J Diarrhoeal Dis Res 1992 Sep;10(3):133 - 138
0253 - 8768/92 $ 1.00 + 0.10

Review Article

Perfusion Studies in Cholera: Methods and Procedures

FPL van Loon[1,2,3], K Gyr[4], AK Banik[1,5]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh; [2]Department of International Health, Johns Hopkins University School of Public Health, Baltimore, MD, USA; [3]Division of Immunization MS E05, Centers for Disease Control, Atlanta, GA 30333, USA; [4]Kantonsspital Liestal, University of Basel, Switzerland; [5]Family Health International, Arlington, VA, USA.

ABSTRACT

This paper reviews the characteristics of perfusion techniques in the study of intestinal functions by specifically examining the methods and procedures of perfusion in patients with diarrhoea due to infection with *V. cholerae* 01. Because of abundant jejunal secretion of water and electrolytes in cholera, perfusion studies require special approaches with regard to patient preparation, use of tubing material, selection of markers, and rate of perfusion. A discussion on specific problems involved in marker perfusion techniques in cholera and on the interpretation of the results is followed by practical recommendations.

Key words: Cholera; *Vibrio cholerae*; Intestinal secretions.

INTRODUCTION

The intestinal perfusion technique using a non-absorbable marker is a procedure for the quantitative assessment of intestinal absorption and secretion (1-7). As such, the technique has been applied to the study of the following conditions: the physiology of the exocrine pancreas (8-17); the physiology of the small and large intestine (18-23); the pathophysiology of the small intestine during cholera and other diarrhoeal diseases (24-26); and the pathophysiology of the colon during diarrhoea due to cholera and shigellosis (27-29). Experimental models for the study of water and solute transport in man have been important for the development of oral rehydration solutions (20,30,31). The ideal model for such investigations provides for rates of gastric emptying and of intestinal absorption and secretion (31). At present, the steady-state perfusion technique is the only satisfactory method for the study of regional absorption of water and solutes in the intact human gut (31).

Correspondence and reprint requests should be addressed to:
Dr. FPL van Loon, Division of Immunization MS E05, Centers for Disease Control, ATLANTA, GA 30333, USA.

Intestinal secretion in cholera constitutes the prototype for any watery diarrhoea, and the use of perfusion technique has been the method of choice in the study of intestinal transport of salt and water in cholera (19,32). In severe cholera, however, the intestinal secretion is so abundant that it poses specific problems to the marker perfusion method (33). This paper reviews some of these problems.

PATIENT PREPARATION

To monitor fluid-losses and fluid-requirements, cholera patients are best managed on a "cholera cot", a specially-designed plastic-lined bed supported by a collapsible wooden frame. The bed has a hole in the middle through which stool is collected into a calibrated bucket. Stool losses are measured every 1 to 4 hours by trained personnel to estimate the patient's requirements for rehydration. Before the patient is intubated orally, fluid losses due to vomiting and diarrhoea must be fully replaced by rehydration fluids. Rehydration will diminish the nausea that usually accompanies acute cholera and will facilitate intubation. For this replacement, a special intravenous rehydration solution (the Dhaka solution) developed for cholera and other diarrhoeal patients is recommended. It contains per litre 133 mmol Na, 13 mmol K, 98 mmol Cl and 48 mmol

bicarbonate, thus reflecting the proportional loss of electrolytes in the rectal effluent of cholera patients (34,35). The usual intravenous rehydration rate is 100 $ml/kg^{-1}.4h^{-1}$, but will depend on the degree of dehydration (36).

Preceding and during the perfusion procedure the patient is given nothing by mouth, but afterward, antibiotics and foods are allowed (37). At no time during perfusion procedures must the patient be left without professional attendance.

INTUBATION

Tubes. The basic method of perfusion studies involves inserting a flexible, radiopaque, oro-intestinal, multilumen tube into the gut at specified levels (4-6). At the tip, these tubes have a mercury weighted balloon to aid in propulsion. The tube is swallowed by the patient in an upright position and then the patient lies down, face up. To ensure that the tube has reached the jejunum, a small amount of fluid is aspirated and is checked for alkalinity. To determine whether the tube lies with its tip 40 cm beyond the duodeno-jejunal junction, the ligament of Treitz, fluoroscopy is performed both before and after each perfusion (33). The test solution that contains a non-absorbable marker is warmed to 37°C and perfused, at a constant rate, into the intestine through the proximal orifice in one lumen of the tube (3,38). The solution passes down the intestine and is sampled by aspiration via a distal orifice in another lumen of the tube. The effluent is collected on ice and kept frozen at −70°C until used.

The tubes should be made of flexible material with an inner diameter of about 2 mm, a length of 90 cm from teeth to duodenojejunal junction-resulting in a dead space of about 6 ml and with several openings at the collection sites to prevent mucus plugs from blocking them (3); de-blocking manoeuvres tend to influence the composition of subsequent samples.

The following types of tubings are commonly used in small intestinal perfusion:

I. *Tubes with proximal occluding-balloon.* The advantage of such a tube is the prevention of artifacts that may occur as a result of reflux and contamination by proximal secretions. Absorption rates of solutes and water that are calculated from aspirates taken with the balloon deflated are generally higher than when the balloon is inflated, because of reflux of the infusion solution proximal to the point of infusion (38,39). A disadvantage of this method is that the balloon might alter the absorption rates by influencing intestinal motility and mucosal blood flow (4,39).

II. *Tubes without occluding-balloons.* There exists some controversy about whether to use a double- or a triple-lumen tube. A double-lumen tube has one infusion port and one collection port, providing for a combined mixing and study segment. This may lead to improper mixing of the marker with the solute, to an unpredictable degree of reflux, and an over-estimation of absorption of substances, glucose in particular (40). Consequently, the two-lumen tube technique used in the study of water and electrolyte absorption tends to result in inaccuracies (2). Therefore, a triple-lumen tube with a 1.6 to 1.8 mm lumen, with an infusion port (IP), a proximal collection port (PP) and a distal port (DP) is preferred, because it separates the mixing segment (between IP and PP) from the study segment (between PP, and DP). In most studies, the length of the mixing segment is 15 cm and that of the study segment is 30 cm (figure). Inserting a separate gastric tube could help prevent proximal secretions from contaminating the study segment, with little discomfort to the patient (23).

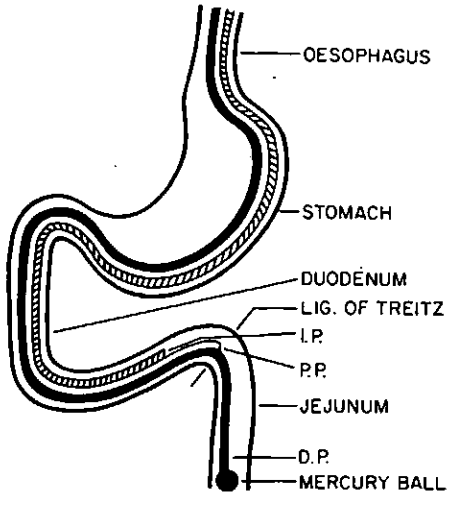

Figure: Diagram of the small intestine with a triple-lumen tube illustrating the triple-lumen perfusion technique.

TIMING

To avoid changes in sampled fluid composition due to diurnal variation, intubation and perfusion of patients within the same study should be carried out at the same hours of the day (4).

RATES OF PERFUSION

The accuracy of perfusion methods is determined by two main factors (1); 1) the degree to which the

infused test solution mixes with endogenous substance; and 2) the degree to which a steady-state can be achieved.

Under the experimental conditions of perfusion, involving fixed and inert tubing material and solutions that are directly perfused into the small intestine, the intestinal motility may differ from normal peristalsis (3). Yet, the method assumes that during a steady-state, homogeneity of intestinal contents and a constant flow-rate are reached at each point of the test segment. To achieve complete equilibrium between infusion solution and intestinal secretion, a minimum period of infusion is required. Conventionally, a 60-minute wash-out period has been recommended after each of successive solutions (3). However, when various glucose-electrolyte formulations were successively perfused in 19 cholera patients, intervals of only 30 minutes proved to be sufficient to achieve steady-state conditions (FPL van Loon, *et al.*, manuscript under review). Intestinal peristalsis, endogenous secretions and reflux of the study solution may account for some variation in the composition of the aspirates, but upper intestinal secretions should not noticeably interfere with jejunal fluid and electrolyte absorption when gastric secretions are aspirated separately (30). To determine whether steady-state conditions are indeed achieved during perfusion, one should calculate the percentage recovery of the marker (41). It is equally important to measure transit times over the study segment, especially when repeat perfusions are performed to compare acute and convalescent phases of illness (41). To assess the flow-rate over the study segment per study period, aspirations should be performed every 5 or 10 minutes, alternatively from the proximal and distal ports (41). This is called "pairwise staggering" and usually requires more than one person to manage.

Absorption as measured from a column of fluid in a state of equilibrium is probably different from the absorption of a meal or a normally ingested solution. In addition, true steady-state conditions are virtually impossible to achieve and in practice collections are made over periods of 10 to 15 minutes, to level out the effect of short-term variations. Steady-state is assumed to have existed during the procedure when, after laboratory analysis, marker concentrations appear to have been constant during four subsequent time intervals (42). Once that is the case, the net absorption rate of water and solutes can be calculated from the perfusion rate on the one hand, and the marker and solute concentrations from the paired proximal and distal aspirates on the other hand (33). Under high infusion rates, however, laminar flows may develop in the perfused segment causing the composition of the central liquid column to differ from the lateral fluid column along the luminal wall (3). Flow-rates, thus, influence the concentrations of the aspirated substances (30,42). Steady-state perfusion is usually performed at a rate of 7 ml per minute or more, so that flow-rate and concentration at each point of the perfusion segment remain almost constant. In whole-gut perfusion studies, initially identical flow-rates of different solutions appear to gradually change as they moved down the intestine (42). For the study of substances with a high turn-over at the enterocyte surface, slow-marker perfusion performed at a rate of 0.5 to 5 ml per minute might, however, be more appropriate, provided that the results are reproducible (24,30).

Net water and electrolyte absorption and flow rates at the proximal and distal collecting sites are calculated with the following non-absorbable marker equations (33), in which F_e = flow entering study segment (ml/min), F_l = flow rate leaving study segment (ml/min), I = infusion rate (ml/min), $M_{i,p,d}$ = marker concentration in the infusion solution and at proximal port (P) and distal port (D) respectively (g/l), and ΔH_2O = net transmural fluid transport rate ($ml.hour^{-1}.cm^{-1}$), A = aspiration rate (ml/min):

$$F_e = I \times \frac{[M]_i}{[M]_p} - A$$

$$F_l = F_e \times \frac{[M]_p}{[M]_d}$$

$$\Delta H_2O = \frac{F_l - F_e}{30} \times 60$$

$$\Delta Na = \frac{F_l \times Na_d - F_e \times Na_d}{30} \times 60 \times 1000$$

Positive values indicate net secretion, while negative values indicate net absorption of fluid. Secretion means a net gain of fluid within the intestinal lumen, but does not indicate whether this process is active or passive. ΔNa, and similarly ΔK, ΔCl, and ΔHCO_3 correspond to net transmural transport rates of sodium, potassium, chloride, and bicarbonate ($\mu mol.hour^{-1}.cm^{-1}$) respectively. Given the circumstances of countries where cholera is studied, battery-run pumps are advised to warrant uninterrupted electric power supply and constant infusion rates.

Manual aspiration of fluid samples is preferred to suction by pump or siphonage, because using a pump may lead to suctioning of the tube onto the luminal wall, and siphoning may influence the composition of the rectal effluent and make study parameters in the stool unreliable.

MARKERS

An ideal internal marker should meet the following requirements (38): it should not be subject to absorption or destruction in the intestine; it should be homogenously distributed within the

intestinal lumen and not be trapped in the intestinal contents, such as mucus; it should be indifferent to intestinal contents or motility; it should not interfere with the digestion of the substance under study; and it should lend itself to easy and accurate estimation in the laboratory. Markers are, therefore, usually water-soluble substances of high molecular weight. Radioactive markers are generally not accepted for studies in countries where cholera occurs.

Bromosulphophthalein (BSP) is a reliable marker when the intestinal fluid pH remains within the narrow range of 6.2 to 6.8. BSP colorimetry is highly accurate in the absence of bile; the measurement of polyethylene glycol (mostly PEG 4000) by turbidimetry is reliable at any pH, but lengthy and cumbersome in practice (4,43). The marker phenolsulphonphthalein (PSP, "phenol red") has the disadvantage of binding to proteins, such as albumin, which results in an uneven distribution within the intestinal content (4).

SOLUTES

Perfusion solutions ought to be isotonic (300 mOsm/l) to prevent osmotic shifts of fluids. In a sequential perfusion procedure, one may want to regain "baseline conditions", by perfusing balanced salt solution (BSS) prior to, and following, the perfusion of the experimental solutions. This "sandwich technique" enables the researcher to correct for temporal shifts in solute concentrations during the natural course of cholera. BSS has a composition similar to plasma (Na 145 mmol/l, K 5 mmol/l, Cl 135 mmol/l, 15 HCO_3 mmol/l) and thus serves as reference for the actual solutions under study.

The following paragraph discusses solutes of relevance for the interpretation of perfusion study results, in particular when performed for the development of new oral rehydration solutions.

Sodium. The intestinal sodium transfer rate from sodium containing solutions is related to the sodium concentration in the lumen and is dependent on water movements predominantly in the proximal small intestine (6). In the jejunum, sodium absorption is stimulated by glucose, bicarbonate, and a number of amino acids, and water, ordinarily coupled in a given proportion to the total solute concentration, follows passively the osmotic gradient (23,45-48). Sodium cotransport, which takes place in the superficial cells, is proportional to the intraluminal bicarbonate concentration, but sodium secretion, which occurs in the crypt cells, is not (6). The luminal concentration of organic solutes is not the only determinant for electrolyte and water absorption; other factors such as the concentration of sodium, and the osmolality and pH of the solutions also play a role (46).

Sugars. Flow-rate and initial glucose concentration determine sodium and water movements (46). Increasing flow rates of glucose containing solutions result in an increase in glucose absorption up to its saturation level of 133mM/l, in an increase of water absorption, and in an increase in sodium absorption and potassium secretion (30). Nearly all ingested glucose is absorbed in the jejunum, irrespective of the initial concentration (41,45,46).

Table I. Recommendations for Small Intestinal Marker Perfusion Studies in Cholera

1. Patients should be placed on a cholera cot in order to accurately monitor fluid losses.
2. Intravenous rehydration (Dhaka solution) should be administered for proper fluid balance maintenance.
3. Detailed explanation to and extensive communication should take place with the patient and his/her attendants prior to and during the procedure; informed written consent must be obtained.
4. The patient should be kept fasting.
5. A flexible radiopaque triple-lumen tube without proximal occluding balloon should be prepared with multiple openings at the collection sites, a mixing segment of 15 cm, a study segment of 30 cm and a mercury weight at the tip; the patency of the tube should be checked before each procedure.
6. The tube should be inserted with its tip 40 cm beyond the ligament of Treitz and its correct position determined based on the alkalinity of the aspirate and on fluoroscopy both before and after the procedure. An additional gastric tube is recommended to prevent gastric juice contamination.
7. A power-checked, battery-run peristaltic pump should be used to warrant a constant perfusion rate.
8. All vials should be sorted and labelled.
9. Samples should be collected on ice and immediately stored at -20° Centigrade. Dry-freezing is advised in case of laboratory analysis abroad.
10. A standard flow sheet should be used; watches should be at hand.
11. Separate syringes are preferred for proximal and distal aspiration.
12. The perfusion fluids and the marker should be freshly prepared as isotonic solutions at a pH of 6 and infused at body temperature.
13. It is recommended to 'sandwich' the experimental and control solutions between 'balanced salt-solutions'.
14. Gastric and jejunal secretions should be separately and manually collected.
15. To warrant steady-state conditions during perfusion of successive solutions, a 30-min equilibration period is recommended at the beginning of the study, and a 30-min wash-out with the subsequent solution is advised in between sampling periods.
16. Pilot studies are advised to adapt the perfusion technique to the specific research questions to be addressed.
17. To determine the percentage recovery and to correct for intestinal volume losses, the concentrations of both marker and electrolytes [sodium, potassium, chloride and bicarbonate] should be measured in each sample.
18. A steady-state perfusion (infusion rate $>= 7$ ml/min) is preferred to slow-marker perfusion (infusion rate $<= 5$ ml/min).
19. A repeat perfusion is advised during convalescence when the patient serves as his/her own control (41).
20. Patient should be left at no time without professional attendance.

Disaccharide absorption also occurs mainly in the proximal small intestine but is preceded by hydrolysis. The processes of hydrolysis and absorption of these carbohydrates are intimately linked as both take place in the brush border of the enterocyte (5).

Amino acids. Studies on intestinal absorption of amino acids in man by intestinal perfusion are still scarce, although the results so far confirm those of animal experiments (47). L – amino acids move across the brush border against a concentration gradient, which indicates active transport. This transport is highly specific, is sodium dependent, and decreases aborally (47). Amino acid absorption in man is a process of inhibitive competition. Dipeptides may be absorbed by separate mechanisms (48).

RESERVATIONS

Sequential small – intestinal perfusion studies in cholera have been important for the development of oral rehydration solutions. However, it is not only the jejunum that is affected in cholera but the large intestine to some extent as well (27). Consequently, conclusions drawn from small – intestinal perfusion studies cannot be entirely extrapolated to clinical cholera and its treatment. Besides sequential perfusions, whole – gut perfusions have recently been performed to evaluate the efficacy of oral rehydration solutions (42).

ACKNOWLEDGMENTS

Funding for this work was provided by grant W79 – 94 of the Netherlands Foundation for the Advancement of Tropical Research (WOTRO). The authors appreciate the helpful suggestions of Dr J Rask – Madsen, University of Copenhagen, Copenhagen, and Dr K Bukhave, Technical University Denmark, Lyngby, Denmark, and Dr J – F Desjeux, INSERM, Paris, France. The authors are grateful to Drs R Eeckels, Leuven, Belgium, and GNJ Tytgat, Amsterdam, the Netherlands, for their reviews.

The International Centre for Diarrhoeal Disease Research, Bangladesh is supported by countries and agencies which share its concern for the health problems of developing countries.

REFERENCES

1. Cooper H, Levitan R, Fordtran JS, et al. A method of studying absorption of water and solute from the human small intestine. *Gastroenterology* 1966;50:1 – 7.
2. Fordtran JS. Marker. perfusion techniques for measuring intestinal absorption in man. *Gastroenterology* 1966;51:1089 – 93.
3. Whalen GE, Harris JA, Geenen JE, et al. Sodium and water absorption from the human small intestine. The accuracy of the perfusion method. *Gastroenterology* 1966;51:975 – 84.
4. Modigliani R,Rambaud JC, Bernier JJ. The method of intraluminal perfusion of the human small intestine. I Principle and technique. *Digestion* 1973;9:176 – 92.
5. Modigliani R,Rambaud JC, Bernier JJ. The method of intra – luminal perfusion of the human small intestine. II Absorption studies in health. *Digestion* 1973;9:264 – 90.
6. Rambaud JC, Modigliani R, Bernier JJ. The method of intra – luminal perfusion of the human small intestine. III Absorption studies in disease. *Digestion* 1973;9:343 – 56.
7. Soergel K H. An evaluation of perfusion techniques in the study of water and electrolyte absorption in man (Comment with reply Sladen GF, Dawson AM) *Gut* 1966;10:601 – 2.
8. Meyer JH, Kelly GA. Canine pancreatic responses to intestinally perfused proteins and protein digests. *Am J Physiol* 1976;231:682 – 91.
9. Krejs G. VIPoma syndrome. *Am J Med* 1987;82:37 – 48.
10. Gyr K, Beglinger C, Koehler E, *et al.* Circulating somatostatin. Physiological regulator of pancreatic function. *J Clin Invest* 1987;79:1595 – 1600.
11. Christ A, Werth B, Hildebrand P, *et al.* Human secretin: biologic effects and plasma kinetics in humans. *Gastroenterology* 1988;94:311 – 6.
12. Fried M, Mayer EA, Jansen JBMJ, Lamers CBHW, Taylor IL, Bloom SR, Meyer JH. Temporal relationship of cholecystokinin release, pancreatobiliary secretion, and gastric emptying of a mixed meal. *Gastroenterology* 1988;95:1344 – 50.
13. Vidon N, Chaussade S, Merite F, *et al.* Inhibitory effect of high caloric load of carbohydrates or lipids on human pancreatic secretions: a jejunal brake. *Am J Clin Nutr* 1989;50:231 – 6.
14. Bardhan PK, Ahmed T, Alam NH, Alam AN, Gyr K, Beglinger C. Exocrine pancreatic function in cholera and acute shigellosis. *Digestion* 1990;46:127.
15. Lin HC, Doty JE, Reedy TJ, Meyer JH. Inhibition of gastric emptying by acids depends on pH, titratable acidity, and length of intestine exposed to acid. *Am J Physiol* 1990;259:1025 – 30.
16. Lin HC, Doty JE, Reedy TJ, Meyer JH. Inhibition of gastric emptying by sodium oleate depends on length of intestine exposed to nutrient. *Am J Physiol* 1990;259:1031 – 6.
17. Hildebrand P, Beglinger C, Gyr K, Jansen JBMJ, Rovati LC, Zuercher M, Lamers CBHW, Setnikar I, Stalder G. Effects of a Cholecystokinin receptor antagonist on intestinal phase of pancreatic and biliary responses in man. *J Clin Invest* 1990;85:640 – 6.
18. Fordtran JS, Rector FC, Ewton MF, *et al.* Permeability characteristics of the human small intestine. *J Clin Invest* 1965;66:1326 – 33.
19. Fordtran JS, Rector FS, Carter NW. The mechanisms of sodium absorption in the human small intestine. *J Clin Invest* 1968;47:884 – 900.
20. Hirschhorn N, Kinzie JL, Sachar DB, *et al.* Decrease in net stool volume in cholera during intestinal perfusion with glucose – containing solutions. *N Eng J Med* 1968;4:176 – 81.
21. Field M, Fromm D, Al – Awqati Q, *et al.* Effect of cholera toxin on ion transport across isolated ileal mucosa. *J Clin Invest* 1972;51:796 – 804.
22. Krejs GJ, Walsh JH, Morawski SG, *et al.* Intractable diarrhea: intestinal perfusion studies and plasma VIP concentrations in patients with pancreatic cholera syndrome and surreptious ingestion of laxatives and diuretics. *Am J Dig Dis* 1977;22:280 – 92.
23. Krejs GJ, Fordtran JS. Effect of VIP infusion on water and ion transport in the human jejunum. *Gastroenterology* 1980;78:722 – 7.
24. Speelman P, Rabbani GH, Bukhave K, Rask – Madsen J. Increased jejunal PGE_2 concentrations in patients with acute cholera. *Gut* 1985;26:188 – 93.
25. Van Loon FPL, Rabbani GH, Bukhave K, Rask – Madsen J. Indomethacin decreases jejunal fluid secretion in addition to luminal release of prostaglandin E_2, in patients with acute cholera. *Gut* 1992;33:643 – 5.
26. Davis GR, Camp RC, Raskin P, Krejs GJ. Effect of somatostatin infusion on jejunal water and electrolyte transport in a patient with secretory diarrhea due to malignant carcinoid syndrome. *Gastroenterology* 1980;78:346 – 9.
27. Krejs GJ, Barkley RM, Read NW, Fordtran JS. Intestinal secretion induced by vasoactive intestinal polypeptide: a comparison with cholera toxin in the canine jejunum in vivo. *J Clin Invest* 1978;501:1337 – 45.

28. Speelman P, Butler T, Kabir I, *et al.* Colonic dysfunction during cholera. *Gastroenterology* 1986;91:1164 – 70.
29. Butler T, Speelman P, Kabir I, *et al.* Colonic dysfunction during shigellosis. *J Infect Dis* 1986;154:817 – 24.
30. Modigliani R, Bernier JJ. Absorption of glucose, sodium and water by the human jejunum studied by intestinal perfusion with a proximal occluding balloon and variable flow rates. *Gut* 1971; 12:184 – 93.
31. Leiper JB, Maughan RJ. Experimental models for the investigation of water and solute transport in man. Implications for oral rehydration solutions. *Drugs* 1988;36:65 – 79.
32. Field M, Rao MC, Chang EB. Intestinal electrolyte transport and diarrheal disease. *N Eng J Med* 1989;321:800 – 6.
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. Gordon RS, Ahmed J, Akbar R *et al.* Treatment of cholera in 1964. *East Pak Med J.* 1964;8:10 – 19.
35. Carpenter CCJ, Mondal A, Sack RB, *et al.* Clinical studies in Asiatic cholera 1,2. Development of 2:1 saline:lactate regimen. Comparison of the regimen with traditional methods of treatment, April and May 1963. *Bull Johns Hopkins Hosp* 1966;118:174.
36. WHO/CDD/SEC/84.2.
37. Greenough WB, Gordon RS, Rosenberg IS, *et al.* Tetra – cycline in the treatment of cholera. *Lancet* 1964;1:355 – 7.
38. Sladen GF, Dawson AM. An evaluation of perfusion techniques in the study of water and electrolyte absorption in man: the problem of endogenous secretions. *Gut* 1968;9:530 – 5.
39. Phillips SF, Summerskill WHJ. Occlusion of the jejunum for intestinal perfusion in man. *Mayo Clin Proc* 1966;41:224 – 31.
40. Sladen GF, Dawson AM. Effects of flow rate on the absorption of glucose in a steady state perfusion system in man. *Clin Sci* 1969;36:132 – 45.
41. Barclay GR, Turnberg LA. Effect of moderate exercise on salt and water transport in the human jejunum. *Gut* 1988;29:816 – 20.
42. Rolston DDK, Zinzuvadia SN, Mathan VI. Evaluation of the efficacy of oral rehydration solutions using whole gut perfusion. *Gut* 1990;31:1115 – 9.
43. Davis GR, Santa Ana CA, Morawski SG, *et al.* Inhibition of water and electrolyte absorption by polyethylene glycol (PEG) *Gastroenterology* 1980;79:35 – 9.
44. Fordtran JS, Rector FC, Ewton MF, *et al.* Permeability characteristics of the human small intestine. *J Clin Invest* 1965;66:1326 – 33.
45. Fordtran JS. Stimulation of active and passive sodium absorption by sugars in human jejunum. *J Clin Invest* 1975; 75:728 – 37.
46. Sladen GF, Dawson AM. Interrelationssships between the absorption of glucose, sodium and water by the normal human jejunum. *Clin Sci* 1969;36:119 – 32.
47. Saunders SJ, Isselbacher KJ. Intestinal absorption of amino acids. *Gastroenterology* 1966;50:586 – 95.
48. Hellier MD, Thirumalai C, Holdswooth CD. The effect of peptides on sodium and water absorption in man. *Gut* 1973;14:41 – 5.