secretory diarrhoea.

(2) Studies on PG may also lead to the development of antisecretory drugs which may be clinically useful.

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 100 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. Only the D.F. negative cases will be included. 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 sgement will be performed, using BSP as non-absorable marker.

In the "slow marker" perfusion technique the test segment will be perfused 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 "steady-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 Thonnell 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, 20 patients will be investigated during acute non-cholera watery diarrhoea and convalescence. In half of the patients the effect of indomethacin will be studied during slow marker perfusion, the other patients will be studied during steady-state perfusion. In order to account for the gap of clinical diagnosis and bacteriological confirmation of E. coli diarrhoea we are keeping plans in the budget for 20 patients.

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. PGE_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.

Stool cultures:

Special attention shall be paid to ST and LT strains of E. coli

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.

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ABSTRACT SUMMARY:

1. Adult male patients with watery diarrhoea, caused by E. coli from the subject population.

2. Introduction of a double or triple limen tube till beyond the ligament of Treitz does not bear any substantial risk. No problems or complications are known. No complication were encountered in the previous study. Perfusion of the small intestine for a few hours with a non absorbable marker as sodium sulfabromphtalein (BSP) is without any risk. Injection of indomethacin (1.0 mg/kg body weight 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 diarrhoeal 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

1. Personnel services

Name	Designation	% time	Project requirement Taka	Dollar
Dr. F.P.L. Van Loon	Pr. Investigator	70	(No cost to ICDDR,B)	
Dr. G.H. Rabbani	Co-Investigator	10	5000	-
3 Senior Staff Nurses		15	11250	-
3 Cleaner		15	4500	-
Biochemist		10	-	670

2. Supplies and materials:

Stool cultures 20 X 4		80
Other supplies I.V. needles		160
Biochemicals		1790

3. Equipment - nil

4. Patient hospitalization 20 X 3 X 150 — 9000 — -

5. Out patients - nil

6. Trasnport: 100

7. Transportation of samples: 200

8. Printing, publication, reporduction: 100

Tk.	30750	$ 3100

Total US $: 3630

Personnel ± $ 1470

Other $ 3100

(Conversion rate US $ 1 = Taka 26)

International Centre for Diarrhoeal Disease Research, Bangladesh

Consent Form : PG-study II

You have watery diarrhoea 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 you 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
of thumb impression

_____
Signature of the Investigator

Date: ----------

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

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আন্তর্জাতিক উদরাময় রোগ গবেষনা কেন্দ্র
(আই, সি, ডি, ডি, আর, বি)

সম্মতি পত্র

(পি, জি, ষ্টাডি - ২)

আপনার উদরাময় (ইকোলাইজনিত) অর্থাৎ ডায়রিয়া হয়েছে। এই রোগে প্রচুর পরিমানে জলীয় পদার্থ ও লবন বের হয়েছে। সেই জন্য আমরা আপনার শিরায় স্যালাইন প্রবেশ করিয়ে চিকিৎসা করবো। আমরা আপনার ক্ষুদ্রান্ত্রের জলীয় পদার্থ পরীক্ষা করবো। এই পরীক্ষার জন্য আপনাকে একটা সরু রবারের নল গিলে ফেলতে হবে এই পরীক্ষা প্রায় অর্ধদিবস চলবে। এবং এর পর রবারের নলটি বের করে ফেলা হবে। এই পরীক্ষা সম্পূর্ন নিরাপদ, কিন্তু নাকে এবং গলায় সামান্য অসুবিধা বোধ করতে পারেন।

আপনাকে ২ সপ্তাহ পর আবার হাসপাতালে আসতে অনুরোধ করা হচ্ছে। সে সময় আমরা আপনাকে যাতায়াত ও পারিশ্রমিক বাবদ কিছু টাকা দেব। আপনি এই গবেষনায় অংশগ্রহন না করলে, কিংবা গবেষনা চলাকালীন হটাৎ পরিত্যাগ করলেও আপনাকে কলেরার চিকিৎসা দেওয়া হবে। আপনি রাজী থাকলে নীচে সই করুন।

গবেষকের স্বাক্ষর

রোগীর স্বাক্ষর/টিপ সহি

তারিখ----------