ttached: 1.

Date March 23, 1988

ETHICAL REVIEW COMMITTEE, ICDDR,B.

rincipal Investigator Dr FPL Van Loon

Trainee Investigator (if any) _____

pplication No. 88-010

Supporting Agency (if Non-ICDDR,B) _____

itle of Study "Evaluation of the effect f alanine plus ~~glucose and glutamine plus~~ ucose on salt and water absorption in e jejunum in acute cholera in adults:

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

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

Source of Population:
(a) Ill subjects — (Yes) No
(b) Non-ill subjects — Yes (No)
(c) Minors or persons under guardianship — Yes (No)

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)

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

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.

(PTO)

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

Principal Investigator

Trainee

SECTION I

1. Title — Evaluation of the effect of alanine plus glucose and glutamine plus glucose on salt and water absorption in the jejunum in acute cholera in adults

2. Principal Investigators — Dr FPL van Loon
Dr FC Patra
Co investigator — Mr WA Wahed

3. Starting date — May 1988

4. Completion date — May 1989

5. Total Direct costs — 31,037 US $
Source of funding — WHO

6. Scientific Division — This protocol has been approved by the Head of the Clinical Sciences Division

Signature: Khaldah's.

Date: 10.3.88

7. Abstract Summary

However effective the current formulas of oral rehydration may be in tackling watery diarrhoeal diseases, still a need is felt for further improvement. As an alternative or adjunct to glucose as the carrier for sodium absorption into the jejunal cells, amino acids have been identified, alanine and glutamine in particular.

In a small intestinal perfusion procedure on forty adults with acute cholera - the prototype of watery diarrhoea -, alanine cum glucose electrolyte solution and glutamine cum glucose electrolyte solution will be compared in their salt absorption promotive capacities with the standard oral glucose electrolyte solution while patients serving as their own control.

8. Reviews

(a) Ethical Review Committee.....

(b) Research Review Committee.....

(c) Director ICDDRB.....

SECTION II - RESEARCH PLAN

Objectives

a. The absorption promoting effect (in terms of salt and water) of alanine and glutamine in acute cholera patients

b. To compare this effect of alanine and glutamine, if any, with that of glucose

Background

Oral rehydration solution (ORS) is the mainstay in the treatment of watery diarrhoea. The concentration of glucose in the standard ORS composition is limited to the 2 % level beyond which it would induce osmotic diarrhoea. On the other hand, enhancing the proportion of salt in the solution would easily lead to hypernatraemia with clinically detrimental effects. In order to nevertheless improve the efficacy of ORS one can look for other vehicles of sodium transport, especially amino acids.

The amino acid alanine has been recognised as one of the potential promotors of sodium transport (1). In a just completed clinical trial at the International Centre for Diarrhoeal Disease Research, Bangladesh a purge rate reduction in acute cholera patients has been demonstrated when alanine in combination with glucose was used as a substrate for the oral rehydration solution (2). Animal studies indicate that the amino acid glutamine may induce an even better absorption of water and sodium than has been claimed for alanine (*' We found that glutamine unlike glucose (..) stimulates piglet jejunal Na and Cl absorption. (..) We now believe that this mechanism involves the metabolic stimulation of intestinal cells, which seems reasonable, as glutamine is their primary metabolic fuel. The other*

promising aspect of glutamine is that it might promote epithelial repair. It has also been shown to prevent gut atrophy associated with chronic total parenteral nutrition in rats ' [J.Marc Rhoads, personal communication, February 19 1988 ; see also appendix I]).

We propose to conduct a segmental jejunal perfusion study on adult cholera patients to investigate what impact alanine and glutamine may have on salt, water and glucose absorption in the jejunum itself where the cholera diarrhoea originates, realizing that the small intestine is the primary site of glucose and amino acid absorption into the blood (3).

In general, small intestinal absorption is known to occur by passive diffusion, facilitated diffusion through parallel or cellular routes, and also by active transport. The jejunal brush border membrane is unique in containing, besides the Na-coupled concentration step for active glucose transport, several specific Na-dependent transport systems for neutral amino acids; alanine and glutamine are likely to share such a Na-dependent carrier for their transport (4,5). Thus the jejunum plays a pivotal role in the interorgan exchange (between muscle, liver and kidney) of glutamine as the major constituent of dietary and endogenous proteins and peptides(6): its mucosa uses glutamine as a respiratory fuel for itself -- equally metabolizing it whether it enters the enterocyte from the luminal side or from the arterial blood across the basolateral cell membrane(7) -- and delivers the majority of the nitrogen as alanine, glutamate, citrulline, proline and ammonia (8); as for the latter, the gut rather than the intestinal flora appears to account for most of the overall ammonia production (9). A non-essential neutral aminoacid, glutamine can be synthesized by virtually all tissues in the body, but its major metabolizing

enzyme, phosphate-dependent glutaminase, is present at the highest levels in the mucosal cells of the jejunum (10). Glutamine is glucogenic and unlike most amino acids has two amine moieties, an alpha-group and an amide group. This characteristic underlies its importance as an nitrogen transporter and a carrier of ammonia from the periphery to the visceral organs (11-14) : together with alanine, it transports more than half of the circulating nitrogen(15-16).

Glutamine is also the precursor of amino sugars and several nucleotides such as ATP, purines and pyrimidines (17). It is avidly consumed in rapidly replicating cells, not only intestinal epithelial cells, but also fibroblasts, lymphocytes, tumor cells and in states of stress and catabolism (18-24). This is of importance in patients with large wounds, inflammation associated with infection, and/or gastrointestinal dysfunction which precludes enteral feeding. Plasma glutamine can even serve as a clinical index of disease severity in some illnesses (25).

Following critical illness such as operation and bacteraemia, or glucocorticoid treatment the enteral glutamine demand rises markedly, thus providing a biochemical rationale, at least in part, for the use of enteral as opposed to parenteral feeding whenever possible (6,15). In case of prolonged starvation intestinal glutamine uptake remains constant while renal utilisation gradually increases, the kidney becoming an important gluconeogenic organ (26,27). Similarly during acidosis glutamine requirements of the kidneys are accelerated since glutamine is the key participant in renal ammoniagenesis (28). Glutamine is then released by the liver in response of low circulating levels. As a result of its antiketogenic and antilipolytic effect glutamine plays a paramount role in regulating substrate and energy metabolism in vivo (29). Finally

of more than 50 g/day have been tolerated in humans in months to years without manifestations of any toxicity (15). During our perfusion procedure only 4.95 g of glutamine will be used (this is based on L-glutamine's molecular weight being 146.5, its concentration 45 mmol/L and its perfusion time of 75 min). Similarly alanine in the amount used, namely 3.0 g, should not give rise to toxicity either (its molecular weight being 89.09, its concentration 45 mmol/L and its perfusion time 75 min) since comparable quantities in our previous study did not cause any adverse reactions(2).

3. Rationale

Glucose electrolyte solutions have been established as the primary treatment for watery diarrhoeal diseases. In this proposal the additive effect of including the amino acids alanine or glutamine to these solutions on small intestinal sodium and water absorption will be studied in acute cholera, in order to come to a further improvement of existing glucose salt formulas.

B. SPECIFIC AIMS

In a small intestinal perfusion in forty adults with acute cholera we propose to study what the promotive effect on salt and water absorption may be of adding the amino acids alanine or glutamine to a glucose salt solution of defined composition as compared to the standard glucose salt solution.

C. METHODS OF PROCEDURE

Experimental design and methodology

Subjects:

On admission at the Treatment Centre of The International Centre for Diarrhoeal Disease Research, Bangladesh forty male acute cholera patients (diagnosis assessed on stool darkfield examination, to be confirmed by culture) over 16 years with a purging rate of more than 5 ml/ kg.hour during a 4 hours period will be invited to participate in the study after giving their written informed consent.

Subject preparation:

Initial severe dehydration will be corrected by intravenous administration of the Dhaka solution (containing Na 133 mmol/L, K 13 mmol/L, Cl 98 mmol/L, Acetate 48 mmol/L) at a rate of 100 ml/kg in 4 hours. Over this period ongoing losses of stool and vomit will be hourly measured and replaced for by infusing equal amounts of intravenous Dhaka solution.

After the patient is completely rehydrated the perfusion will commence. During the perfusion the above intravenous infusion will be kept and ongoing losses of stool and urine will be replaced for during the following hour.

Tetracycline (500 mg every 6 hours) will be given after the perfusion is over (30).

Steady state segmental intestinal perfusion:

After complete rehydration the subjects are to swallow a quadruple lumen radiopaque polyvinyl tube with a mercury bulb at the tip. Immediately thereafter the patient will be asked to lie down in right lateral position. Subsequently the tube's position will from time to time be checked under fluoroscopy. Once the infusion port reaches the ligament of Treitz the tube will be fixed by means of adhesive tape to the patient's nose. Thus the tube will have its infusion port located at the ligament of Treitz, the proximal and the distal collection site 30 cm and 60 cm downwards beyond the infusion port allowing for a 30 cm mixing segment and a 30 cm study segment subsequently (31,32) ; the extra tube will have its tip in the antrum for intermittent manual gastric suction.

The perfusion fluid will be infused at a rate of 10 ml/min, i.e. 600 ml per hour and 3.6 liter in 6 hours. Even if 50 % of the infused fluid would be absorbed the quantity of the absorbed fluid will be 1-2 liter over a period of 6 hours which might just be sufficient to compensate for the stool output during that period. Since we will be including patients with a baseline purging rate of 5 ml/kg.hour or more and assuming that an average Bangladeshi adult weighs around 40 kg, we expect that the patient will pass around 200 ml of stool every hour, i.e. 1200 ml over a period of 6 hours. If 50 % of the infused fluid will be absorbed then the rest 50 % or 2-3 liter will come out in the stool. That means that the actual stool output during the perfusion period will be 1200 ml plus 2-3 liter or 3-4 liter in 6 hours or 500 ml every hour. We plan to measure the stool output every hour during the perfusion period. If the stool output exceeds 500 ml per hour then the excess amount of stool will be replaced by intravenous Dhaka solution during the following hour.

Each patient will be perfused with three solutions in sequence. BSP will be used as the non-absorbable marker, dissolved in either of the solutions in a concentration of 750 mg/L.

The composition of the three perfusion solutions is as follows :

-- glucose electrolyte solution (A)

Na	100	mmol / L
K	5	mmol / L
Cl	75	mmol / L
HCO3	30	mmol / L
glucose	90	mmol / L

-- alanine & glucose electrolyte solution (B)

Na	100	mmol / L
K	5	mmol / L
Cl	75	mmol / L
HCO3	30	mmol / L
glucose	45	mmol / L
alanine	45	mmol / L

-- glutamine & glucose electrolyte solution (C)

Na	100	mmol / L
K	5	mmol / L
Cl	75	mmol / L
HCO3	30	mmol / L
glucose	45	mmol / L
glutamine	45	mmol / L

In the course of the perfusion the glucose electrolyte(A) solution will be used, kept as the control in between the experimental alanine-cum-glucose electrolyte(B) and glutamine-cum-glucose electrolyte(C) solutions which are randomly chosen to either proceed or follow the glucose electrolyte solution.

After a 45 min equilibration period during which the first of the experimental solutions is perfused, the perfusion time for each of the three solutions is 75 min. During the perfusion of each solution a 10 ml sample is taken every 10 min from both the proximal and the distal collection port at a rate of 1.5 ml/min. Samples from the proximal port are taken 15 min prior to the distal one (33). After the last sample from the distal port has been taken during perfusion of each solution, a 30 min wash out interval is observed during which the subsequent solution is being perfused. This adds up to a 6 hour perfusion time in total for each patient (see appendix II).

Preparations of salt mixtures:

The glucose electrolytes solution, and the two glucose cum alanine and glucose cum glutamine electrolyte solutions will be prepared as listed above in the ICDDRB laboratory just before the study begins, the osmolality being kept at 300 mOsm/L (isotonic solution): Na 100 mmol/L, K 5 mmol/L, Cl 75 mmol/L, bicarbonate 30 mmol/L, and either glucose plus alanine, or glucose plus glutamine 90 mmol/L -- 45 mmol/L of each. The control solution will contain 90 mmol/L of glucose. In order to reduce the difference in concentration of electrolytes between perfusion solution and plasma we have increased the sodium concentration to 100 mmol/L and decreased the potassium concentration to 5 mmol/L. For the same reason we have decreased the substrate concentration to 90 mmol/L.

Sample Analysis

Electrolytes will be measured by ionselective electrodes. Serum osmolality will be determined by the freezing point depression method. Specific gravity will measured by refractometry, BSP by the colorimetric method, amino acids by the colorimetric method, glucose by the glucose oxidase method, and nitrogen by the micro-Kjeldahlmethod.

Analysis of samples, calculations, and statistics:

After measuring BSP and electrolytes concentrations of each sample, net water and electrolyte absorption and flow rates at the proximal and distal collecting sites will be calculated with nonabsorbable marker equations (33-35). We will be using the following formulae :

$$Fe = I \times \frac{[BSP]i}{[BSP]p} - 0.5$$

$$Fi = Fe \times \frac{[BSP]p}{[BSP]d}$$

$$\text{delta } H_2O = \frac{Fl - Fe}{30} \times 60$$

$$\text{delta Na} = \frac{[\text{ Fl Na }]\,d - [\text{ Fe Na }]\,p}{30} \times 60 \times 1000$$

in which Fe = flow entering study segment (ml/min), Fl = flow rate leaving study segment (ml/min), I = infusion rate (ml/min), BSPi,p,d = BSP concentration in the infusion solution and at P and D respectively (mg/100 ml), and deltaH_2O = net transmucosal fluid transport rate (ml/hour.cm) Positive values indicate net secretion and negative values, net absorption of fluid. Secretion means net gain of fluid within the intestinal lumen ; its use provides no indication whether this is an active or passive process. Delta Na, K, Cl, and HCO3 = sodium, potassium, chloride, and bicarbonate net transmucosal transport rates (umol/hour.cm).

The primary aim will be just to compare either experimental solution with the control. We randomise the sequence of the experimental solutions to precede or to follow the control solution so that any difference between the solutions over the 5 hour study period should not be ascribed to the natural course of the disease. Appropriate parametric and non-parametric statistical tests will be applied.

Results:

Using the segmental small intestinal perfusion technique we hope to demonstrate the advantage, if any, of partly replacing glucose by glutamine over alanine in potentiating ORS absorption in acute cholera. Taking the glucose absorption in WHO ORS (solution A) for 100 %, we may expect an increased absorption rate for the glutamine containing solution(B) of 100-120 % ; the absorption rate of the alanine containing solution(C) is then expected to be 70 % (36).

We have used the following formula for sample size calculation (37):

$$n = 2 \times \frac{(\text{standard deviation})^2}{(\text{worthwile difference})^2} \times 7.9$$

at a 0.05 significance level / 80 % power.

Using Fordtran's perfusion study data on glucose containing electrolyte solutions (38) -- net mean water absorption of 97 ml/h/20 cm with a standard deviation of 27 -- and taking a worthwile decrease of 20 % i.e. 19.4 ml, this results in a sample size of 31 patients. Taking into account possible non-compliance of patients, procedure failures, or negative cultures in some cases we plan to include 40 patients.

D. SIGNIFICANCE

see under Rationale

E. FACILITIES REQUIRED

1. Office space : the present commodities will do
2. Laboratory space : the existing space is sufficient
3. Hospital beds : the current study ward is suitable.
4. Rent, Communication and Utilities : the present facilities are sufficient.

References:

1. Rhoads JM, MacLeod RJ, and Hamilton JR. Alanine enhances jejunal sodium absorption in the presence of glucose: studies in piglet viral diarrhoea. Ped Res 1986;20(9):879-83

2. Patra FC, Sack DA, Islam A et al. Oral rehydration therapy with alanine - glucose ORS : A controlled clinical trial (Manuscript in preparation)

3. Stevens RC, Kaunitz JD, Wright EM. Intestinal transport of amino acids and sugars: advances using membrane vesicles Am Rev Physiol 1984;46:417-33

4. Crane RK, Miller D, Bihler I. The instructions on possible mechanisms of intestinal active transport of sugars. In: Membrane Transport and Metabolism, 1961; pp 439-449. Ed A Kleinzeller & A Kotyk. Academic Press London

5. Bradford NM, McGivan JD. The transport of alanine and glutamine into isolated rat intestinal epithelial cells. Bioch Bioph Acta 1982;689:55-62

6. Souba WW, Roughneen PT, Goldwater DL et al. Postoperative alterations in interorgan glutamine exchange in enterectomized dogs. J Surg Res 1987;42:117-25

7. Hanson PJ, Parsons DS. Metabolism and transport of glutamine and glucose in vascularly perfused small intestine rat. Biochem J 1977;166:509-19

8. Soeters PB, Van Leeuwen PAM. Ammoniak- und Glutaminstoffwechsel des Darms. Infusionstherapy 1986;13:186-90

9. Windmueller HG Glutamine utilization by the small intestine. Adv Enzymol 1982;53:202

11. Marliss EB, Aoki TT, Pozefsky T, et al: Muscle and splachnic glutamine and glutamate metabolism in postabsorptive and starved man. J Clin Invest 1971;50:814

12. Muhlbacher F, Kapadia CR, Colpoys MF, et al: Effects of glucocorticoids on glutamine metabolism in skeletal muscle. Am J Physiol 1984;247:E75

13. Souba WW: Glutamine metabolism in catabolic states: Role of the intestinal tract. Thesis, Harvard School of Public Health, Dep of Nutritional Biochemistry. June 1984

14. Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. J Biol Chem 1980;255:107

15. Ruderman N. Muscle amino acid metabolism and gluconeogenesis. Ann Rev Med 1975;26:245

16. Roediger WEW. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 1982;83:424

17. Martin DW. Metabolism of purine and pyrimidine nucleotides. In Harper's Review of Biochemistry, Martin DW, Mayes PA, Rodwell VW(eds). Lange Book series, New York, 1980 p331

18. Windmueller HG, Spaeth AE. Identification of ketone bodies and glutamine as major respiratory fuels in vivo in postabsorptive rat small intestine. J Biol Chem 1978;253:69-76

19. Cahill GF, Aoki TT. Renal gluconeogenesis and amino acid metabolism in man. Med Clin North Am 1975;59:751.

20. Kapadia CR, Muehbacher F, Smith RJ et al. Alterations in glutamine metabolism in response to operative stress and food deprivation. Surg Forum 1982;33:19

24. Felig P, Wahren J, Raf I. Evidence of interorgan amino acid transport by blood cells in humans. Proc Natl Acad Sci USA 1973;70:1775

25. Glassman M, Yudkoff M, Nissim I et al. Plasma glutamine correlates with growth retardation in Crohn's disease. Ped Res 1983;17(supp):614

26. Cahill F. Starvation in man. N Engl J Med 1970;282:668

27. Felig P, Owen OE, Wahren J et al. Amino acid metabolism during prolonged starvation J Clin Invest 1969;48:584.

28. Owen OE, Robinson RR. Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration J Clin Invest 1963;42:263

29. Phromphet-Charat V, Jackson A, Dass PD, Welbourne TC Ammonia partitioning between glutamine and urea. Interorgan participation in metabolic acidosis. Kidney Int 1981;20:598-605.

30. Greenough WB, Gordon RS, Rosenberg IS, Davies BI, Benenson AS. Tetracycline in the treatment of cholera. Lancet 1964;1:355-7

31. Fordtran JS. Marker perfusion techniques for measuring intestinal absorption in man. Gastroenterology 1966; 51:1089-93

32. Fordtran JS, Rector FS, Carter NW. The mechanisms of sodium absorption in the human small intestine. J Clin Invest 1968;47:884-900

33. Banwell JG, Pierce NF, Mitra RC, et al. Intestinal fluid and electrolyte transport in human cholera. J Clin Invest 1970;49:183-95

34. Dillard RL, Eastman H, Fordtran JS. Volume flow relationship during the transport of fluid through the human small intestine. Gastroenterology 1965;49:58-66

35. Fordtran JS, Rector FC, Ewton MF, et al. Permeability characteristics of the human small intestine. J Clin Invest 1965;66:1326-33

36. Hellier MD, Thirumalai C, Holdswooth CD. The effect of aminoacid and dipeptides on sodium and water absorption in man. Gut 1973;14:41-5.

37. WHO/CDD/CMT/87.2:6

38. Fordtran JS. Stimulation of active and passive sodium absorption by sugars in human jejunum. J Clin Invest 1975;75:728-37

Budget (all figures in US $)

Investigators/ Personnel	% time		Salary/costs
FPL van Loon	70		---
FC Patra	10		---
MA Wahed	20	NOB 6	1820
Clinical Assistant	100	NOD	7500
Clerk	10	GS 6	630
Coding assistent	10	GS 3	265
Hospitalisation	30/p x 3 d x 40 pts		3600
Microbiology of stool samples			
Dark field	1.85 x 40		74
Culture for *Vibrio cholerae*	3.50 x 40		140
Culture for *E.coli*	10 x 2.75		28
Biochemistry :			
1. Patients' blood samples :			
Electrolytes (Na,K,Cl,Bicarbonate)	4.40		
Ht	1.60		
Specific gravity	1.10		
	40 x 7.10		284
2. ORS preparations :			
Electrolytes (Na,K,Cl)	4.40		
Osmolality, specific gravity	2.00		
Glucose	2.75		
Nitrogens	12.00		
	40 x 21.15		846

3. Aspirates (in duplicate)

both from distal and from proximal ports and at the beginning and the end of the perfusion (26 samples pro patient) :

Electrolytes(id)	4.40 x 26 x 40	4576
BSP (in triplicate)	3.00 x 26 x 40	3120

Supplies

Office supplies		300
Glucose (a,D glucose [dextrose]) Aldrich 15,896-2	6 kg	29
L-alanine Aldrich A 2680-2	2.5 kg	625
L-glutamine Aldrich G 320-2	3.5 kg	400
Battery backed peristaltic pumps (2)		6000
Data analysis		500
Xerox		300
Total costs		31037

সম্মতি-পত্র

মানুষের প্রচলিত মুখে খাওয়ার স্যালাইন, পাতলা পায়খানার চিকিৎসার জন্য কার্যকরী প্রমাণিত হয়েছে, তবুও আরো উন্নতমানের খাওয়ার স্যালাইন আবিষ্কারের জন্য চেষ্টা চালানো উচিৎ। আপনি কলেরা রোগে আক্রান্ত হয়েছেন এবং আমরা আপনাকে এই গবেষনায় অংশ গ্রহন করতে অনুরোধ করবো। এই গবেষনায় প্রচলিত মুখে খাওয়ার স্যালাইন এবং অম্লমেহর সারাংশ অ্যালানিন এবং গ্লুটামিন স্যালাইন কোনটা বেশী কার্যকরী, পরীক্ষা করে জানা যাবে। অন্যান্য প্রাণীতে গবেষনা করে দেখা গিয়েছে, অম্লমেহ প্রসিড স্যালাইন ক্ষুদ্রান্ত্র থেকে পানি ও লবন জাতীয় পদার্থ আরো কার্যকরীভাবে শোষন করতে পারে।

যদি আপনি রাজী থাকেন তাহলে আপনাকে চারটি সংযুক্ত নল মুখে প্রবেশ করিয়ে (নল দেখানো হবে) ক্ষুদ্রান্ত্রের দ্বিতীয় অংশে রাখা হবে। এক্সরে করে নলের অবস্থান ঠিক থাকার পর, তিন ধরনের স্যালাইন নলে প্রবেশ করিয়ে পরীক্ষা করা হবে। এই পরীক্ষা ৬ ঘন্টা ধরে করা হবে এবং পাকস্থলী ও ক্ষুদ্রান্ত্র থেকে তরল পদার্থ পরীক্ষার জন্য নেয়া নেয়া হবে। প্রাথমিক অবস্থায় রোগীর হাত থেকে ৫ মিঃলিঃ রক্ত নেয়া হবে পরীক্ষার জন্য. এবং সুস্থ রোগীকে সর্বক্ষণ কলেরা স্যালাইন দিয়ে চিকিৎসা করা হবে। এবং গবেষনা শেষ হওয়ার পর প্রয়োজনীয় এন্টিবায়োটিক দিয়ে চিকিৎসা করা হবে।

আপনি যদি রাজী থাকেন তাহলে নিচে নাম সহি অথবা আঙ্গুলের ছাপ দিন। আপনি যদি গবেষনায় অংশ গ্রহন নাও করেন অথবা যে কোন সময় গবেষনা থেকে প্রত্যাহার করেন তবুও আপনাকে যথাযথ চিকিৎসা করা হবে।

গবেষকের স্বাক্ষর

রোগী সহি / স্বাক্ষর

তারিখ : ——

The effect of alanine and glutamine on salt and water absorption in cholera
Consent form (translation of the Bangla original)

Although you may be familiar with the standard salt sugar solution that is currently advocated for the treatment of watery diarroea still a need is felt trying to develop more effective formulas. Since you are suffering from cholera -- the prototype of watery diarrhoeal diseases --, we are inviting you to participate in a study in which the absorption of the standard sugar salt ORS is compared with two other ORS formulas where the sugar component is partly replaced by amino acids -- the constituants of proteins -- namely alanine and glutamine consecutively. Animal studies have confirmed the promoting effect of these amino acids on absorption of salt solutions.

If you agree the following procedure will be performed: A quadruple tube will be let down to the second part of your small intestine *[tube is shown]*. After checking the tube's position under fluoroscopy the three solutions will consecutively be perfused during a 6 hour period and both gastric juice and small intestinal fluid aspirated. After an initial 5ml blood sample, continuous rehydration will take place by intravenous administration of the Dhaka solution especially designed for cholera. Once the procedure is over you will get the usual antibiotics for cholera treatment(tetracycline).

Please sign or indicate by thumb print below if this explanation is clear to you and you are willing to participate. You should realize that you are free to withdraw from the study at any time without compromising the medical care you need.

Date .._/.._/.. ....

Signature/thumbprint patient/legal guardian Investigator

SUMMARY FOR THE ETHICAL REVIEW COMMITTEE

1. Although glucose salt solutions have been established as the primary treatment for watery diarrhoea, still a need is felt for a further improvement of the existing formulas.

Enhancing the glucose component of the current glucose salt solution however, is bound to lead to osmotic diarrhoea ; increasing the salt content leads to hypernatraemia.

Besides glucose also some amino acids - the components of proteins - have been identified as carriers for sodium and water influx into the gut cells. Both in animals and in clinical studies in humans (such as a just completed one at the ICDDRB) they could account for a considerable reduction in purge rate. Besides an effect on salt and water absorption the pivotal role of these amino acids appears from the fact that glutamine in particular acts as a nutritient and fuel for gut cells. This is even more the case under circumstances such as stress, severe illness, starvation, acidosis and malnourishment in which the uptake of glutamine counteracts the breakdown of the glucose/glucogen stores in the liver and the protein stores of the muscles, and supports the kidney in regulating the acid-base balance. In addition it is preeminently the jejunum -- the very site of the cholera toxin attachment -- where glutamine is absorbed and metabolized.

As a follow up on the previous ICDDRB study, we propose to investigate in a physiological design the exact effect on sodium and water absorption of adding the amino acids alanine or glutamine to a glucose salt solution of defined composition, as compared to the standard glucose salt solution.

Since cholera is the prototype of any watery diarrhoeal illness, adult acute cholera patients are invited to participate, in such a setting that they serve as their own control.

2. The introduction of a polyvenyl quadruple lumen tube with a mercury bulb on its tip till beyond the ligament of Treitz does not bear any substantial risk. No complications were previously encountered in similar studies in the Centre. Small intestinal perfusion with a non-absorbable marker like sodium sulphaphthaleine (BSP) is a well established device without any risk. As constituents of protein, both the amino acids alanine and glutamine form part of daily food requirements ; no toxicity has been described in doses even of a multifold higher than administered here.

3. In the procedure selected - the small intestinal segmental perfusion - both principal investigators are experienced.

4. Data collection will be handled confidentially. Only patient identification numbers will be used during analysis of the results.

5. Informed signed consent will be obtained from all patients.

6. Interview will be taken in the hospital to obtain clinical history.

7. The use of hospital records, jejunal fluids and a 5 ml venous blood sample will be required.

8. Continuous rehydration will be provided by intravenous administration of the Dhaka solution ; after the six hour procedure the patients will additionally be offered the usual antibiotic treatment of tetracycline (500 mg every 6 hours).

9. Results of this study may help to modify the future approach to oral rehydration and establish a more prominent role for aminoacids in it.

ABSTRACT FORM FOR: AMERICAN GASTROENTEROLOGICAL ASSOCIATION
ew Orleans, Louisiana AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES
lay 15-18, 1988 GASTROENTEROLOGY RESEARCH GROUP

Appendix - 1

(For office use)

ype name, address, and telephone number of author who should receive
rrespondence in Box G and complete boxes A through F.

ABSTRACT RECEIPT DEADLINE: DECEMBER 18, 1987

TYPE ABSTRACT BELOW. BE SURE TO STAY WITHIN BORDER

L-GLUTAMINE AND L-PHENYLALANINE ENHANCE JEJUNAL ION TRANSPORT MORE THAN D-GLUCOSE IN NEWBORN FORMULA-FED PIGS

M Rhoads, E Keku, J Lecce. Dept of Pediatrics Univ of North Carolina at Chapel Hill NC, Core Center in Diarrheal Diseases & NC State Univ Dept of Animal Science Raleigh NC.

Determining the effects of amino acids on intestinal ion transport in newborn animals may facilitate formulation of improved oral rehydration solutions (ORS) for infant diarrhea. We studied effects of glutamine (L-gln), the primary fuel of the small bowel; phenylalanine (L-phe); and glucose (D-glc), in Ussing chambered jejunum from pigs 3d-3wks old. We measured fluxes under short-circuit conditions during a basal 40 min period with 10 mM glucose as fuel in serosal buffer, with 10 mM mannitol mucosally. We then added 30 mM amino acid or glucose mucosally, 30 mM mannitol serosally, and measured fluxes for 60 min.

Results: As animals matured, mucosal-to-serosal Na flux responses (ΔJ_{net}^{Na}) to D-glc and to L-gln increased ($p<0.01$); jejunal villus height increased ($p<0.01$); and specific activities of sucrase ($p<0.001$) and Na,K-ATPase ($p<0.001$) increased. L-gln, L-phe, and D-glc stimulated net Na flux and short-circuit current above basal values. L-gln enhanced net Cl absorption (Table).

	N	ΔJ_{net}^{Na} ($\mu Eq.cm^{-2}.h^{-1}$)	ΔJ_{net}^{Cl} ($\mu Eq.cm^{-2}.h^{-1}$)	ΔIsc ($\mu Eq.cm^{-2}.h^{-1}$)	
L-gln	11	2.70±0.49*	1.27±0.50*	1.17±0.32*	*p<0.01
L-phe	5	3.92±0.95+	0.09±0.58	2.91±0.76+	+p<0.05
D-glc	10	1.36±0.26*	0.47±0.49	2.32±0.43*	

Increments in J_{net}^{Na} after L-gln and L-phe exceeded increments after D-glc in age-matched pigs ($P<0.05$). Replacement of buffer Na with choline eliminated the Cl-absorptive response to L-gln. J_{net}^{Cl} also increased after we added 30 mM L-gln serosally, balanced by 30 mM mannitol mucosally ($p<0.01$).

Conclusions: (1) Jejunal Na transport response to amino acid increases in concert with recognized increases in mucosal enzyme activities and villus height during development. (2) L-gln enhances electrogenic Na transport; and it also increases electroneutral NaCl uptake. Specific amino acids are more potent stimulators of Na and solute transport than glucose in healthy neonatal animals and should continue to be studied in animal models of epithelial injury.

A

AGA CATEGORIES: Circle one only.

Molecular and Cell Biology
Morphology/Pathology
Hormones
Immunology/Microbiology
Intestine:
- Salt and Water Transport
- Nutrient Transport
- Other

Splanchnic Blood Flow
Stomach:
- Secretion
- Cytoprotection
- Other

Pancreas
Motility:
- Basic Science
- Clinical

Oncology
Growth and Development
Nutrition
Aging
Clinical Epidemiology
Inflammatory Bowel Disease
Clinical:
- Esophageal Disease
- Acid Peptic Disease
- Endoscopy
- GI Bleeding
- Campylobacter
- Other

AGA/AASLD LIVER CATEGORIES:

Immunology & RE System Transplantation, Hepatobiliary Surgery
Morphology, Regeneration, Cancer
Molecular & Cell Biology, Collagen, Fibrosis
Chronic Hepatitis & Primary Biliary Cirrhosis
Viral Hepatitis
Ethanol, Nutrition, Metabolism (including genetic diseases)
Complications of Cirrhosis: Portal Hypertension, Portal Circulation
Complications of Cirrhosis: Ascites, Portosystemic Encephalopathy
Anion Transport, Bile Formation, Cholestasis
Biliary Lipids, Proteins, Bile Acids, Gallstone Formation
Cell Injury, Hepatotoxicity
Drug Metabolism & Kinetics

B

COST OF PUBLICATION

Payment enclosed ($45.00)
Check no. _____
(payable to Elsevier Science Publishing Co.)

Payment delayed ($50.00)
Purchase order no. 546109
Issued by University of North Carolina at Chapel Hill
(Institution name)

A copy of this abstract *must* be attached to original purchase order for identification. Payment must be made in U.S. currency. No payment required for student research abstracts.

C

IMPORTANT

The principal author affirms that the material herein: 1) will not have been published as an article by May 15, 1988 or have been presented in the U.S. at a national meeting of a national society, or abroad at a major foreign or international meeting; 2) that if human subjects were exposed to risks not required by their medical needs, the study was approved by an appropriate committee or, if no such committee was available and informed consent was needed, it was obtained in accordance with the principles enunciated in "The Institutional Guide to DHEW Policy on Protection of Human Subjects"; or 3) any animal studies conform with the "Guiding Principles in the Care and Use of Animals" of the American Physiological Society.

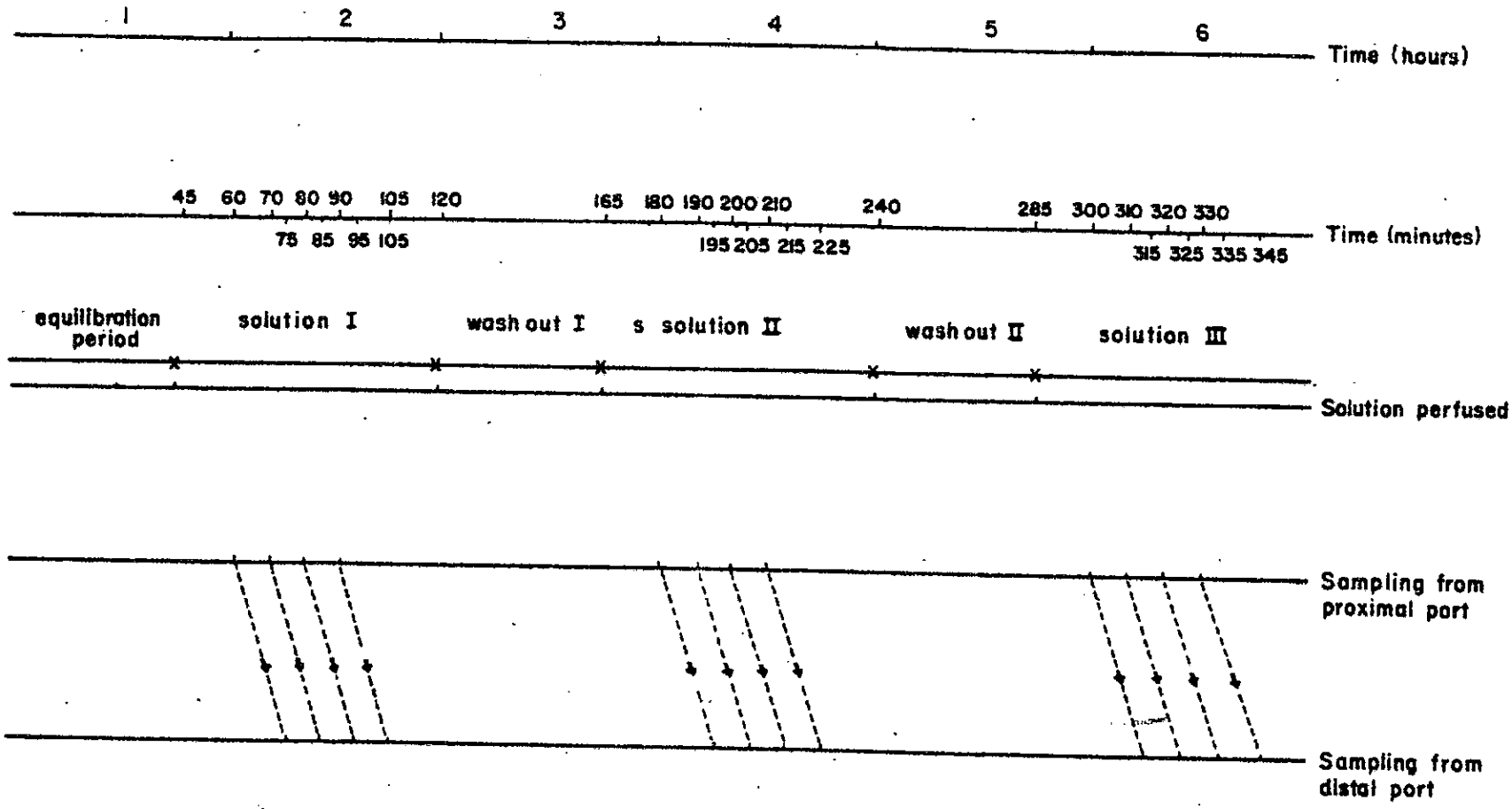
J. Marc Rhoads

(Signature of Principal Author)

D. Movie Projection Required for Oral Presentation ☐ | E. Poster Presentation Only ☐

F. If submitted for Student Research Prize competition check here: _____
(Sponsor's Name) _____

G. Name J. Marc Rhoads, M.D.
Address Department of Pediatrics CB# 7220
Burnett-Womack Bldg, Univ of North Carolina
Chapel Hill, NC 27599-7220
Telephone, Office: (919)966-1343 (area code) Home: (919)929-4891 (area code)


1
2
3
4
5
6
Time (hours)
45 60 70 80 90 105 120
75 85 95 105
165 180 190 200 210 240
195 205 215 225
285 300 310 320 330
315 325 335 345
Time (minutes)
equilibration period
solution I
wash out I
s solution II
wash out II
solution III
Solution perfused
Sampling from proximal port
Sampling from distal port

Appendix II