FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B

Principal Investigator: Dr. M. A. Salam & ~~Dr. Hans Lindblad~~

Trainee Investigator (if any)

Application No. 94-005

Supporting Agency (if Non-ICDDR,B)

Title of Study: Exploratory study on salt ~~water homeostasis and renal~~ function in children with and without hyponatraemia in association with shigellosis

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 NA
   (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 NA
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.

RCG

I 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

Trainee

A-032052

1.	Title	:	Exploratory study on salt and water homeostasis and renal function in children with and without hyponatraemia in association with shigellosis.
2.	Collaboration	:	This study will be performed in collaboration with the Karolinska Institute, Sweden.
3.	Principal Investigator	:	Dr. Mohammed Abdus Salam : ICDDR,B Dr. Hans Lindblad : Huskvarna Health Care Centre, Sweden
4.	Coinvestigator	:	Dr. Ali Miraj Khan
5.	Consultants	:	Prof. Ulla Berg Dr. Dilip Mahalanabis Prof. Rolf Zetterstrom
6.	Starting date	:	01 March, 1994.
7.	Completion date	:	30 June, 1994.
8.	Total Direct Cost	:	US$ 14,106.00
9.	Funding source	:	
10.	Scientific Programme	:	This protocol has been approved by the Clinical Sciences Division.

February 01, 1994

Associate Director
Clinical Sciences Division
ICDDR,B

10. Abstract Summary

Serum electrolyte abnormalities are very frequently observed among diarrhoeal children and hyponatraemia is quite common. Hyponatraemia and other complications such as hypoglycaemia, toxic megacolon, haemolytic-uraemic syndrome, convulsions are, however, seen more often in association with infections due to *Shigella* compared to other infectious diarrhoea. Alterations in CNS functions in hyponatraemia interfere with oral rehydration and food intake, making management of such patients difficult. Hyponatraemia and other factors such as fever, increased catabolism and loss of enteric protein will have a great impact on their nutrition. Moreover, death rates are significantly higher in hyponatraemic children compared to children with normonatraemia and hypernatraemia. The cause of hyponatraemia in shigellosis is not fully understood. Loss of sodium in stool and urine are not important determinants. There are indications that inappropriate release of antidiuretic hormone (SIADH) play a significant role. It is possible that other factors, such as disturbed water homeostasis, decreased glomerular filtration and disturbances of renal tubular sodium handling may also be involved in the pathogenesis of hyponatraemia in shigellosis. Also, there are suggestions that the hyponatraemia, at least in part, may be related to protein energy malnutrition (PEM) and its associated disturbances of sodium and water metabolism. Infants and children with PEM suffer from failure to increase fractional excretion of sodium in response to sodium load which may lead to expansion of extracellular volume and hyponatraemia. That dysfunction at the proximal tubules may be another factor for development of hyponatraemia is indicated by aminoaciduria in such patients. In shigellosis, hyponatraemia is seen more commonly in patients with complications such as toxic colitis and haemolytic-uraemic syndrome (HUS), which in turn, are seen more often in association with infections due to *S. dysenteriae* type 1. This suggests that factors such as "Shiga toxin" or endotoxin, which are produced in increased amounts by this strain may play a role. It appears that the development of hyponatraemia in shigellosis is multifactorial and its pathogenesis is not yet clear. A better understanding of the pathogenesis of this complication may help development of better interventional strategies and thereby reducing deaths from shigellosis. This study proposes to study the relationship between hyponatraemia in shigellosis and a number of factors, such as nutritional status of children, hydration status, total body water and body water metabolism, GFR, and salt and water handling and other renal parameters.

11 Review : _____

12. Ethical Review Committee : _____

13. Research Review Committee: _____

14. Introduction

A. Objective

The objectives of this exploratory study are;

A.1 To create facilities at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B to) study body water metabolism and renal physiology in collaboration with a Swedish group.

A.2 To train ICDDR,B personnel to undertake such studies, and

A.3 To understand the mechanisms of hyponatraemia in a limited number of children with shigellosis.

15. Background

Hyponatraemia

Hyponatraemia is generally defined as a serum sodium concentration of <130 mmol/L. It is important to recognize, however, that this lower than normal serum sodium concentrations may be associated with a low total body sodium, a high total body sodium or a normal total body sodium [1].

Hyponatraemia may be a consequence of disorders of diluting capacity of the kidneys which can occur due to;

1. Continued inappropriate secretion of antidiuretic hormone (ADH) in spite of serum hypo-osmolality, which by itself would suppress ADH secretion.

2. Some internal factors in the kidney, such as decreased glomerular filtration rate (GFR), increased proximal tubular sodium and fluid reabsorption, or both, which diminish the delivery of fluid to the distal diluting segments of the nephron, including the cortical and medullary ascending limbs of the loop of Henle and impair the capacity of the nephrons to dilute urine.

A. Hyponatraemia associated with low total body sodium

A.1 Increased secretion of ADH: The ADH release is controlled by the net effect of the actions of osmoreceptors and volume receptors. However, the role of osmoreceptors in relation to volume receptors remains to be elucidated.

A.2 Gastrointestinal loss: In the case of gastrointestinal losses causing severe hypovolaemia with initial normal renal function, the urine sodium concentration is <10 mmol/L and usually in the range of 1-3 mmol/L. In presence of vomiting and metabolic alkalosis with bicarbonaturea, urinary bicarbonate anions necessitates obligatory cationic loss leading to >10 mmol/L of urinary sodium. Ketonuria with starvation or diabetes mellitus will also result in obligatory renal

sodium loss despite presence of hypovolaemia. In the presence of renal disease, examination of urinary sodium chloride concentrations and osmolality may be misleading.

A.3 Due to diuretic abuse.

A.4 In salt-losing nephropathy.

A.5 In adrenal insufficiency: The association of hyponatraemia and depleted extracellular fluid (ECF) suggests the possibility of adrenal insufficiency. Urinary sodium in such instances ia >20 mmol/L, and hyperkalaemia results from its decreased urinary excretion consequent to decreased mineralocorticoid activity in adrenal insufficiency. .

A.6 Osmotic diuresis: The urinary sodium is usually >20 mmol/L because of the fact that osmotic diuresis obligates cation excretion in spite of concomitant volume depletion. Hyponatraemia is also contributed by enhancement of the osmotic movement of water from intracellular to extracellular compartments (e.g. for 100 mg/dL increase of glucose there is a 1.6 mmol/L fall in serum sodium concentration).

B. Hyponatraemia associated with increased total body sodium

This occurs in oedematous disorders when sodium and water both are retained, however, the retention of water occur to a greater extent, such as in cardiac failure, hepatic failure and nephrotic syndrome. Principle mechanisms involved in this type of hyponatraemia are increased release of ADH and decreased GFR.

C. Hyponatraemia associated with normal total body sodium

In such instances, patients develop hyponatraemia without evidence of hypovolaemia or oedema. Various stimuli for ADH release, such as pain or emotion if sustained for a prolonged period and water intake is continued can lead to this type of hyponatraemia.

C.1 Hypothyroidism: Increase in ADH is perhaps responsible for hyponatraemia in such cases.

C.2 SIADH: Usually a diagnosis of exclusion, and is characterized by absence of hypovolaemia, oedematous disorders and endocrine dysfunction (primary and secondary adrenal insufficiency, hypothyroidism), renal failure, drugs etc.

D. Hyponatraemia and diarrhoeal diseases

Diarrhoeal diseases are important causes of childhood morbidity and mortality in the developing countries, and hypovolaemic shock is the leading cause of death among diarrhoeal children. Different mortality rates are observed among children infected with specific enteropathogens; infection due to *Shigella* which usually is not associated with severe dehydration have higher mortality than watery diarrhoeas caused by pathogens such as *V.*

cholerae 01, enterotoxigenic *Escherichia coli*, rotavirus etc. There are a number of complications such as electrolyte disturbances, hypoglycaemia, bacteraemia, haemolytic-uraemic syndrome, toxic colitis, encephalopathy, convulsions etc. which are seen more in patients with shigellosis than in other conditions, and association with these complications carry higher mortality.

E. Electrolyte disturbances in diarrhoeal diseases

Electrolyte abnormalities are the most common complications of diarrhoeal diseases, and among them hyponatraemia is very common. In one study, retrospective chart analysis of 1,330 children admitted in the inpatient ward of the Clinical Research and Service Centre (CRSC) of ICDDR,B revealed that about 21% of them had a serum sodium concentrations of <130 mmol/L [2]. The incidence of hyponatraemia was higher in shigellosis compared to other types of diarrhoeas. In a prospective study including 815 children admitted to the inpatient ward of the CRSC of ICDDR,B with bacteriologically confirmed shigellosis, serum sodium was <130 mmol/L in 433 (53%) of the patients [3]. The incidences of hyponatraemia of <125 mmol/L was observed in about 50% of the children with infection due to *S. dysenteriae* type 1, compared to 20% children with infections due to other species of *Shigella* [3]. Haemolytic-uraemic syndrome (HUS) is another complication of shigellosis which in typical cases is characterized by leukemoid peripheral blood picture, thrombocytopenia, microangiopathic haemolytic anaemia, and renal failure [4]. This syndrome is particularly noted in association with *S. dysenteriae* type 1 strains, and hyponatraemia is very common in patients with haemolytic-uraemic syndrome [3,5]. .pa

F. Deaths in association with hyponatraemia

Hyponatraemic patients have been found to carry a relative risk of death of 2.5 and 10 compared to hypernatraemics and normonatraemics respectively [2].

G. Pathogenesis of hyponatraemia in shigellosis

The pathogenesis of hyponatraemia in shigellosis, especially its association with infection due to *S. dysenteriae* type 1 strains is not completely understood. However, the following factors deserve consideration;

G.1 Role of stool and urinary loss of sodium

Unlike cholera, stool concentration of sodium in patients with shigellosis is much lower, about 80 mmol/L [6]. This, along with lower stool volume in shigellosis makes stool losses of sodium unlikely to be a major factor in the development of hyponatraemia [7]. Also, hyponatraemia can not be explained by urinary sodium loss [6].

G.2 Role of microbial factors

Hyponatraemia is more common in association with infection due to *Shigella* compared to diarrhoea due to other aetiology, and the incidence of

hyponatraemia in shigellosis is higher in association with infection due to *S. dysenteriae* type 1. This would suggest contribution of microbial features in the development of hyponatraemia in such cases. One such factor could be "Shiga toxin" which is produced in larger amounts by this strain of *Shigella* [8], and higher levels have been documented in patients with shigellosis and haemolytic-uraemic syndrome [8]. Endotoxin may be another factor. The colitis is more severe with *S. dysenteriae* type 1 infections where the extensive ulcerations may permit absorption of larger amounts of endotoxin in the circulation [5,9]. Endotoxin is known to cause damage to the vascular endothelium including those of the glomerular capillaries; the changes may lead to decreased GFR and expansion of body fluids, and dilutional hyponatraemia. Patients with shigellosis and haemolytic-uraemic syndrome have low serum sodium, and a higher amount of circulating endotoxin has been documented in such patients in a study conducted at the ICDDR,B [4]. A recent prospective study has confirmed the earlier observations [5]. The extensive ulcerations and the associated more intense inflammatory response in the intestine association with volume depletion may be responsible for triggering excessive release of ADH (SIADH) leading to expansion of extracellular volume and dilutional hyponatraemia.

G3. Inappropriate secretion of ADH (SIADH) and hyponatraemia

In one study conducted at ICDDR,B, the serum levels of ADH has been found to be significantly higher in patients with hyponatraemia compared to patients who did not develop this complication [3]. The ADH release is controlled by the net effect of the actions of the volume receptors and osmoreceptors. This may raise the hypothesis that the inappropriate release of ADH is the result of volume depletion (although it may be subclinical) in shigellosis. Patients with cholera with severe dehydration have been shown to have higher amounts of ADH and creatinine. About 24 hours later, after correction of dehydration, both the level of ADH and creatinine normalized in such patients. On the contrary, children with shigellosis were found to maintain higher amounts of ADH and creatinine in their serum 24 hours after admission [3]. The study indicate that inappropriate release of ADH and hyponatraemia can not be explained by volume depletion alone [3].

G4. Compromised GFR and hyponatraemia

There are several factors that can modify GFR, such as renal blood flow, afferent and efferent arteriolar constriction, sympathetic stimulation, and arterial pressure.

Diarrhoeal patients with some to severe dehydration have lower GFR consequent to volume depletion, reflected in addition to clinical features by elevation of serum creatinine. In one study, highest levels of serum creatinine were observed in patients suffering from cholera with some to severe dehydration. Although severe dehydration is uncommon in shigellosis, higher creatinine levels were also observed in patients with shigellosis and hyponatraemia [3]. After hydration, serum creatinine concentrations in cholera patients normalized, but even 24 hours after admission patients with shigellosis maintained higher serum levels of creatinine [3]. These findings suggest that the decreased GFR and consequent reduction in renal excretion

of sodium and water is likely to be one of the factors responsible for elevated creatinine, expansion of extracellular volume, and dilutional hyponatraemia. Such abnormal conservation of fluid may partly explain, why patients with shigellosis do not usually show signs of dehydration. The pathogenesis of hyponatraemia in shigellosis may, thus, in a way, be similar to that of acute post-streptococcal glomerulonephritis.

H. Protein-energy malnutrition (PEM), altered renal function and hyponatraemia

Severe PEM has two extreme pictures which represent different stages of "adaptation" to nutritional and infective stress. At one end, there are infantile marasmus or "dry" PEM; at the other, there is the oedematous type, mostly occurring in toddlers, universally known as kwashiorkor. Whether these are two different diseases is a subject of discussion. In a recent study of oral rehydration, some well nourished children developed osmotic diarrhoea and some malnourished children developed oedema [10]. The reasons behind these complications are not understood. Infant and children with PEM have a tendency to retain sodium and water irrespective of presence or absence of oedema. Although the mechanism for this retention remains unknown, altered renal function may be a factor. In one study, an association has been noted between the decline in glomerular filtration rate and degree of malnutrition, albeit with large intra-individual variations, indicating that, perhaps, decreased GFR is one of many factors responsible for this hyponatraemia [11]. Samadi *et al* noted that there was an association of hyponatraemia with increasing age and malnutrition, and serum sodium concentrations were lower in malnourished children, irrespective of type of dehydration [2]. This association of hyponatraemia with increasing age may be due to gradual deterioration of nutritional status [12]. In the pathogenesis of oedema in PEM, two main principles have to be considered. According to the Starling equilibrium, the balance between plasma and interstitial fluid is regulated by opposing hydrostatic and oncotic forces in the capillaries. The other regulating principle is the renal handling of salt and water. Oncotic pressure, unlikely to be the only cause of oedema in PEM, is markedly reduced in oedematous children and the relationship between hypo-albuminaemia and oedema formation is obvious in kwashiorkor, even if there is no cross relation between the plasma albumin concentrations and the presence of oedema. Because of the expansion of the extracellular fluid (ECV) in kwashiorkor, the low plasma albumin concentrations seems partly to be the result of dilution and partly due to reduction in total amount of albumin. In one study conducted in adults with shigellosis, the concentration of prealbumin was found to be depressed during the acute stage which increased gradually when infection was cured by treatment with appropriate antimicrobials [Wasif *et al*, unpublished observations]. Decreased production of albumin in children with PEM (already in a state of hypo-albuminaemia) and shigellosis may result in reduced oncotic pressure and consequently lead to an increase in GFR. GFR has, however, been reported to be reduced in PEM due to hypovolaemia and reduced renal plasma flow [11]. As the water balance is regulated by the ADH, it is not surprising that this hormone is high in plasma and urine of children with kwashiorkor, while the levels are not elevated in comparable marasmic patients. Renin activity in plasma is also increased in kwashiorkor and this is particularly true for

very ill patients many of whom subsequently will die. Surviving patients show normal values after rehabilitation. Low delivery of urea to the counter-current system in the loop of Henlè in the malnourished children may give rise to a deficient ability to concentrate urine. Another phenomenon observed in PEM is the failure to increase fractional excretion of sodium in response to salt load, an entity also seen in neonates. The reason for this disturbance remains unknown. Additionally, aminoaciduria in PEM points to a proximal tubular dysfunction. In PEM, there is a tendency to hyponatraemia although sodium may be retained; a situation similar to that found in SIADH, and the situation may be aggravated in shigellosis.

I. Assessment of dehydration in PEM

The criteria for assessment of degree of dehydration in infants and children with acute diarrhoea can not be applied to malnourished children, since plasma volume and extracellular volume may remain high in these children in spite of volume depletion. Therefore, estimation of total body water, plasma volume and extracellular volume at different stages of diarrhoea may provide important information regarding relative contribution of these compartments in dehydration in malnourished children.

16. Rationale

Hyponatraemia is a well recognized complication of infection due to *Shigella*, which may lead to impairment of general well-being, depression of mental status, loss of appetite, nausea and vomiting, seizures, and prolonged comatose state [1]. The CNS abnormalities interfere with oral rehydration and feeding practices. This low nutrient intake along with loss of endogenous protein may be critical for children who are already malnourished [13,14]. Additionally, these CNS manifestations may precipitate other complications like aspiration pneumonia, leading to death. The presence of hyponatraemia in diarrhoeal patients has been shown to be associated with higher death rates compared to patients with normal serum sodium or hypernatraemia [2]. An in-depth pathophysiologic study will contribute to our understanding of the problem, and may help prevention and/or better management of such patients.

17. Hypotheses

In the light of the background information, we hypothesize that the development of hyponatraemia in association with infection due to *Shigella* is multifactorial, and include;

A. Unrecognized loss of salt and water during the early phase of shigellosis in addition to low salt intake (due to severe anorexia) and increased salt-free fluid intake.

B. Adrenal insufficiency, particularly in a subgroup of patients who are very sick with or without bacteraemia.

C. Inappropriate release of antidiuretic hormone (SIADH).

D. Decreased GFR as a consequence of endothelial damage due to endotoxin or "Shiga toxin".

E. Protein energy malnutrition with its effect on salt and water homeostasis.

18. Specific aims

The specific aims of this study are;

A. To study GFR and effective renal plasma flow (ERPF) in children with shigellosis with normonatraemia in three nutritional groups viz. undernourished, marasmic and marasmic-kwashiorkor, before and after hydration, and also after nutritional rehabilitation.

B. To study GFR and ERPF in children with shigellosis with hyponatraemia (serum sodium $\leq$125 mmol/L) in two nutritional groups viz. marasmus and marasmic kwashiorkor, before and after rehydration, and before and after nutritional rehabilitation.

C. To study renal sodium and water homeostasis in groups mentioned above trying to clarify the mechanisms behind the development of oedema and the risk of overhydration.

D. To study renal concentrating capacity (maximum urine osmolality) through the renal handling of sodium and urea after administration of DDAVP intranasally in groups of children mentioned above after rehydration, and before and after nutritional rehabilitation.

E. To assess total body water, plasma volume, and extracellular and interstitial fluid volume, as well as different regulatory hormones such as plasma renin, aldosterone and ADH in groups of children mentioned above.

19 Methods of procedure

A. Enrollment criteria

The study population will be selected from children who are admitted in the inpatient ward of the CRSC with a clinical diagnosis of acute dysentery of <5 days duration. Children who are documented to have bloody-mucoid stools and who have not been treated with effective antimicrobials will be monitored to see if they fulfill the following criteria for inclusion into the study:

A.1 Age : 3-5 years

A.2 Sex : Males only (to facilitate collection of urine). Females can be accepted if capable of voluntary bladder emptying.

A.3 Weight : $\geq$10 kg.

A.4 Patient groups

Group 1: 6 children with shigellosis with weight for age of >60% without oedema, and with serum sodium concentration between 125 - 129 mmol/L.

Group 2: 6 children with marasmus and shigellosis, with serum sodium of between 131 mmol/L to 149 mmol/L.

Group 3: 6 children with marasmus and shigellosis, with serum sodium of ≤125 mmol/L.

Group 4: 6 children with marasmic kwashiorkor and shigellosis, with serum sodium of between 131 mmol/L to 149 mmol/L.

Group 5: 6 children with marasmic kwashiorkor and shigellosis, with serum sodium of ≤125 mmol/L.

A.5 Informed consent: Written informed consent will be required from either of the parents, or legal guardians of the children.

B. Exclusion criteria

B.1 Female sex, unless as mentioned under inclusion criteria 2.B2. Patients with suspected septicaemic shock

B.2 Age: <3 years

B.3 Weight: <10 kg on admission

B.4 Dehydration: Patients with severe dehydration requiring intravenous rehydration.

B.5 Patients with septicaemic shock

B.6 Patients with high fever (rectal temperature of >40.0°C)

B.7 Patients with pneumonia

B.8 Patients with convulsions, or history of convulsive disorders

C. Methods

C.1 Study of renal functions

Renal function will be measured by GFR and ERPF determined by clearances of inulin and Para-amino hippuric acid (PAH) respectively. To avoid catheterization of bladder, the investigation will be performed under ORS (during diarrhoea) or water (during diarrhoea-free time) diuresis, so that the patients can empty bladder regularly through spontaneous

micturition. The time required for these tests is about 4 hours.

C.1.1 **Preparation of patients:** Patients undergoing examination should ideally be fasting, as protein intake elevates GFR. However, patients who have taken a light meal will be considered eligible for this study.

C.1.2 Weight, height/length and blood pressures will be measured and recorded. Blood pressure will be measured by doppler method (equipment available at the CRSC of ICDDR,B). Two intravenous cannulas will be applied: one at each arm. This is required to avoid repeated pricking for time sampling (description follows), as renal function is influenced by discomfort.

C.1.3 After establishment of cannulae, blood sample "B0" (2 ml of whole blood collected in a serum test tube) will be drawn.

C.1.4 The patients will be asked to void urine, and urine will be collected in a paediatric urine collector (PUC) bag at the time blood is drawn. This urine sample will be designated as "U0". Time and volume of urine will be recorded. A sample will be taken from the PUC, labeled, and stored at -20°C for subsequent assays.

C.1.5 The inulin will be administered as Inutest^{R} 25% (Laevosan-Gesellschaft) and it will be mixed with PAH-20% (amino-hippurate sodium, 20%, MSD) solution in the proportion of 17 ml inulin^{R} to 3 ml PAH. The patient will be given a prime-dose equal to 0.3 ml/kg of body weight of this solution followed by a continuous infusion using a infusion pump (Digitinfusion, Switzerland) according to the following schedule;

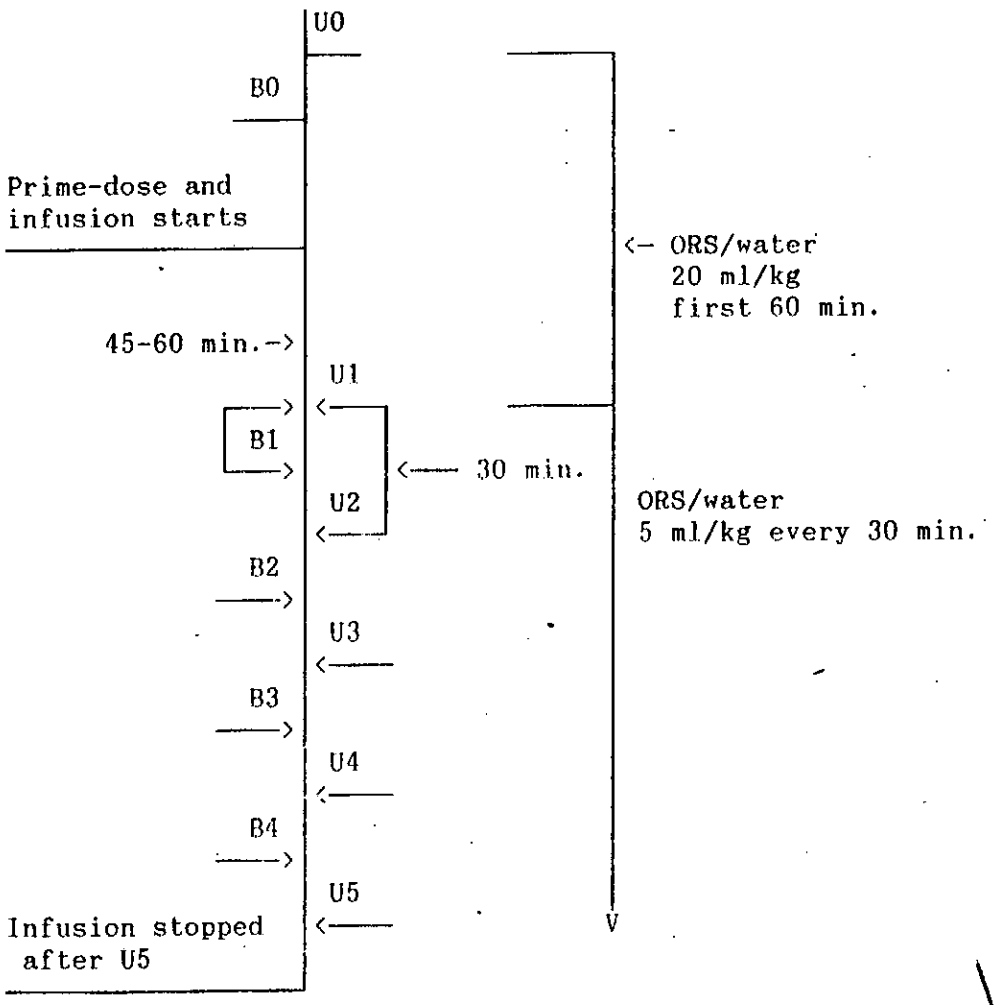
Children with body weight 10-20 kg : 0.1 ml/mi. = 6 ml/hour

Children with body weight >20 kg : 0.2 ml/mi. =12 ml/hour

Note: In cases with known reduction of GFR, the amount will be reduced.

C.1.6 The infusion mentioned above will continues until end of examination, and the examination starts by collection of second sample of urine designated as "U1", at least 45-60 min. after initiation of infusion of inulin^{R} and PAH.

C.1.7 About 15 minutes after the "U1" sample, blood will be drawn from another cannula (sample B1). The sequence of urine and blood sampling is shown in the follcwing plot;



needle in vein no extra prick.

A total of 1 ml x 5 times = 5 ml of blood will be required for the purpose of this test. If a patient can not empty the bladder at exact times, or experience urgency before time, he should be allowed to empty his bladder on demand. In all instances the exact time of urine collection will be recorded. The urine samples will be frozen to -20°C until assay. .pa

C.1.8 The patients will be encouraged to drink ORS solution (for patients with diarrhoea) or water (for diarrhoea-free time) @ 20 ml/kg during the first hour (maximum 1200 ml) and thereafter 5 ml/kg every 30 minutes (maximum 300 ml) until the examination is over.

C.1.9 Determination of extracellular space by iohexol (Omnipaque^{R} 300 mg/ml, Nycomed, 0.1 ml/kg), and plasma volume by dye dilution technique using Evan's blue given intravenously (exact time noted) in a dose of 0.1ml/kg body weight and blood will be sampled after 15, 30, 90, 120 and 150 minutes in connection with blood samplings for inulin and PAH clearance (please see section C.1.7).

C.2 GFR and its determination

GFR is the amount of glomerular filtrate formed in each minute in all the nephrons of both the kidneys together, and can be estimated by clearance studies.

C.3 Plasma clearance

Clearance is a measure of effectiveness of the kidneys to remove substances from extracellular fluid (in ml/min.), and plasma clearance (PC) of a substance can be expressed as;

$$PC = \frac{\text{Quantity of urine (ml/min.)} \times \text{Concentration of substance in urine}}{\text{Concentration of substance in plasma}}$$

C.3.1 Inulin clearance

Plasma clearance can be measured by measuring inulin clearance. Inulin is a polysaccharide with a molecular weight of about 5,200. It is totally filtered through glomerular capillary wall like water, and neither reabsorbed, nor secreted by the tubules. Therefore, the concentration of inulin in glomerular filtrate is equivalent to that of plasma. Filtered inulin is carried into the urine, hence, all the glomerular filtrate formed is cleared of inulin, which is equal to glomerular filtration rate. For example;

If inulin concentration in plasma is 0.1 g/100 ml and 0.125 g of inulin passes in to the urine/min., then 0.125/0.1 = 1.25 100 ml portions of glomerular filtrate must have been formed, or 1.25 x 100 = 125 ml is the inulin clearance and GFR.

C.3.2 Para-Aminohippuric Acid (PAH) clearance as a measure of plasma flow

Like inulin, PAH is filtered through glomerular capillary wall, but is also secreted from the proximal tubular cells. The plasma PAH concentration should be kept low. PAH clearance can therefore, be used as a measure of effective renal plasma flow. For example;

If plasma concentration of PAH is 1 mg/100 ml and 5.85 mg of PAH passes in the urine every minute, then, using the formula mentioned under section C.3, 585 ml of plasma is cleared of PAH every minute. However, for more accuracy, the amount of PAH remaining in the plasma when it leaves the kidneys must be accounted. This fraction has been measured to be about 9% [15]. Therefore, a more accurate measure of plasma flow would be (585 x 100)/91 = 650 ml. This 91% extraction of PAH when it passes through the

kidneys is termed as "extraction ratio".

C.4 Total renal blood flow

Total blood flow can be measured from Inulin clearance or PAH clearance if the Hct% (packed cell volume) of the person is known, e.g. for PAH clearance of 685 ml/min. of a person with a Hct% of 45%, the renal blood flow will be (685 x 100)/55 = 1182 ml/min.

C.5 Calculation of filtration fraction from plasma clearance

Filtration fraction can be estimated from clearance of Inulin and PAH. The following informations are required to do that;

a. Effective renal plasma flow (clearance of PAH)

b. Glomerular filtration rate/min. (clearance of inulin).

Example: Effective renal plasma flow = 650 ml/min.

GFR = 125 ml/min.,

Filtration fraction (FF) = 125/650 = 19%.

C.6 Renal sodium and water handling

This will be studied during hydration in connection with the clearances of inulin and PAH (please see section C.1.5).

C.7 Renal concentrating ability

Will be studied following intranasal administration of desmopressin (vasopressin: Minitrin^{R}, Ferring). Desmopressin will not be given to the dehydrated patients, in whom the first spontaneously voided urine sample will be analyzed for osmolality, sodium, potassium, urea and creatinine.

A sample of spontaneously voided urine will be collected. Desmopressin (Minitrin^{R}) will be administered in a dose of 20 ug intranasally, and 2-3 urine samples will be obtained during the next 3-5 hours. Urine will be analyzed for osmolality, urea, creatinine, sodium, chloride, and potassium.

D. Body surface area: Body surface area (BSA) is measured according to the formula of Haycock *et al.* According to the formula;

$$BSA = Weight^{0.5378} \times Height^{0.3964} \times 0.024265$$

E. Determination of total body water

Total body water will be measured both by the deuterium dilution technique and measurement of bioelectrical impedance.

E.1 Deuterium dilution

Total body water can be estimated by deuterium oxide (^2H_2O) dilution [10,11]. For this study, natural abundance of the stable isotope in deionized water will be determined, and also the background abundance in body fluid will be determined in a sample of spontaneously voided urine. Then, the children will be given a dose of 0.08 g of deuterium oxide (99.9 atom %; Sigma Chemical Company Ltd., Poole, UK) per kilogram of body weight dissolving it in 20 ml of deionized water by mouth. The container will then be rinsed with 20 ml of water for complete wash-out and administered to the patients. Spontaneously voided urine will be collected during 4-5 hours following the dose. Isotopic enrichment of urine sample will be measured using isotope ratio mass spectrometer (such as type Aqua Sira, VG Isogas). Total body water (TBW) will be calculated as;

Deuterium space = 1.04 x TBW, or

TBW = Deuterium space/1.04

The above correction (1.04 x TBW) is required for exchange of deuterium label with non-aqueous hydrogen of body solids [12].

E.2 Bioelectrical Impedance

The measurement of total body water and fat free mass is through measurement of bioelectric impedance is a recently developed method which is non-invasive and can be implemented with little training. This method of measurement of TBW is based on the principle that bioelectric impedance (Z) to the flow of an applied current is related to the length and cross-sectional area of conductor when signal frequency and conductor configurations are constant[19,20] and the relationship can be shown by the following equation;

$$Z = p\ (L^2/Z)$$

where $Z = (R^2 + Xc^2)^{1/2}$

R = resistance

Xc = reactance

V = TBW volume

L = length of the conductor, and

p = volume resistivity in ohm-cm (a constant)

R and Xc are measured after introducing an insensible current through one pair of electrodes (I) attached to the skin and then reading the voltage drop between the pairs of detector electrodes (E). The technique has been used at the CRSC for over two years. For the measurements of impedance a body composition analyzer (BIA 101, RJL System, Detroit, MI) will be used.

F. Measurements of body fluid volumes

Volume in any compartment of body fluids can be estimated through the use of dilution principle (marker dilution technique). Following this principle, known amount of substance (marker) is allowed to disperse evenly in the field of interest and its concentration in the fluid is measured using the following equation;

$$\text{Volume in ml} = \frac{\text{Quantity of test substance instilled}}{\text{Concentration per ml of dispersed fluid}}$$

F.1 Measurements of plasma volume

This can be done using radioactive proteins such as I^{131}, or using a dye. For the purpose of this study, to avoid radioactive material, we propose to use the dye dilution technique using "Evans blue", also known as T-1824. Upon injection of this dye in a vein, a tight union is formed with proteins and the dye disperse throughout the circulatory system [21]. A dose of 0.1 ml/kg. body weight of Evans blue is injected intravenously and allowed to disperse for about 15 minutes. Two blood samples, 1.0 ml each will be drawn at 15 and 30 minutes in heparinized tubes, red cells separated by centrifugation to obtain plasma, and the concentration of the dye in plasma is determined by spectrophotometric analysis [21]. This dye leaks out through the capillary walls with the bound protein at a rate of about 5% per hour. Therefore, for a more precise estimation, two samples can be drawn at 15 and 30 minutes after injection of dye, and a semi-logarithmic plot can be constructed to determine its concentration at "0" time [21].

F.2 Measurement of blood volume

The blood volume can be measured if the plasma volume if the haematocrit is known according to the following equation;

$$\text{Blood volume} = \frac{\text{Plasma volume} \times 100}{100 - 0.87 \text{ haematocrit}}$$

F.3 Measurement of extracellular fluid volume

To do this, a substance must be used that readily disperse throughout the extracellular compartment and pass easily through the capillary membranes, but not through the cell membrane into the cells [21]. After allowing about half of an hour, a blood sample is drawn to estimate its concentration in plasma. A number of radioactive substances have been used, such as radioactive sodium and bromide etc. [21]. We do not intend to use radioactive materials for our study. Other substances that can be used are inulin, sucrose and iohexol. We propose to use iohexol for this purpose, in addition to inulin that will be used to determine plasma volume and glomerular filtration rate. This will enable us to determine "Inulin space" as well as "Iohexol space". It may be mentioned that because of the fact that different substances used for this purpose gives different estimates of the volume of the extracellular fluid compartment, the term such as "Inulin

space" is used to denote the methods used.

F.4 Measurement of interstitial fluid volume

Direct measurement of interstitial fluid volume is not possible at the present time, necessitating use of indirect methods. The method that can be used is to deduct plasma volume (PV) from the extracellular volume (ECV) to obtain interstitial fluid volume;

Interstitial fluid volume = ECV - PV

G. Determination of nutritional status

Nutritional status of the study patients will be determined by using Welcome classification system [22]. Additionally, anthropometric measurements such as weight, height, head circumference, left mid upper arm circumference (LMUAC); triceps and biceps skin-fold measurements, and degree of oedema through measurement of pitting depths (after application of firm pressure on dorsum of the foot for 15 seconds) will be made. Assessment of dehydration status, pulse rate, blood pressure (by doppler technique), liver size, skin and hair colour and changes will also be done.

H. Assessment of dehydration

Will be done following WHO classification system [23].

I. Haematological and blood biochemical assays

Will include determination of complete blood count (haematocrit, and total and differential blood counts); serum sodium, potassium, chloride and total carbon dioxide which are performed on routine basis for patients admitted in the inpatient wards of the CRSC. Additionally, for the purpose of this study, serum albumin, osmolality, magnesium, urea, creatinine, ASAT, ALAT, ALP, plasma renin, aldosterone and AVP will be determined. Estimation of these will involve a total of about 5.0 ml of blood. These tests will be performed on three times on every patients; on entry into the study, after recovery of their illness, and after nutritional rehabilitation.

J. Urine examination

Will include determinations of urine volume at specified intervals (please see flow chart; page-18), albumin, sodium, potassium, osmolality, magnesium, chloride, pH, urea, creatinine, aldosterone, and 24 hours urine volume and total urinary sodium. These tests will be repeated after correction of hyponatraemia in hyponatraemic patients and after resolution of illness in others, and also after nutritional rehabilitation with other tests. Additional tests that would be performed after desmopressin administration are, maximum urine osmolality, sodium, potassium, urea and creatinine.

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Abstract Summary

Hyponatraemia is a common complication of shigellosis. Children with milder hyponatraemia may develop altered mentation that may interfere with oral rehydration and feeding, and may also contribute to other complications such as pneumonia. Severe hyponatraemia may lead to convulsions with or without neurological sequelae, and even death. The exact mechanism for development of this complication is not well understood. A number of factors such as alteration of water homeostasis, early and unrecognized loss of sodium in stools, inappropriate secretion of antidiuretic hormone, actions of endotoxin and/or Shiga toxin on glomerular endothelium with altered glomerular filtration, and altered handling of sodium by kidneys may be involved in the pathogenesis of hyponatraemia in shigellosis. This study aims to study the role of some of these factors in the pathogenesis of hyponatraemia in shigellosis.

1. Children are the primary sufferers of this complication of shigellosis. Children in the age group 3-5 years has been selected for this study because of ease of conduction of this study where some cooperation is required from the patients, and also because of requirement of adequate amount of blood for different laboratory tests. Water homeostatis is likely to be different in children with different nutritional status which is the reason for selection of patients with different nutritional status.

2. Intravenous administration of inulin, para-aminohippuric acid, iohexol and Evans blue using infusion pumps, administration of deuterium orally, and intranasal administration desmopressin will be required for this study. The procedures of setting two venous cannulae are associated with some pain and discomfort, but not associated with significant risks. Pain will be minimized by avoiding multiple venipunctures and using indwelling intravenous cannulae. Drawing of 15 ml of blood on two occasions in a span of 4-5 days period is unlikely to pose significant physical risks to the children of this age.

3. Children with shigellosis and complications such as severe dehydration, respiratory infections, toxic colitis, haemolytic uraemic syndrome, suspected septicaemia etc. will not be enrolled into the study. Adequate aseptic precautions will be taken to minimize risk of infections, including use of disposable sterile equipments. Intranasal administration of desmopressin will be avoided in children with signs of dehydration to avoid possible renal complications related to administration of desmopressin.

4. Upon enrollment into the study, children will be assigned to a study number, and this number along with the hospital registration numbers will be used in the data extraction forms. All information will be kept in locked cabinets so that only the investigators will have access to the records.

5. Written informed consent will be required from either of the parents, or their legal guardians for enrollment into the study. The parents, or guardians will be approached for their permission following admission of the children in the general ward and after determining their suitability for the

study. Upon request, they will be provided with all available information of their children.

6. The parents will be interviewed to obtain history related to illness of their children. Typical interview takes about 10 minutes.

7. The study may reveal important information regarding pathogenesis of hyponatraemia in association with shigellosis which may play important role in their prevention or treatment benefiting the society. Individual benefit to the patients is not substantial. However, the tests performed on them may disclose previously unrecognized problems such as renal functional problems.

8. This study will use pertinent patient information from hospital records and body fluids such as blood, stool and urine.

BUDGET SECTION

A. 3100 Local Personnel

Name	Level	% time	Rate/month	Duration	Amount (US$)
Dr. M. A. Salam	ND	10%	1,400/m	3 months	420.00
Dr. Ali Miraj Khan	NB	20%	960/m	3 months	576.00
				Sub-total= US$	996.00

B. 3200 International Personnel

Name	% time	Duration	Amount
Dr. Hans Lindblad	100%	1 month	0*
Study Nurse (to be named)	100%	1 month	0*
		Sub-total = US$	0*

Travel costs: Sweden-Dhaka-Sweden for 2 persons: US$ 0*

C. 4809 Biochemistry tests

	Test	Cost/test	Amount (US$)
C1.	Serum albumin, electrolytes, osmolality, Mg+, urea, creatinine, ASAT, ALAT, ALP, protein	40.0	
	Cost for 3 tests/patient	120.0	
	Cost for 30 patients		3,600.00
C2.	Measurement of plasma conc. by Evans blue	5.0	
	Cost for 3 tests/patient	15.0	
	Cost for 30 patients		450.00

C3.	Cost for urine pH, osmolality, glucose, electrolyte, urea, creatinine, Mg, total protein	25.0		
	Cost for 3 tests/patient	75.0		
	Cost for 30 patients			2,250.00
			Sub-total= US$	6,300.00
D.	**4807 Pathology tests**			
D1.	Hct%, Total WBC, Differential	4.0		
	Cost for 3 tests/patient	12.0		
	Cost for 30 patients			360.00
			Subtotal US$	360.00
E.	**4813 Patient study**			
	30 patients x 3 days/pt = 90 days @ US$ 20/day			US$ 1,350.00
E.	**Tests to carried out abroad**			
E1.	Deuterium for 30 patients x 3 times/patient			US$ 5,000.00
E2.	Iohexol, PAH, Inulin			0*
E3.	Plasma renin, aldosterone			0*
			Subtotal US$	5,000
E4.	Supplies etc.			US$ 1,000

Total Direct Cost : US$ 14,106.00

Note: Costs marked with (*) will be supported by the Swedish group.

OK. Shaming Hou
01. Feb 1994

CONSENT FORM

Your child is suffering from bacillary dysentery caused by infection due to a germ called *Shigella*. This illness is an important cause of childhood death and malnutrition in the developing countries including Bangladesh, and is associated with many complications which are not usually seen in other types of diarrhoea. One such complication is hyponatraemia, a condition where there is decrease in the concentration of blood sodium. When severe, this can lead to convulsions and even death. In the milder form, this is associated with altered mentation which can interfere with feeding and may pose other management problems. The cause of this complication is not fully understood. We are conducting a study at this centre to understand why some children with bacillary dysentery develop this complications and other do not. A better understanding of the cause of this complication, we believe, may help development of policies for it's prevention and treatment.

We request your permission for participation of your child in this study. If you agree to the participation of your child in the study:

1. He will be required to be hospitalized until he recovers from his diseases (usually <5 days is required). During this period he will receive standard care of this hospital.

2. We will take him to the study ward for close observation and performing of special tests for this study where he will stay for about 26 hours. This will not interfere with his standard treatment.

3. In the study ward we will take medical history and perform thorough physical examination, including measurements of his height, and weight, left mid upper arm circumference, and blood pressure. His pulse, temperature and respiratory rates will be recorded every 6 hours.

4. Next, we will attach wires to his four limbs and connect those to an instrument to measure the amount of his body water. The procedure is painless and totally harmless.

5. Next, your child will be given 0.08g/kg body weight of deuterium oxide (a harmless substance) to drink with 20 ml of water, followed by another 20 ml of water after rinsing the container; urine passed by your child over the next 5 hours will be collected and stored.

6. Next, two cannulae (like a plastic needle) will be introduced into the veins on each of his elbows to avoid multiple pricks and they will be removed after the special tests are over within the next 8 hours. After introducing the cannulae, small amounts of blood will be drawn on 6 occasions, however, the total amount of blood drawn for special tests would be about 15.0 ml (appx.3 teaspoon). Blood samples would be tested to determine his total body water, extracellular water, plasma volume and to assess his kidney functions. His urine will be similarly collected throughout his stay in the study ward and small samples would be stored for different laboratory tests.

7. For the above mentioned tests small amounts of a mixture of inulin, para-aminohippuric acid and iohexol, and small amount of a dye- Evans blue, will be injected slowly into his vein using an infusion pump. Injection of these substances into his vein will not cause any harm. Evans blue, when injected too frequently has been reported to cause temporary discolouration of eyes in persons with fair skin. Your child will be offered ORS solution for the whole duration of carrying out the above mentioned studies.

8. Only if your child is found to be have no signs of dehydration, 20 microgram of desmopressin will be administered into his nostrils and urine will be collected during the next 4-5 hours to determine concentrating capacity of his kidneys.

9. On the following day your child will be taken back to the general ward where he will receive standard treatment for his illness until he recovers. He will again be taken to the study to repeat the same tests done on the first day. He will be discharged from the hospital the next day.

10. We would appreciate your bringing the child to the study ward where he will be kept for one day to repeat the tests as done before.

11. You are the one to decide participation of your child into this study. Your child will receive standard care and treatment of this hospital, even if you decide against participation of your child in this study.

12. All information obtained from your child will be kept confidential, and only the investigators of this study will have access to those information. Subject to availability of results, we would be happy to share them, if you so desire.

12. You may contribute to our understanding on the cause of hyponatraemia in association with acute bacillary dysentery through participation of your child in this study, and thereby contribute to the society through allowing participation of your child in this study.

If you agree to our proposal of participation of your child in this study please put your signature/left thumb impression below.

Thank you for your kind assistance.

Investigator's signature	Parent's/guardian's signature/LTI	Witness's signature
Date	Date	Date

INTERNATIONAL CENTRE FOR
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To : Chairman, RRC

Date: February 01, 1994

Thorugh : Associate Director, CSD

From : Dr. Mohammed Abdus Salam
Principal investigator,
Exploratory study on salt and water homeostasis, and renal function in children with and without hyponatraemia in association with shigellosis.

Subject : **Submission of Research Protocol for Review by the RRC, and Response to External Reviewers' Comments**

I am submitting the above mentioned research protocol for review by the RRC.

The protocol has been developed in collaboration with a Swedish group of scientists and has been reviewed by two external reviewers (comments enclosed). Both the reviewers have been supportive of the study; one of them supported the study without qualification. The second reviewer had two specific suggestions. His suggestions and our response to those are as follows:

1. Incorporation of a group of children with shigellosis but without marasmic kwashiorkor as controls

   We have accepted the suggestion and made appropriate changes in the current version of the protocol.

2. Determination of urine and plasma osmolality, and urine glucose and amino acids, before and after control of hyponatraemia; 24 hours urinary excretion of sodium; and total body sodium.

   We have incorporated suggested tests in the protocol, except determination of total body sodium. It could be interesting to estimate total body sodium, however, the procedure involves administration of radioisotopes which we do not intend to use for our study.

I hope that the RRC will find the response as appropriate, and would consider the protocol for approval.

Thank you.

Title: Exploration study of Pathogenesis of Hyponatraemia, and Renal Functions in Shigellosis

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	X		
Adequacy of Project Design	X		
Suitability of Methodology	X		
Feasibility within time period		X	
Appropriateness of budget	-	-	-
Potential value of field of knowledge	X		

CONCLUSIONS

I support the application:

a) without qualification ☒

b) with qualification

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

- the application ☐

The planned study promises significant progress in some unsolved scientific questions and will certainly help to improve practical health care in Bangladesh.

The objectives and the underlying hypotheses are convincing. The methods of procedure are adequate to answer the underlying questions.

I consider this project as a model for international and cooperative research on diarrhoeal disease.

Title: Exploratory study on salt and water homeostasis and renal function in children with and without hyponatraemia in association with shigellosis

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 ☐

November 24, 1993

Associate Director
Clinical Sciences Division
ICDDR,B

Detailed comments

please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project,its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications(scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

Title :"Exploratory study on salt and water homostasis and renal function with and without hyponatraemia in association with shigellosis".

Reviewer:The study subjects are marasmic/kwashioker children 10 in group I and 6 in other 3 groups. I suggest inclusion control i.e.children without marasmic kwashioker but having shigellosis and hyponatraemia as control.

In methodology all children(study subjects)should have urine and plasma osmolality done before and after correction of hyponatraemia which will give an idea about the type of hyponatraemia,urine should be examined for glucose and aminoacids and also 24 hours urinary Na+ before and correcti
If possible total body sodium estimation should also be included in the study subjects.

Remarks:I support the study as it will add some new knowledge on the salt and water metabolism in this particular situation in children.

PROCEDURE FOR MAINTAINING CONFIDENTIALITY

Questionnaires will be provided with a code which matches a master sheet where the name of the interviewee and location of the house are noted. Only the principal investigators and the field supervisor will have access to this sheet.
After each interview, the name and code number of the interviewee will be checked by the field supervisor, then the name of the interviewee will be crossed out of the questionnaire and all data entry will use the code for identification.

All the interviewers will be female; they will be counselled on the sensitive nature of some of the information to be collected and on the need for confidentiality.