

Gut

Leading article – Tropical infection of the gastrointestinal tract and liver series

Cholera and severe toxigenic diarrhoeas

The reintroduction of cholera into South and Central America,¹ and its rapid spread through the region, illustrate the continued susceptibility of most of the world's population to this often lethal disease. This susceptibility is based on attributes of the pathogen, *Vibrio cholerae*, and its bioecology, and on the human host. Host factors include the strength (and weaknesses) of the gastric acid barrier, the faecalisation of the environment leading to contamination of drinking water and food with the pathogen, and the continued lack of education or habit formation sufficient to curtail intestinal contamination and colonisation by *V. cholerae*. The host immune response which develops too late to protect the victim and gives variable protection to the survivor and to vaccinees.

Cholera can maintain itself and increase when epidemic strains of *V. cholerae* persist longer in the environment and multiply to higher concentrations in water, other beverages or food, notably if chitin is present.² The pathogen can pass through the stomach because of the absence of normal acidity or, when acid is present, by attachment to or within a particle. The vehicular particles may be of chitin² zoo- or phytoplankton³ or possibly algae,^{3,4} protozoa,⁵ cellular debris,⁶ parasitic nematodes and their eggs⁷ or other substrates offering protection from acidity or other noxious environmental influences, such as heat,⁸ cold,⁹ and low salinity.⁸ *V. cholerae*'s environmental survival and transmission may be linked to these vehicles.

Vaccine development has consistently produced vaccines giving only a modest protection from disease (at best 50–85%) for periods typically too short (six months – one year and after this period of time, a protection of 0 to 50%) to be of practical significance in all age groups.^{10–13} Treatment remains the main area of progress in research, with repeated findings of the success of intravenous and oral replacement of the water and electrolyte losses characterising the disease. This article will attempt at analysing how recent and future research could help to understand and to better control the cholera problem.

The causative vibrio and its bioecology

After the initial hypothesis^{14,15} based on analogy with Japanese and American studies of *V. parahaemolyticus* coupled with *V. cholerae*'s then unexplained chitinase production, that an estuarine or other aquatic niche for *V. cholerae* might exist in copepods or other chitinaceous fauna, a number of confirmatory studies have been published.¹⁶ Such a niche is firmly established,^{3,16} and vibrio

adhesion to chitin from such organisms, or ingestion of vibrios in certain foods, can provide safe passage through the gastric acid barrier.² Further study will define the comparative rank importance of copepods, free living^{3,17} or parasitic on fish,¹⁸ molluscs^{19,20} or mammals²¹; isopods,²² other plankton,³ crabs^{16,23} prawns^{9,24} or other organisms, which are chitinaceous or chitinophagous, or parasitised by copepods or other vibrio-carrying organisms, in maintaining and disseminating epidemic *V. cholerae* 01 serovar strains in the environment and in human ingesta. The role of birds in transferring *V. cholerae* to new aquatic sites has also been suggested.²⁵

The recognition that dormant, viable but non-culturable forms of *V. cholerae* may persist in aquatic environments is potentially of great significance.¹⁶ Infection of two volunteers challenged with a dormant form of a live gene deletional attenuated vaccine strain of *V. cholerae* has been shown,³ but the comparative importance of wild, dormant forms in cholera pathogenesis and epidemiology, including their ability to cause disease directly in the human host, needs to be determined.

The full range of potential extrahuman hosts of *V. cholerae* awaits definition. Findings on *V. cholerae* colonisation of intestinal nematodes and attachment to their eggs⁷ and multiplication (with lethal effect) in earthworms²⁶ remain unpursued despite potentially important bioecological implications. Study of the fate of colonised expelled parasitic nematodes, and of earthworms naturally infected by consumption of faecally contaminated soil, after ingestion of infected worms by natural predators and scavengers, could show additional survival or multiplication niches. The possible persistence or multiplication of *V. cholerae* in other chitin enriched natural microenvironments, such as in water suitable for oviposition and larval development by mosquitoes, for example, in crab holes,²⁷ or in the intestinal tracts of fish harbouring vibrios,²⁸ some of which maintain ingested chitin residues in their intestines,²⁹ or in other chitinivorous marine animals, will probably be a useful line of research. There is a need for additional studies of seasonal variations of *V. cholerae* 01 counts (culturable and 'non-culturable') in flora,³⁰ chitinaceous fauna, sediments and water columns of freshwater sources, and the relation of counts to variations in salinity, temperature, and nutrient content³¹ of surface and other drinking water sources in endemic areas.⁸ Given that one copepod may harbour 10⁵ vibrios,³ ≥one infective dose₅₀,³² it is not hard to imagine how small numbers of such aquatic organisms, if imbibed in drinking water, could provide a vehicle for gut contamination, colonisation, and multiplica-

tion leading, with or without prior multiplication in food contaminated by such water, to infection of humans. Ingestion in a chitin vehicle is the mode of infection of ayu fish with *V anguillarum* and offers a possible vehicle (live or killed) for oral vaccines against enteric vibrio infections of humans as for ayu.³³

The fate of the ingested pathogen, bound or unbound to a copepod or to other microfauna or flora occurring in local drinking water, also revolves around the still underinvestigated phenomenon of tropical hypochlorhydria. Indications that early malnutrition can cause hypochlorhydria and, in turn, lead to gastric bacterial overgrowth, gastritis, parietal cell loss, and subsequent lifelong hypochlorhydria, have yet to be followed up to identify potentially preventive early nutritional interventions. The role of particular gastric pathogens such as *Helicobacter pylori* in the causation of tropical hypochlorhydria and ultimately, in the susceptibility to cholera, is currently under study.³⁴

Given the prevalence of hypochlorhydria in developing countries, and the finding of *V cholerae* serovar O1 free swimming, adherent to or inside copepods and algae,³⁴ amoebae,⁵ and nematodes and their eggs,⁷ it is easy to imagine how most of the human population in endemic areas acquires vibriocidal antibody titres early in life.

Pathophysiology

Study of pathogenesis of disease after colonisation has chiefly focused on the role of increased adenylyl cyclase and cyclic AMP (cAMP) concentrations after cell intoxication by classic cholera toxin.³⁵⁻³⁶ Recent elucidation of other toxins that the pathogen can produce may explain some aspects of early pathogenesis,³⁷⁻³⁸ but seems unlikely to supplant the central importance of classic cholera toxin. Nevertheless, conceptualisation of pathophysiology should move beyond the findings of increased adenylyl cyclase and cAMP. The mechanism by which cAMP might cause (and not merely parallel) the changed electrolyte and water movement in cholera patients remains unknown. In the light of evidence of multiple vibrio toxins³⁷⁻³⁸ apparently non-A subunit³⁹⁻⁴⁰ or non-cAMP related⁴¹⁻⁴² toxic effects, and little studied differences in effects of cholera toxin on the numerous different intestinal epithelial and neuronal gut cell types, current concepts of causation seem simplistic.

In fact, evidence has mounted suggesting that adenylyl cyclase and cAMP may participate only partially, transiently or indirectly in that their inhibition or antagonism have not consistently paralleled G_s or electrolyte flux changes⁴¹⁻⁴³⁻⁴⁴ or diminished diarrhoea in experimental situations or in cholera patients.⁴⁵⁻⁴⁷ Adenylyl cyclase activity in some cholera patients may not parallel diarrhoea,⁴⁸ and in cell models adenylyl cyclase activity is stimulated at low, but not at high concentrations of cholera toxin or with prolonged exposure to it.⁴⁴

Experimental anti-secretory effects of phenothiazines were not associated with changes in ADP ribosylation, though a parallel was found with adenylyl cyclase inhibition, and phenothiazine effects on calcium mediated pathways, which can also change sodium fluxes, are possibly responsible for anti-secretory effects in experimental models.⁴⁵ Studies showing modest cholera diarrhoea reduction by the cyclase antagonist chlorpromazine without tetracycline,⁴⁹ but not with tetracycline,⁵⁰ probably represent only the antibacterial efficacy of chlorpromazine, not an anti-cyclase effect on diarrhoea rate. Other cyclase inhibitors, such as chloroquine⁴⁶ are ineffective in patients.⁴⁷

In vivo, even chloride transport inhibitors may not inhibit choleraic fluid secretion⁵¹; in a cell model, maximum promotion of chloride secretion did not require an increase in cAMP activity,⁵²⁻⁵³ and the reduction of secretion can occur without diminishing cAMP activity.⁵⁴ Perhaps excised,

avascular, and enervated short circuited epithelial strips or isolated cells³⁵⁻³⁶⁻⁵³ do not faithfully reflect events in intact living patients. Causal links between cAMP and the augmented villous tip hypertonicity after cholera toxin,⁵⁵ or the non-absorbability of tritiated water after cholera toxin⁵⁶ are needed if cAMP's central role is to be retained.

A cascade of cholera toxin-associated changes in G proteins, 5-hydroxytryptamine receptors,⁵⁷ possible release of prostaglandins,⁵⁸ vasoactive intestinal polypeptide⁵⁹⁻⁶⁰ (by neuronal mechanisms) and other hormones has been shown, but not integrated. A clear chain of cause and effect explaining exactly how sodium, potassium, chloride, and water movement (and performance of related transporters) are changed to produce diarrhoea in the patient has not been shown. Whether some of the change in adenylyl cyclase or cAMP activities in vivo might be a cell reaction to other effects of cholera toxin (compared with a direct cholera toxin effect) has not been ruled out. cAMP has appeared in the role of chloride secretion inducer,³⁵⁻³⁶ absorption promoter of sodium-glucose cotransport and other substances,⁶¹⁻⁶³ protein kinase phosphorylator⁶⁴ or bystander.⁴⁴⁻⁴⁵⁻⁵² The comparative importance in cholera genesis of other secondary messengers, or of effects of the cholera toxin cascade on cell nuclei and DNA,⁶⁵ is not yet established.

Additional cAMP pathway inhibitors with reported anti-cholera activity exist (for example, progesterone,⁶⁶ retinoic acid,⁶⁷ barium salts,⁵³ glucagon,⁶⁸ and serotonin antagonists),⁶⁹ which have not been clinically tested, but one must conclude that no inhibitors of adenylyl cyclase, or cAMP antagonists tested have proved of therapeutic value against cholera diarrhoea. It is time to look further.

Treatment

Experimental anti-cholera agents, like acids, unsuitable for treatment but capable of removing cholera toxin affected intestinal mucosal cells, have been the only agents shown to abruptly stop the diarrhoeagenic effect of cholera toxin⁷⁰ when given after diarrhoea starts (in the dog model). This therapeutic approach has not been pursued despite the availability of potentially usable, tolerable agents such as epidermal growth factor or glutamine,⁷¹⁻⁷² capable of speeding up intestinal cell turnover and thereby replacing toxin affected cells sooner. Concurrent antibiotics could, in such a regimen, prevent newly generated cells from being exposed to the toxin by eliminating vibrios concurrently. Neuropeptide Y seems capable of reducing diarrhoea in the cat model⁶⁰ and may merit further study, along with anti-secretory hormones found in milk (and bile) of rats and sows.⁷³⁻⁷⁴ Zinc replenishment has also shown therapeutic promise,⁷⁵⁻⁷⁶ but may interfere with oral glucose and amino acid absorption.⁷⁷

The studies first showing that oral glucose electrolytes solution treatment (ORT) could reduce by 80% therapeutic intravenous fluid needs of cholera patients already in shock,⁷⁸ and could be used for the entire rehydration and maintenance phases in most patients not in shock⁷⁹⁻⁸⁰ were followed by similar studies showing the utility of the amino acid glycine as a substrate.⁸¹ Greater absorptive efficiency and reduced diarrhoea duration accompanied use of solutions containing both glucose and glycine in cholera and cholera like toxigenic diarrhoeas.⁸²⁻⁸⁸ Other amino acids, for example, alanine and glutamine, can also do this⁸⁹⁻⁹⁰ but are costlier and less available than glycine.

The practical importance of the glucose plus glycine solution in terms of reduced duration of cholera treatment and stay in hospital⁸¹⁻⁸³ has been underestimated, because of the apparent lack of reproducible advantage in non-enterotoxigenic diarrhoeas,⁹¹ which are generally much milder than cholera and, in contrast with acute cholera,

can be treated with a very wide variety of oral fluids. Some of these studies have changed sodium concentrations and substituted polysaccharides for glucose or glycylglycine for glycine, clouding comparison with the original studies.^{81-87 92 93} Some oligosaccharides may inhibit glucose absorption,⁹⁴ and polysaccharides may promote hypernatraemia.⁹⁵ Advantages seen in some studies of solutions with glucose plus glycine, or with rice powder, which is rich in glycine and other amino acids as well as starch,⁹⁶ may relate to enhancement by cholera toxin of cell sugar and amino acid absorption, perhaps mediated by cAMP.^{62 63}

Subsequent studies have shown the utility, though not consistent superiority, of cereal and other complex substrates.⁹⁷ It remains unclear if the variability in amino acid content of rice used for different studies may account for variability in results of different studies of rice ORT.⁹⁸ The numerous studies of different complex substrates, ranging from rice preparations to mung bean and chicken soup⁹⁹⁻¹⁰¹ have yielded many complex solutions, which can support absorption during cholera or other related diarrhoeas, depending on local availability, but most of these studies have not been conducted in patients admitted in shock, in whom the choice of the most highly absorbable oral solutions (after initial intravenous rehydration) is of critical importance. Contradictory results have been obtained chiefly in numerous small studies unstratified by pretreatment diarrhoea rate, unblinded, and lacking sufficient statistical power to provide trustworthy conclusions. These have left uncertain the evidence that any of these solutions is truly superior to glucose ORT or to glucose and glycine ORT in cholera.^{97 98} Rice malabsorption has been reported as a potential problem.¹⁰²

In cholera in particular, the question whether the WHO ORT has optimal sodium and potassium content has been repeatedly asked, but in the interests of preserving a solution formulation usable for milder diarrhoeas as well, the WHO formula remains slightly suboptimal for cholera in its sodium concentration and generally too low in potassium concentration.^{103 104} This has a potential influence on amino acid absorption¹⁰⁵ and hence on outcome of oral treatment studies. Studies evaluating the comparative longterm effects of more complete potassium replacement are much needed, especially for use in paediatric cholera and related non-cholera watery diarrhoeas.

Many formulas in use lack the optimal substrate to sodium ratio,¹⁰⁶ necessary to promote maximal sodium and water absorption. Claims (still unbacked by scientific data) that starch can promote more net absorption without osmotic penalty¹⁰⁷ have overlooked the issue of the substrate to sodium ratio and the fact that luminal starch hydrolysis is a prerequisite for absorption¹⁰⁸ and that starch malabsorption may occur.^{102 109} Findings of increased water absorption from hypotonic oral solutions,¹¹⁰ usable for treating low volume, short duration, non-life threatening diarrhoeas, need to include measurements of net sodium balance, which is generally noticeably negative when such solutions are used to treat cholera or cholera like diarrhoeas.¹¹¹ Similarly, base free oral solutions^{112 113} may suffice in mild non-cholera diarrhoeas, but can lead to life threatening haemodynamic events during rehydration of acidotic cholera patients in shock on arrival.¹¹⁴

In recent years, many new substances have been discovered to be capable of enhancing intestinal absorptive capacity of glucose, amino acids, water or salts, and studies are needed to find out if such substances (for example, vitamin B-6, ethylacetate, 17 α methyltestosterone, glucagon, epidermal growth factor), might enhance the efficacy or efficiency of ORT.¹¹⁵⁻¹¹⁹ ORT remains grossly underused in many countries and improvement will require continuation

of the excellent programme implementation of World Health Organisation, Unicef, USAID and other voluntary agencies, including a focus on Western developed countries.¹²⁰

Prevention

The immune response to cholera infection, and the mechanism by which immunosuppressive effects of cholera toxin and related enterotoxins may block or diminish it in vivo¹²¹⁻¹²⁶ need further definition. Critical questions are the optimal modes of antigen delivery, comparative influence on protective efficacy of different routes of administration, immunogenicity and tolerability of megadoses of purer, more potent immunogens, including protein conjugates, and newer modes of adjuvantation. A mode of immunising those with group O blood type needs to be developed.¹²⁷ Despite a great deal of research,^{10-13 128-130} the 80%, two year protection afforded by parenteral somatic antigen in peanut oil adjuvant still matches the best protection ever achieved by any cholera vaccine,¹³¹ and may point to the best future approach, using more powerful, better tolerated novel adjuvants, and purer preparations containing more precisely defined key antigenic epitopes.¹²⁹ Pili,¹³² outer membrane proteins,¹³³ and protein conjugates using enterotoxin moieties¹³⁴ are of current research interest. Live attenuated vaccines offer promise,¹²⁸ but have not yet been proved superior to older vaccines, and will depend on cold chain maintenance.

Other severe toxicogenic diarrhoeas

Toxins closely related to cholera toxin are produced by non-O1 vibrios, other vibrio species, *Escherichia coli*, and some strains of salmonella.¹³⁵⁻¹⁴¹ These organisms can produce a clinical syndrome of severe enterotoxigenic diarrhoea strongly resembling cholera. *E. coli* causing severe cholera like disease often produce both heat labile and heat stable (ST_a or ST_b) enterotoxins.¹⁴² Other organisms (aeromonas, etc) have been reported to produce cross reacting enterotoxins,¹⁴³ but are rarely if ever associated with diarrhoea as severe as cholera.

Details of the epidemiology and bioecology of these organisms are beyond the scope of this paper, but in general the vibrios tend to follow the pattern of a marine, estuarine or freshwater extra human niche^{144 145} (for which *V. parahaemolyticus* is the original paradigm). These organisms may also produce other virulence factors, including various other heat labile and heat stable toxins, shiga or shiga like toxins, haemolysins, etc. Association of outbreaks with faecally or marine contaminated food or water is common. Of special interest is the discovery of guanylin, an intestinal hormone resembling ST_a and the EAST-1 *E. coli* enterotoxin which is possibly the harbinger of other toxin hormone analogues to come.¹⁴⁶

D R NALIN

Merck Research Laboratories,
PO Box 4, West Point, PA 19486, USA

- 1 Sepulveda J, Gomez-Dantes H, Bronfman M. Cholera in the Americas - an overview. *Infection* 1992; 20: 243-8.
- 2 Nalin DR, Daya V, Reid A, Levine MM, Cisneros L. Adsorption and growth of Vibrio cholerae on chitin. *Infect Immun* 1979; 25: 768-70.
- 3 Colwell RR, Tamplin ML, Brayton PR, Glauzens AL, Tall BD, Herrington D, et al. Environmental aspects of Vibrio cholerae in transmission of cholera. In: Sack RB, Zinnaka Y, eds. *Advances in research on cholera and related diarrheas*, 7. Tokyo; KTK Scientific Publishers, 1990: 327-43.
- 4 Islam S, Drasar BS, Bradley DJ. Long-term persistence of toxigenic Vibrio cholerae 01 in the mucilaginous sheath of a blue-green alga, *Anabaena variabilis*. *J Trop Med Hyg* 1990; 39: 133-9.
- 5 Thom S, Warhurst D, Drasar BS. Association of Vibrio cholerae with fresh-water amoebae. *J Med Microbiol* 1992; 36: 303-6.
- 6 Sasnal D, Guhatakurta B, Datta A. Adhesion of Vibrio cholerae 01 to human buccal epithelial-cells in vitro. *FEMS Microbiol Lett* 1987; 48: 335-8.
- 7 Nalin DR, McLaughlin J. Vibrio cholerae colonizes Ascaris lumbricoides of cholera patients. *J Parasitol* 1976; 62: 839-41.
- 8 Huq A, West PA, Small EB, Huq MI, Colwell RR. Influence of water temperature, salinity, and pH on survival and growth of toxigenic Vibrio cholerae serovar 01 associated with live copepods in laboratory microcosms. *Appl Environ Microbiol* 1984; 48: 420-4.
- 9 Shimodori S, Moriya T, Kohashi O, Fanning D, Amako K. Extraction from prawn shells of substances cryoprotective for Vibrio cholerae. *Appl Environ Microbiol* 1989; 55: 2726-8.

- 10 Clemens JD, Sack DA, Harris JR, van Loon F, Chakraborty J, Ahmed F, *et al.* Field trial of oral cholera vaccines in Bangladesh: results from three year follow-up. *Lancet* 1990; 335: 270-3.
- 11 Holmgren J, Czerkins C. Cholera as a model for research on mucosal immunity and development of oral vaccines. *Curr Opin Immunol* 1992; 4: 387-91.
- 12 Levine MM, Pierce NF. Immunity and vaccine development. In: D Barua, WB Greenough III, eds. *Cholera*. New York: Plenum Publishing, 1992: 285-320.
- 13 Finkelstein RA. Combating epidemic cholera [Letter]. *Science* 1992; 257: 862.
- 14 Nalin DR. Cholera, copepods and chitinase. *Lancet* 1976; ii: 958.
- 15 Nalin DR. Cholera research: what next? *Lancet* 1976; ii: 1283-4.
- 16 Colwell RR, Spira WM. The ecology of *Vibrio cholerae*. In: D Barua, WB Greenough III, eds. *Cholera*. New York: Plenum Publishing, 1992: 107-23.
- 17 Huq A, Small EB, West PA, Huq MI, Rahman R, Colwell RR. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. *Appl Environ Microbiol* 1983; 45: 275-83.
- 18 Ali MY. Investigation on fish diseases and parasites in East Pakistan. *Bulletin de l'Office International des Epizooties* 1968; 69: 1517-21.
- 19 Gee JM, Davey JT. Experimental studies on the infestation of *Mytilus-edulis* (L) by *Mytilicola-intestinalis* Steuer (Copepoda, Cyclopoida). *Journal du Conseil International pour l'exploration de la Mer* 1986; 42: 265-71.
- 20 Salmaso S, Greco D, Bonfiglio B, *et al.* Recurrence of pelecypod-associated cholera in Sardinia. *Lancet* 1980; ii: 1124-7.
- 21 Hogans WE. Morphological variation in *Pennella-balaenoptera* and *Pennella-filosa* copepoda pennellidae with a review of the genus *Pennella* oken 1816 parasitic on cetacea. *Bulletin of Marine Science* 1987; 40: 442-53.
- 22 Ueki N, Sugiyama T, Muroga K. *Vibrio* infection in the freshwater shrimp (Palaemon pauceidace) predisposed by a parasitic isopod (*Tachea chinensis*) infestation. *Fish Pathology* 1988; 23: 175-8.
- 23 Huq A, Huq SA, Grimes DJ, O'Brien M, Chu KH, McDowell, *et al.* Colonization of the gut of the blue crab (*Callinectes sapidus*) by *Vibrio cholerae*. *Appl Environ Microbiol* 1986; 52: 586-8.
- 24 Nair GB, Bhadra RK, Ramamurti T, Ramesh A, Pal SC. *Vibrio cholerae* and other vibrios associated with paddy field cultured prawns. *Food Microbiology* 1991; 8: 203-8.
- 25 Ogg JE, Ryder RA, Smith HL. Isolation of *Vibrio cholerae* from aquatic birds in Colorado and Utah. *Appl Environ Microbiol* 1989; 55: 95-9.
- 26 Nalin DR, Robbins-Browne R, Levine MM, Daya V, Noble P. Multiplication of *Vibrio cholerae* in earthworms. *Current chemotherapy and infectious disease: proceedings of the 11th ICC and the 19th ICAAC*. Washington, DC: American Society of Microbiology, 1980: 936-7.
- 27 Riviere F, Kay BH, Klein JM, Sechan Y. Mesocyclops-aspericornis (copepoda) and *Bacillus-thuringiensis* var *israelensis* for the biological control of aedes and culex vectors (Diptera, culicidae) breeding in crab holes, tree holes, and artificial containers. *J Med Entomol* 1987; 24: 425-30.
- 28 Kiiyukia C, Nakajima A, Nakai T, Muroga K, Kawakami H, Hashimoto H. *Vibrio cholerae* non-01 isolated from ayu fish (*Plecoglossus altivelis*) in Japan. *Appl Environ Microbiol* 1992; 58: 3078-82.
- 29 Sakata T, Okabayashi J, Kakimoto D. Variations in the intestinal micro flora of *Tilapia zillii* reared in fresh water and sea water. *Bulletin of the Japanese Society of Scientific Fisheries* 1980; 46: 313-8.
- 30 Islam MS, Drasar BS, Bradley DJ. Survival of toxigenic *Vibrio cholerae* 01 with a common duckweed, *Lemna minor*, in artificial aquatic ecosystems. *Trans R Soc Trop Med Hyg* 1990; 84: 422-4.
- 31 Rojas YA, Hazen TC. Survival of *Vibrio cholerae* in treated and untreated rum distillery effluents. *Water Research* 1989; 23: 103-13.
- 32 Suntharasamaj P, Migasena S, Vongsthongsi U, Supanaranond W, Pitisuttitham P, Suerperanan L, *et al.* Clinical and bacteriological studies of El Tor cholera after ingestion of known inocula in Thai volunteers. *Vaccine* 1992; 10: 502-5.
- 33 Kawai K, Yamamoto S, Kusuda R. Plankton-mediated oral delivery of *Vibrio anguillarum* vaccine to juvenile ayu. *Nippon Suisan Gakkaishi* 1989; 55: 35-40.
- 34 Sack RB. *Helicobacter pylori* infection in developing world [Letter]. *Lancet* 1993; 341: 1274-5.
- 35 Field M. Intestinal electrolyte secretion - history of a paradigm. *Arch Surg* 1993; 128: 273-8.
- 36 Field M, Semrad CE. Toxigenic diarrheas, congenital diarrheas, and cystic fibrosis: disorders of intestinal ion transport. *Ann Rev Physiol* 1993; 55: 631-55.
- 37 Fasano A, Trucksis M, Comstock L, Fiorenti C, Donelli G, Guandali S, *et al.* Cholera-toxin (CT), zonula-occludens toxin (ZOT), and accessory cholera enterotoxin (ACE) - 3 distinct toxins elaborated by the same pathogen. *Gastroenterology* 1993; 104: A247.
- 38 Tamplin ML, Colwell RR, Hall S, Kogure K, Strichartz GR. Sodium-channel inhibitors produced by enteropathogenic *Vibrio cholerae* and *Aeromonas hydrophila* [Letter]. *Lancet* 1987; ii: 975.
- 39 Krasinikov OV, Muratkhodjaev JN, Voronov SE, Yezepchuk YV. The ionic channels formed by cholera toxin in planar bilayer lipid membranes are entirely attributable to its B-subunit. *Biochim Biophys Acta* 1991; 1067: 166-70.
- 40 Muton T, Tokuda A, Guroff G, Fujiki N. The effect of the B-subunit of cholera-toxin on the action of nerve growth-factor on PC12 cells. *J Neurochem* 1993; 60: 1540-7.
- 41 Chang FH, Bourne HR. Cholera-toxin induces cAMP-independent degradation of G_s. *J Biol Chem* 1989; 264: 5352-7.
- 42 Aksamit RR, Backlund PS, Cantoni GL. Cholera toxin inhibits chemotaxis by a cAMP-independent mechanism. *Proc Natl Acad Sci USA* 1985; 82: 7475-9.
- 43 Hyun CS, Kimmich GA. Effect of cholera toxin on cAMP levels and Na⁺ influx in isolated intestinal epithelial cells. *Am J Physiol* 1982; 243: C107-15.
- 44 Macleod KG, Milligan G. Biphasic regulation of adenylate cyclase by cholera toxin in neuroblastoma X glioma hybrid cells is due to the activation and subsequent loss of the α subunit of the stimulatory GTP binding protein (G_s). *Cell Signal* 1990; 2: 139-51.
- 45 Longbottom D, van Heyningen S. Effect of antipsychotic drugs on the molecular action of cholera toxin in rabbit intestinal epithelial cells. *Federation of European Biochemical Societies Letters* 1990; 272: 41-4.
- 46 Janicot M, Clot JP, Desbuquois B. Interactions of cholera toxin with isolated hepatocytes - effects of low pH, chloroquine and monensin on toxin internalization, processing and action. *Biochem J* 1988; 253: 735-43.
- 47 Rabbani GH, Butler T. Indomethacin and chloroquine fail to inhibit fluid loss in cholera. *Gastroenterology* 1985; 89: 1035-7.
- 48 Chen LC, Rohde JE, Sharp GW. Intestinal adenyl-cyclase activity in human cholera. *Lancet* 1971; ii: 939-41.
- 49 Rabbani GH, Greenough WB, Holmgren, Kirkwood B. Controlled trial of chlorpromazine as antisecretory agent in patients with cholera hydrated intravenously. *BMJ* 1982; 284: 1361-4.
- 50 Islam MR, Sack DA, Holmgren J, Bardhan PK, Rabbani GH. Use of chlorpromazine in the treatment of cholera and other severe acute watery diarrheal diseases. *Gastroenterology* 1982; 82: 1335-40.
- 51 Larsson H, Berglund ML, Fryklund J. Chloride-transport inhibitors in vitro do not inhibit intestinal fluid secretion in vivo. *Ann NY Acad Sci* 1989; 574: 491-3.
- 52 Barrett KE, Bigby TD. Involvement of arachidonic acid in the chloride secretory response of intestinal epithelial cells. *Am J Physiol* 1993; 264: C446-51.
- 53 Barret KE, Dharmasathaporn K. Mechanisms of chloride secretion in a colonic epithelial cell line. In: Leberthal E, Duffey M. *Textbook of secretory diarrhea*. New York: Raven Press, 1990: 59-66.
- 54 Farack UM, Kautz U, Loeschke K. Loperamide reduces the intestinal secretion but not the mucosal cAMP accumulation induced by cholera toxin. *Naunyn-Schmiedeberg Arch Pharmacol* 1981; 317: 178-9.
- 55 Hallback DA, Jodal M, Sjoqvist A, Lundgren O. Evidence for cholera secretion emanating from crypts. *Gastroenterology* 1982; 83: 1051-6.
- 56 Nalin DR, Ally K, Hare K, Hare R. Effects of cholera enterotoxin on jejunal osmotic regulation of mannitol solutions in dogs. *J Infect Dis* 1972; 125: 528-32.
- 57 Beubler E, Horina G. 5-HT₂ AND 5-HT₁ receptor subtypes mediate cholera toxin-induced intestinal fluid secretion in the rat. *Gastroenterology* 1990; 99: 83-9.
- 58 Van Loon FPL, Rabbani GH, Bukhake R, Rask-Madsen J. Indomethacin decreases jejunal fluid secretion in addition to luminal release of prostaglandin E₂ in patients with acute cholera. *Gut* 1992; 33: 643-5.
- 59 Cassuto J, Fahrenkrug J, Jodal M, Tuttle R, Lundgren O. Release of vasoactive intestinal polypeptide from the rat small intestine exposed to cholera toxin. *Gut* 1981; 22: 958-63.
- 60 Sjoqvist A, Fahrenkrug J, Jodal M, Lundgren O. The effect of splanchnic nerve stimulation and neuropeptide Y on cholera secretion and release of vasoactive intestinal polypeptide in the feline small intestine. *Acta Physiol Scand* 1988; 133: 289-95.
- 61 Briseid G, Briseid K, Kirkevold K. Increased intestinal absorption in the rat caused by sodium lauryl sulphate, and its possible relation to the cAMP system. *Naunyn-Schmiedeberg Arch Pharmacol* 1976; 292: 137-44.
- 62 Clancy BM, Czech MP. Hexose transport stimulation and membrane redistribution of glucose transporter isoforms in response to cholera toxin, dibutyryl cyclic AMP, and insulin in 3T3-L1 Adipocytes. *J Biol Chem* 1990; 265: 12434-43.
- 63 Kinzie JL, Ferrendelli JA, Alpers DH. Adenosine cyclic 3':5'-Monophosphate-mediated transport of neutral and dibasic amino acids in jejunal mucosa. *J Biol Chem* 1973; 248: 7018-24.
- 64 Hirayama T, Noda M, Ito H, Takeda Y. Stimulation of phosphorylation of rat brush-border membrane-proteins by *Escherichia coli* heat-stable enterotoxin, cholera enterotoxin and cyclic-nucleotides, and its inhibition by protein-kinase inhibitors, isouquinolinesulfonamides. *Microbial Pathogenesis* 1990; 8: 421-31.
- 65 Pines M, Ashkenazi A, Cohen-Chapnik N, Binder L, Gertler A. Inhibition of the proliferation of Nb2 cells by femtomolar concentrations of cholera toxin and partial reversal of the effect by 12-O-tetradecanoyl-phorbol-13-acetate. *J Cell Biochem* 1988; 37: 119-29.
- 66 Schorderet-Slatkine S, Schorderet M, Baulieu EE. Cyclic AMP-mediated control of meiosis: effects of progesterone, cholera toxin, and membrane-active drugs in *Xenopus laevis* oocytes. *Proc Natl Acad Sci USA (Developmental Biology)* 1982; 79: 850-4.
- 67 De Cremonux P, Zimmer A, Calvo F, Lanotte M, Mercken L, Abita JP. G_s availability to cholera toxin-catalysed ADP-riposylation is decreased in membranes of retinoic acid-treated leukemic cell lines HL-60 and THP-1. *Biochem Pharmacol* 1991; 42: 2141-6.
- 68 Irvine FJ, Houslay MD. Insulin and glucagon attenuate the ability of cholera-toxin to activate adenylate-cyclase in intact hepatocytes. *Biochem J* 1988; 251: 447-52.
- 69 Sjoqvist A, Cassuto J, Jodal M, Lundgren O. Actions of serotonin antagonists on cholera-toxin-induced intestinal fluid secretion. *Acta Physiologica Scandinavica* 1992; 145: 229-37.
- 70 Nalin DR, Richardson SH, Islam N, Yardley J. Decrease in enterotoxin-induced jejunal fluid accumulation by acids in dogs. In: Fukumi H, Ohashi M, eds. *Symposium on cholera, Kyoto, Japan, 1974*. Tokyo: Japanese Cholera Panel, US-Japan Cooperative Medical Science Program, NIH. 169-78.
- 71 Challacombe DN, Wheeler EE. Tropic action of epidermal growth factor on human duodenal mucosa cultured in vitro. *Gut* 1991; 32: 991-3.
- 72 Karlstad M, Klindt R, Jones J, Miller R, Fuhr J. Stimulation of intestinal epithelial-cell proliferation with glutamine enriched enteral nutrition in injured rats. [Abstract]. *FASEB J* 1991; 5: 1724.
- 73 Lonroth I, Martinsson K, Lange S. Evidence of protection against diarrhoea in suckling piglets by a hormone-like protein in the sow's milk. *J Vet Med* 1988; B35: 628-35.
- 74 Lange S, Lonroth I. Bile and milk from cholera-toxin treated rats contain a hormone-like factor which inhibits diarrhoea induced by the toxin. *International Archives of Allergy and Applied Immunology* 1986; 79: 270-5.
- 75 Sachdev HPS, Mittal NK, Mittal SK, Yadav HS. A controlled trial on utility of oral zinc supplementation in acute dehydrating diarrhoea in infants. *J Pediatr Gastroenterol Nutr* 1988; 7(6): 877-81.
- 76 Roy S, Drasar BS, Tomkins AM. The impact of zinc-deficiency on the intestinal response to cholera-toxin. *Proc Nutr Soc* 1986; 45: A39.
- 77 Watkins DW, Chenu C, Ripoch P. Zinc inhibition of Na⁺ stimulated glucose-uptake by intestinal brush-border membrane-vesicles [Abstract]. *Fed Proc* 1987; 46: 1081.
- 78 Nalin DR, Cash RA, Islam R, Molla M, Phillips RA. Oral Maintenance therapy for cholera in adults. *Lancet* 1968; ii: 370-3.
- 79 Cash RA, Nalin DR, Rochat R, Reller B, Haque E, Rahman M. A clinical trial of oral therapy in a rural cholera treatment center. *Am J Trop Med Hyg* 1970; 19: 653-6.
- 80 Cash RA, Nalin DR, Forrest J, Abrutyn E. Rapid correction of the acidosis and dehydration of cholera with an oral solution. *Lancet* 1970; ii: 549-50.
- 81 Nalin DR, Cash RA, Rahaman M, Yunus M. Effect of glycine and glucose on sodium and water absorption in patients with cholera. *Gut* 1970; 11: 768-72.
- 82 Nalin DR, Cash RA. Oral or nasogastric maintenance therapy for diarrheas of unknown etiology resembling cholera. *Trans R Soc Trop Med Hyg* 1970; 64: 769-71.
- 83 Pizarro D, Posada G, Mahalanabis D, Sandi L. Comparison of efficacy of a glucose/glycine/glycylglycine electrolyte solution versus the standard WHO/ORS in diarrheic dehydrated children. *J Pediatr Gastroenterol Nutr* 1988; 7: 882-8.
- 84 Myint YY, Maung-U K, Kyaw A, Khine ZT, May KK. Effect of cholera toxin on L-[¹⁴C]glycine uptake and intestinal cell enzymes in rabbit. *Molecular Biology and Medicine* 1991; 8: 129-33.
- 85 Nalin DR. Comparison of oral rehydration solutions [Letter]. *J Pediatr Gastroenterol Nutr* 1989; 8: 272-7.
- 86 Khin-Maung-U, Myo-Khin, Nyunt-Nyunt-Wai, Mu-Mu-Khin, Mya-Thi, Thein-Thein-Myint. Comparison of glucose/electrolyte and maltodextrin/glycine/glycyl-glycine/electrolyte oral rehydration solutions in acute diarrhea in children. *J Pediatr Gastroenterol Nutr* 1991; 13: 397-401.
- 87 Khin-Maung-U, Myo-Khin, Nyunt-Nyunt-Wai. Comparison of glucose/electrolyte and maltodextrin/glycine/glycyl-glycine/electrolyte oral rehydration solutions in cholera and watery diarrhoea in adults. *Ann Trop Med Parasitol* 1991; 85: 645-50.
- 88 Mahalanabis D, Patra FC. In search of a superior oral rehydration solution: can optimum use of organic solute-mediated sodium absorption lead to the development of an absorption promoting drug? *J Diarrhoeal Dis Res* 1983; 1: 76-81.
- 89 Patra FC, Sack DA, Islam A, Alam AN, Mazumder RN. Oral rehydration formula containing alanine and glucose for treatment of diarrhoea: a controlled trial. *BMJ* 1989; 298: 1352-6.
- 90 Agero MET, Uicich R, Carmuega E, O'Donnell AM. Super glutamine oral rehydration solution - its effect on sodium and water-absorption in perfused rat gut [Abstract]. *Pediatr Res* 1989; 26: 165.
- 91 Bhan MK, Gore SM, Grange AN, Jalan KN, Kassem AS, U KM, Mahalanabis D, *et al.* Impact of glycine-containing ORS solutions on stool output and duration of diarrhoea - a metaanalysis of 7 clinical-trials. *Bull World Health Organ* 1991; 69: 541-8.
- 92 Akbar MS, Baker KM, Aziz MA, Khan WA, Salim AFM. A randomized, double-blind clinical-trial of a maltodextrin containing oral rehydration solution in acute infantile diarrhoea. *J Diarrhoeal Dis Res* 1991; 9: 33-7.
- 93 Santos Ocampo PD, Bravo LC, Rogacion JM, Battad GR. A randomized double-blind clinical trial of a maltodextrin-containing oral rehydration solution in acute infantile diarrhoea. *J Pediatr Gastroenterol Nutr* 1993; 16: 23-8.
- 94 Bartels H, Link A, Daniel H, Rehner G. Intestinale freisetzung und resorption von monosacchariden aus kohlenhydraten unterschiedlichen polymerisationsgrades. *Z Ernahrungswiss* 1987; 26: 179-93.
- 95 Lindfors A, Lundberg B, Stenhammar L. Glucose polymers in diarrhoea-risk of hypernatraemia. *Acta Paediatr* 1992; 81: 73-4.
- 96 Food composition table for use in East Asia. A research project sponsored by US Department of Health, Education, and Welfare and the Centers for Disease Control, Health Services and Mental Health Administration and Food and Agriculture Organiza-

- tion of the United Nations, Washington, DC: DHEW Publ No. (NIH) 75-465, Dec 1972, 198-9. Washington DC: US Government Printing Office.
- 97 Kenya PR, Odongo HW, Oundo G, Waswa K, Muttunga J, Molla AM, *et al*. Cereal based oral rehydration solutions. *Arch Dis Child* 1989; **64**: 1032-5.
 - 98 Fore SM, Fontaine O, Pierce NF. Impact of rice based oral rehydration solution on stool output and duration of diarrhoea; meta-analysis of 13 clinical trials. *BMJ* 1992; **304**: 287-91.
 - 99 Khin-Maung-U, Nyunt-Nyunt-Wai, Myo-Khin, Mu-Mu-Khin, Tin-U, Thane-Toe. Effect of boiled-rice feeding in childhood cholera on clinical outcome. *Human Nutr-Clin Nutr* 1986; **40C**: 249-54.
 - 100 Bhan MK, Ghai OP, Khoshoo V, Vasudev AS, Bhatnager S, Arora NK, *et al*. Efficacy of mung bean (lentil) and pop rice based rehydration solutions in comparison with the standard glucose electrolyte solution. *J Pediatr Gastroenterol Nutr* 1987; **6**: 392-9.
 - 101 Auricchio S, DeVizia B, Cucchiara S, D'Antonio AM, deRitis G, Iaccarino E. Use of diet with chicken meat, rice flour, oil, mineral and vitamin in the therapy of severe chronic diarrhoea of newborn with food intolerance. *Rivista Italiana di Pediatria (IJP)* 1985; **11**: 383-92.
 - 102 Khin-Maung-U, Bolin TD, Duncombe VM, Myo-Khin, Nyunt-Nyunt-Wai, Pereira SP, *et al*. Epidemiology of small bowel bacterial overgrowth and rice carbohydrate malabsorption in Burmese (Myanmar) village children. *Am J Trop Med Hyg* 1991; **47**: 298-304.
 - 103 Nalin DR, Harland E, Ramlal A, Swaby D, McDonald J, Gangarosa R, *et al*. Comparison of low and high sodium and potassium content in oral rehydration solutions. *J Pediatr* 1980; **97**: 848-53.
 - 104 Nalin DR. Nutritional benefits related to oral therapy. In: *Acute diarrhea; its nutritional consequences in children*. New York: Bellanti JA, ed. Nestle, Vevey/Raven Press, 1983; 193-7.
 - 105 Cremash D, James PS, Meyer G, Rossetti C, Smith MW. Intracellular potassium as a possible inducer of amino-acid transport across hamster jejunal enterocytes. *J Physiol (Lond)* 1986; **375**: 107-19.
 - 106 Nalin DR. Oral therapy of diarrheal diseases. Projects for future research and development. *Symposium proceedings. Cereal-based oral rehydration therapy: theory and practice*. National Academy of Sciences, Washington, DC: US. February 1987: 37-46. Also published in *J Diarrhoeal Dis Res* 1987; **5**: 283-92.
 - 107 Anonymous. Cereal-based oral rehydration solutions - bridging the gap between fluid and food. [Editorial]. *Lancet* 1992; **339**: 219-20.
 - 108 Heitlinger LA, Sloan HR, DeVore DR, Lee P, Leberthal E, Duffey ME. Transport of glucose polymer-derived glucose by rabbit jejunum. *Gastroenterology* 1992; **102**: 443-7.
 - 109 Khoshoo V, Bhan MK, Jain R, Jayashre S, Bhandari N, Sazawal S, *et al*. Intestinal glucoamylase and other disaccharidases in children with protracted diarrhea. *Indian J Med Res* 1990; **92**: 1-4.
 - 110 Hunt JB, Carnaby S, Farthing MJ. Assessment of water and solute absorption from experimental hypotonic and established oral rehydration solutions in secreting rat intestine. *Aliment Pharmacol* 1991; **5**: 273-81.
 - 111 Nalin DR, Cash RA. The optimal oral therapy formula for cholera and cholera-like diseases. In: *Uses of epidemiology in planning health services: proceedings of the 6th International Epidemiologic Association Meeting, Primosten, Yugoslavia*. Savremena Administracija, Yugoslavia: 1973: 1048-57.
 - 112 Islam MR, Ahmed SM. Oral rehydration solution without bicarbonate. *Arch Dis Child* 1984; **59**: 1072-5.
 - 113 Price HV, Dodge JA, Thomas MK. Oral rehydration without added bicarbonate for childhood gastroenteritis. *BMJ* 1984; **289**: 532.
 - 114 Harvey RM, Enson Y, Lewis ML, Greenough WB, Ally KM, Panno RA. Hemodynamic effects of dehydration and metabolic acidosis in Asiatic cholera. *Trans Assoc Am Physicians* 1966; **Ixxix**: 177-86.
 - 115 Akedo H, Sugawa T, Yoshikawa S, Suda M. Intestinal absorption of amino acids. *J Biochem* 1960; **47**: 124-30.
 - 116 Csaky TZ, Esposito G, Faelli A, Capraro V. Stimulation of the water transport in the jejunum of the rat by ethyl acetate. *Proc Soc Exp Biol Med* 1971; **136**: 242-4.
 - 117 Hazzard CE, Ahearn GA. Rapid stimulation of intestinal D-glucose transport in teleosts by 17 α -methyltestosterone. *Am J Physiol* 1992; **262**: R412-8.
 - 118 Opleta-Madsen K, Hardin J, Gall DG. Epidermal growth-factor up-regulates intestinal electrolyte and nutrient transport. *Am J Physiol* 1991; **260**: G807-14.
 - 119 Debnam ES, Sharp PA. Acute and chronic effects of pancreatic glucagon on sugar transport across the brush-border and basolateral membranes of rat jejunal enterocytes. *Exp Physiol* 1993; **78**: 197-207.
 - 120 Walker-Smith JA. Underutilisation of oral rehydration in the treatment of gastroenteritis. *Drugs* 1988; **36**(S4): 61-4.
 - 121 Niemialtowski M, Klucinski W, Malicki K, Spohr de Faundez I. Cholera toxin (cholera) - polymorphonuclear leukocyte interactions: effect on migration in vitro and Fc γ R - dependent phagocytic and bactericidal activity. *Microbiol Immunol* 1993; **37**: 55-62.
 - 122 Haack BM, Emmrich F, Resch K. Cholera toxin inhibits T cell receptor signaling by covalent modification of the CD3 ζ subunit. *J Immunol* 1993; **150**: 2599-606.
 - 123 Iwaz J, Lafont S, Revillard JP. Elevation of cyclic 3'5' adenosine monophosphate levels by cholera toxin inhibits the generation of interleukin 2 activity. *Cell Immunol* 1986; **103**: 455-61.
 - 124 Kim DK, Nau GJ, Lancki DW, Dawson G, Fitch FW. Cholera toxin discriminates between murine T lymphocyte proliferation stimulated by activators by protein kinase C and proliferation stimulated by IL-2. *J Immunol* 1988; **141**: 3429-37.
 - 125 Asano T, Murai M, Nakamura H. Synergistic effect of cholera toxin with cyclosporine and azathioprine on survival of rat renal allografts. *Transplant Proc* 1993; **25**: 761-2.
 - 126 Patke CL, Orson FM, Barron KS, Rosenblatt HM, Shearer WT. Cholera toxin inhibits immunoglobulin production in a resting and phorbol ester stimulated B cell line by a cyclic AMP dependent mechanism [Abstract]. *Federation Proceedings* 1987; **46**: 1202 (Abs 5158).
 - 127 Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan MR, Huda S, *et al*. ABO blood groups and cholera: new observations on specificity of risk and modification of vaccine efficacy. *J Infect Dis* 1989; **159**: 770-3.
 - 128 Levine MM, Kaper JB. Live oral vaccines against cholera: an update. *Vaccine* 1993; **11**: 207-12.
 - 129 Pierre PG, Lucas G, Van Damme M, Vaerman JP. Recent progress in cholera vaccination. *Acta Gastroenterol Belg* 1992; **LV**: 430-6.
 - 130 Manning PA. Molecular design of cholera vaccines. *Vaccine* 1992; **10**: 1015-21.
 - 131 Azurin JC, Cruz A, Pesigan TP, *et al*. A controlled trial of the effectiveness of cholera and cholera E1 Tor vaccines in the Philippines. *Bull World Health Organ* 1967; **37**: 703-27.
 - 132 Iwanaga M, Nakasone N, Yamashiro T, Higa N. Pili of *Vibrio cholerae* widely distributed in serogroup-O1 strains. *Microbiol Immunol* 1993; **37**: 23-8.
 - 133 Sengupta DK, Sengupta TK, Ghose AC. Major outer membrane proteins of *Vibrio cholerae* and their role in induction of protective immunity through inhibition of intestinal colonization. *Infect Immun* 1992; **60**: 4848-55.
 - 134 Nashar TO, Amin T, Marcello A, Hirst TR. Current progress in the development of the B subunits of cholera toxin and *Escherichia coli* heat-labile enterotoxin as carriers for the oral delivery of heterologous antigens and epitopes. *Vaccine* 1993; **11**: 235-40.
 - 135 Albert MJ, Siddique AK, Islam MS, Faruque ASG, Ansaruzzaman M, Faruque SM, *et al*. Large outbreak of clinical cholera due to *Vibrio cholerae* non-O1 in Bangladesh [Letter]. *Lancet* 1993; **341**: 704.
 - 136 Ramamurthy T, Garg S, Sharma R, Bhattacharya SK, Nair GB, Shimada T, *et al*. Emergence of novel strain of *Vibrio cholerae* with epidemic potential in southern and eastern India. *Lancet* 1993; **341**: 703-4.
 - 137 Kothary MH, Richardson SH. Fluid accumulation in infant mice caused by *Vibrio hollisae* and its extracellular enterotoxin. *Infect Immun* 1987; **55**: 626-30.
 - 138 Rhodes JB, Ogg JE. Isolation of *Vibrio cholerae* from ruminants in western Colorado [Abstract]. *85th Annual Meeting of The American Society for Microbiology*. Las Vegas, Nevada, March 3-7, 1985: 320.
 - 139 Honda T, Arita M, Takeda T, Yoh M, Miwatani T. Non-O1 *Vibrio cholerae* produces 2 newly identified toxins related to *Vibrio parahemolyticus* hemolysin and *Escherichia coli* heat-stable enterotoxin [Letter]. *Lancet* 1985; **2**: 163-4.
 - 140 Prasad R, Chopra AK, Peterson JW, Pericas R and Houston CW. Biological and immunological characterization of a cloned cholera toxin-like enterotoxin from *Salmonella typhimurium*. *Microbial Pathogenesis* 1990; **9**: 315-29.
 - 141 Nalin DR, Bhattacharjee AK, Richardson SH. Cholera-like toxic effect of culture filtrates of *Escherichia coli*. *J Infect Dis* 1974; **130**: 595-601.
 - 142 Nalin DR, McLaughlin JC, Rahman M, Yunus M, Curlin G. Enterotoxigenic *Escherichia coli* and idiopathic diarrhea in Bangladesh. *Lancet* 1975; **ii**: 1116-9.
 - 143 Rose JM, Houston CW, Copenhaver DH, Dixon JD, Kurosky A. Purification and chemical characterization of a cholera toxin-cross-reactive cytolytic enterotoxin produced by a human isolate of *Aeromonas hydrophila*. *Infect Immun* 1989; **57**: 1165-9.
 - 144 Lupiani B, Baya AM, Magarinos B, Romalde JL, Li T, Roberson BS, *et al*. *Vibrio mimicus* and *Vibrio cholerae* non-O1 isolated from wild and hatchery-reared fish. *Gyobyo Kenkyu* 1993; **28**: 15-26.
 - 145 Islam MS, Alam MJ, Neogi PKB. Seasonality and toxigenicity of *Vibrio cholerae* non-O1 isolated from different components of pond ecosystems of Dhaka City, Bangladesh. *World Journal of Microbiology and Biotechnology* 1992; **8**: 160-3.
 - 146 Savarino SJ, Fasano A, Watson J, Martin BM, Levine MM, Guandalini S, *et al*. Enteroregative *Escherichia coli* heat-stable toxin I represents another family of E coli heat stable toxin. *Proc Natl Acad Sci USA* 1993; **90**: 3093-7.