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Mutant *V. cholerae* Strains Found To Cause Disease

In a discovery with vital implications for human health, vaccine development and environmental contamination, scientists at an international research institute have isolated a new potent cholera toxin.

Though unlike known cholera toxin in most respects, the newly-found substance produces cholera-like effects in laboratory tests and infant rabbits–suggesting it may do likewise in man.

The new toxin is manufactured by marine-living mutant strains of *Vibrio cholerae* bacteria, strains that had been widely considered incapable of causing cholera, because they lack the gene which produces previously recognized cholera toxin. Consequently, in recent years, these strains–as well as similar, genetically engineered varieties–have been considered prime candidates for an oral cholera vaccine.

However, say scientists at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) where the discovery was made, such mutant, so-called "cholera-toxin gene-deficient" varieties appear not to be useful for a cholera vaccine–since they may cause diarrhoea in humans.

Moreover, they add, the mutant strains may be doubly dangerous. For tests show that each time these varieties pass through an animal system, they produce a more potent toxin, as well as more of it. Thus, the mutants potentially could pose an ever increasing hazard, were they to become a widespread human affliction.

Unfortunately, this last point is possible, the scientists maintain. For over the past decade, perhaps 80 of these mutant strains have been found in such diverse places as the U.S. Gulf Coast, Great Britain, the U.S.S.R., Guam, Brazil, Japan, India and Australia. The strains have been isolated from such marine sources as sewage, oysters and brackish water, as well as from intestinal and other human infections.

"Unfortunately," says Dr. S.C. Sanyal, an Indian microbiologist who headed the ICDDR,B research team, "until now such strains had not been recognized as a cause of cholera–even though, for almost a decade, numerous cholera cases had been attributed to unexplainable contamination of the very types of marine environments where the mutant *V. cholerae* varieties were known to exist.

"Especially in Western Europe and the U.S., which have been free of cholera epidemics for decades, minor cholera outbreaks were thought to have been caused by bacteria that somehow had been imported to the area. No one believed the bacteria to be indigenous. This was the case with minor outbreaks in Louisiana and Texas that began in 1973, continued each year, and culminated

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Mutant *V. cholerae* strains, so far believed to be harmless to humans, not only produce disease, but also gain virulence as they pass through animal hosts.

in a total of 31 cases in 1981 alone.

"The first scientific report to the contrary appeared in 1980, concerning a mini-outbreak in Louisiana in 1978. There the disease was traced to cooked crabs from local marshes. Investigators concluded that the responsible bacteria had been indigenous to the area for at least five years.

"Nevertheless," he continues, "most scientists did not connect this finding with the toxin gene-deficient, mutant cholera strains found in marine environments. In fact, based on certain standard tests for known cholera toxin, it was widely believed that such *V. cholerae* strains were non-toxigenic to man–and thus critically different from the varieties long known to be a major cause of human death and debility."

Questioning this premise were Dr. Sanyal and other scientists at the ICDDR,B–the only international facility devoted solely to studying the causes, preventives, treatments and cures for the many diarrhoeal diseases which annually claim an estimated five million lives worldwide, mostly of young children. Cholera is the most deadly of the diarrhoeal diseases.

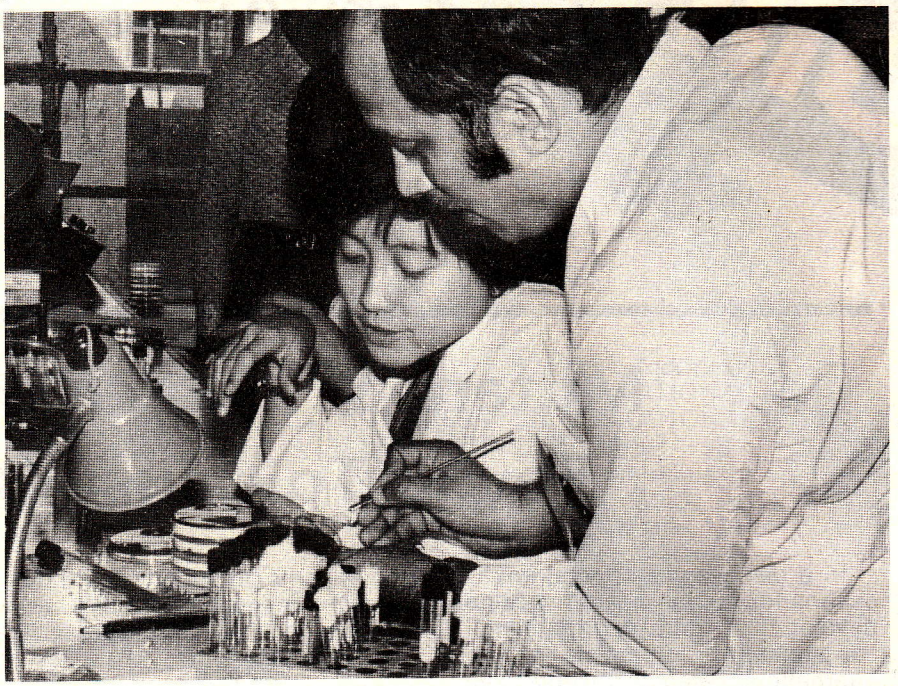
About a year ago, Dr. Sanyal's team discovered the new toxin, and subsequently their findings were confirmed by scientists at Johns Hopkins University. On June 11, 1983, the ICDDR,B team outlined its findings in a letter to the editor of the prestigious British medical journal "Lancet." More details are in press.

"We discovered the mutant strains toxigenicity or toxin-producing capacity," says Dr. Sanyal, "because we started with a controversial hypothesis: that *V. cholerae* strains found in marine environments might not be harmless to humans. To understand our work, some background is necessary."

As Dr. Sanyal explains, before the advent of genetic analysis and manipulation, different researchers used diverse methods to measure a given bacterium's toxigenicity. For various reasons there was no international standard technique; and, depending on diverse factors, the results varied from laboratory to laboratory. Hence, the results were unreliable.

In recent years genetic techniques, widely considered more reliable, were developed as toxigenicity indicators. Thus, scientists sought to discover the gene responsible for a given toxin; and then to determine toxigenicity based on the presence or absence of such a gene.

"Previous researchers began with an erroneous assumption, and therefore reached a wrong conclusion," he contends. "They assumed the existence of only one type of enterotoxin, produced by a specific *V. cholerae* gene.



Dr. S.C. Sanyal (right), an Indian microbiologist now working at the ICDDR,B, found that a mutant strain of *V. cholerae*—widely considered to be incapable of causing cholera and a serious candidate for oral vaccine—produces toxin, and causes cholera in humans.

"The gene responsible for the toxin then known to cause cholera in man thus was found," says Dr. Sanyal. "The next step, many researchers believed, was to discover, or engineer, *V. cholerae* strains that lacked the culprit toxin gene–but had another important gene, one that would allow the toxin to attach itself inside the human gut, thus mimicking cholera and stimulating an antibody response capable of preventing the disease."

A few years ago, he continues, it was discovered that diverse mutant *V. cholerae* strains, found in various marine environments, did not contain the only recognized cholera toxin gene; and, thus, failed to produce the known cholera toxin. However, these mutant strains did have the gene necessary for the toxin to colonize the human intestines.

Therefore, it was concluded that such toxin gene-deficient, marine-borne *V. cholerae* varieties were ideal candidates for a cholera vaccine. However, at that point, says Dr. Sanyal, a crucial error was made. Thus, they tested for the known toxigenic gene and the known toxin–thereby missing the presence of a different gene, producing a different toxin.

"Given the mini-cholera outbreaks in various parts of the world, and extensive work with oral cholera vaccines using mutant varieties," Dr. Sanyal explains, "we decided to more closely examine some of the toxin gene-deficient strains."

The scientists tested 12 marine-related mutants, using bacteria from a variety of sources, including from an apparently contaminated seafood-related diarrhoea case in Florida. They also tested one laboratory-engineered mutant variety, Texas Star-SR, considered a possible vaccine candidate.

Using various animal, tissue culture and immunological assays, they sought to determine whether the supposedly non-toxic strains would produce cholera symptoms: a secretory response in the small

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intestine, leading to fluid accumulation and/or diarrhoea.

All 13 strains caused fluid accumulation in rabbit tissue tests, indicating that the bacteria produce a toxin. Moreover, all strains caused fluid accumulation in infant rabbits' large intestines; and most of the strains caused diarrhoea in rabbits, leading to dehydration—the diarrhoea consequence that often kills. Other tests for cholera, including increased permeability factor (PF) of rabbits' bare skin, confirmed the presence of cholera.

The scientists also studied the new toxin's nature:

*To compare the old and new toxins' antigenicity, a proven antibody against known cholera toxin was used with the new toxin. Attempts were made to neutralize fluid accumulation, and skin PF, as well as to precipitate the toxin. Both tests failed, showing the new toxin to be antigenically different.

*To test relative modes of action, morphological changes in tissue cultures were compared. The new toxin did not cause the types of cell changes associated with the known toxin.

*Using an ELISA test for receptor site, the toxins were shown to bind to different points on animal gut cells.

*Culture filtrates were tested in a suckling mice assay, and found to be heat-labile. This, combined with an amonium sulfate precipitation test, shows the new toxin to be a heat-destroyable protein. Only in this last way are the two toxins similar.

Reviewing his team's findings, Dr. Sanyal has the following to say.

"Our results indicate that the toxin gene-negative cholera strains isolated from marine sources and human infections do produce a toxin, albeit a previously unknown one. Therefore, while these mutant strains lack the gene for known cholera toxin, they must have a different toxin-governing gene, located at an unknown point on the bacterial chromosome.

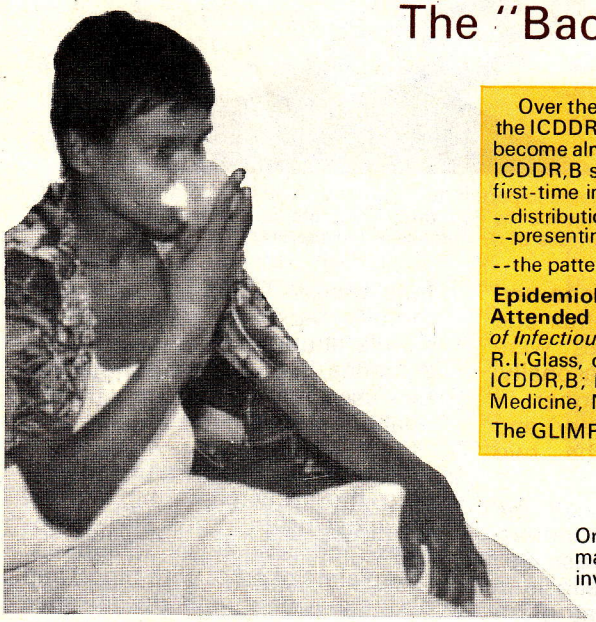
"As to the effects of the mutant bacteria on humans, we believe that the Florida diarrhoea case may have been caused by seafood contaminated by the toxin gene-deficient strain we tested. Moreover, we found that the lone laboratory-tailored strain caused diarrhoea in a number of human volunteers fed the bacteria. This would indicate that such man-made strains also must be examined more closely."

Finally, he explains, the ICDDR,B researchers tested for "enterotoxigenic activity" or virulence. The result: the virulence of toxins produced by all strains increased greatly after the organisms had been passed twice through the animals guts—and the total toxin produced each time also went up.

"This enhancement of enterotoxigenicity may suggest," maintains Dr. Sanyal, "that if such an organism is fed to humans in a vaccine and is excreted in nature, it may be ingested by others—resulting in further increases in virulence.

"Overall," Dr. Sanyal concludes, "I think our findings are a warning that the immunobiologic and genetic aspects of gene-deficient *V. cholerae* organisms must be studied in much greater detail, in order to develop a safe, effective living vaccine."

The "Backlash" Diarrhoeas



Oral rehydration therapy alone may not be successful in cases of invasive diarrhoeas.

Over the past 10 years, the number of patients coming to the Dhaka Treatment Centre of the ICDDR,B, has gone up from more than 10,000 to more than 100,000 per year. It now has become almost impossible to collect useful information on all these patients for surveillance. ICDDR,B scientists examined a representative sample of all these patients, perhaps for the first-time in a hospital-based epidemiological study, to examine:

--distribution of patients with shigellosis, by age, sex, and season of infection;
--presenting signs and symptoms, the course of illness and treatment during hospitalization;
--the patterns of antibiotic resistance of *Shigella* isolates.

Epidemiological and Clinical Features of Patients Infected with *Shigella* Who Attended a Diarrhoeal Disease Hospital in Bangladesh was published in the *Journal of Infectious Diseases*. Vol. 146, No. 2: August 1982. It was authored by B.J. Stoll, ICDDR,B; R.I. Glass, on assignment to ICDDR,B from Centers for Disease Control, USA; M.I. Huq, ICDDR,B; M.U. Khan, ICDDR,B; H. Banu, ICDDR,B; and J. Holt, Mount Sinai School of Medicine, New York, USA.

The GLIMPSE version appearing in this issue has been adapted from the published paper.

With the development of oral rehydration therapy, deaths due to diarrhoea have been drastically reduced. However, while ORT is very effective in cases of watery, dehydrating diarrhoea, additional antibiotic therapy may be crucial for dysentery-type "backlash" diarrhoeas, and a simple, reliable way of identifying the responsible pathogen is important.

So conclude ICDDR,B researchers in a study that not only pinpoints at least some shigellosis as a "backlash" diarrhoea, but makes two other interesting contributions.

The researchers note that *Shigella's* importance as an enteric pathogen in Bangladesh only has been recognized relatively recently, as more emphasis has been given to non-cholera diarrhoeas. Thus, detailing the results of a study of 3,550 hospitalized diarrhoea patients from Dhaka city, the scientists report finding *Shigella* the second most common pathogen in all patients over age two and the fourth most common overall.

Moreover, their most important finding was that:

Shigellosis may manifest intself one of two ways: as a watery diarrhoea characterized by much

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vomiting and severe dehydration for a relatively short time before hospitalization; or as the dysentery-type, "invasive" diarrhoeal disease it's classified as, causing severe abdominal pain, and blood and mucous in the stool for a relatively longer time before hospitalization.

Whether these are two distinct disease mechanisms or two stages in the same disease, the scientists say, it's important to note that different treatments—and hence fast, accurate diagnosis—are needed. To facilitate such diagnosis the researchers describe useful signs and symptoms.

Meanwhile, in an interesting sidelight, the scientists found that breast feeding appears to reduce the incidence and severity of shigellosis in infants under age one–thereby apparently explaining a considerably lower *Shigella* prevalence found in this age group.

Moreover, their research protocol may be an important first for hospital-based studies in a developing country. For in what is perhaps a unique experiment, the researchers successfully used a 4 percent systematic sample of about 100,000 persons hospitalized over a year's time, to assess the epidemiologic and clinical characteristics of patients suffering from a group of related ailments.

Three things thus were accomplished. Surveillance helped define the range of diseases among patients; and identified areas where patient care could be improved, thus generating new research ideas. In addition, the study showed that successful hospital-based epidemiology is possible, using a sampling technique that takes less time and money than traditional hospital experiments.

The investigators sought to identify the relative importance of various enteropathogens in urban Dhaka, and to determine the seasonality and range of diseases associated with these agents.

To do this, a surveillance system was set up at the ICDDR,B's Dhaka Hospital; and for a year beginning December 1, 1979, a representative sample of 3,550 diarrhoea patients was studied. Of these, 25 percent were under age one, 37 percent between one and five, and 38 percent five or older. Sixty percent were male.

A recognized pathogen was found in 66 percent of patients screened for all pathogens, one-third of whom had a mixed infection of two or more agents. *E. coli* was most often detected (20%), followed by rotavirus (19%), *Campylobacter jejuni* (14%) and *Shigella* (12%).

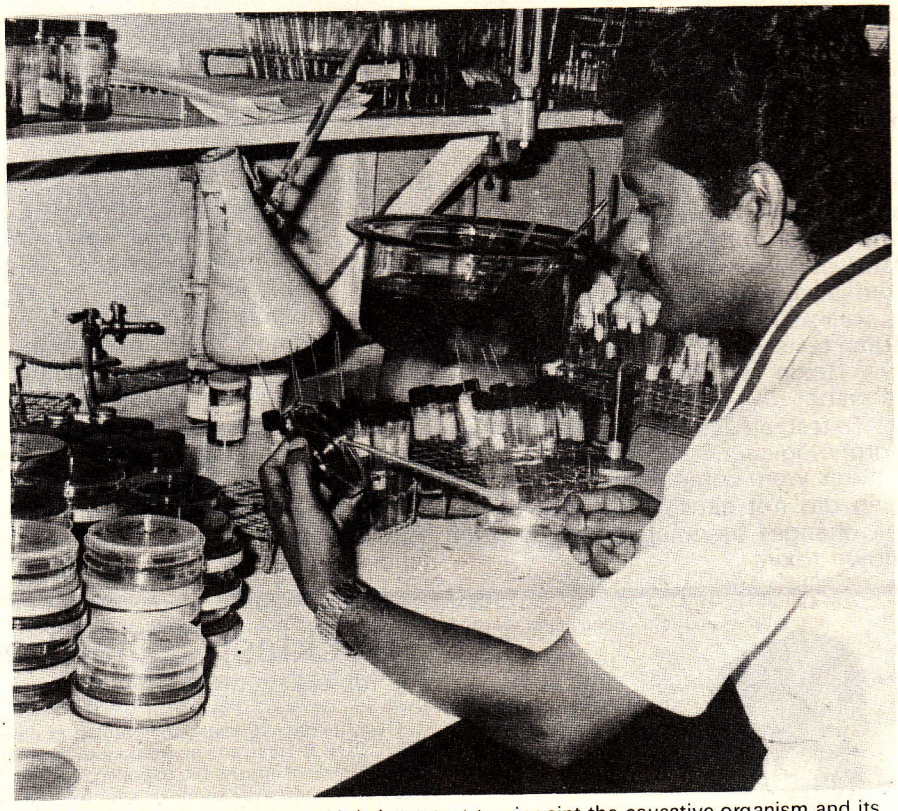
Shigella was found most frequently in people over age 60, least in children under age one, and equally frequently in all other age groups. Also, both sexes were similarly affected. Seasonally, *Shigella* infections were higher in the winter (October–January) and summer (April–May), the two driest periods.

The researchers examined the widely-held theory that poor nutrition and failure to breast-feed increase the risk of shigellosis in children. They found that nutritional status apparently is unrelated to shigellosis in children under age eight–but that children under two with *Shigella* were less often breast-fed than children without *Shigella* (59% versus 78%).

Moreover, in under-two children with shigellosis, those who were breast–fed were less ill: fewer needed IV therapy (16% versus 38%), fewer were hospitalized (5% versus 19%), and fewer were significantly malnourished (34% versus 48%).

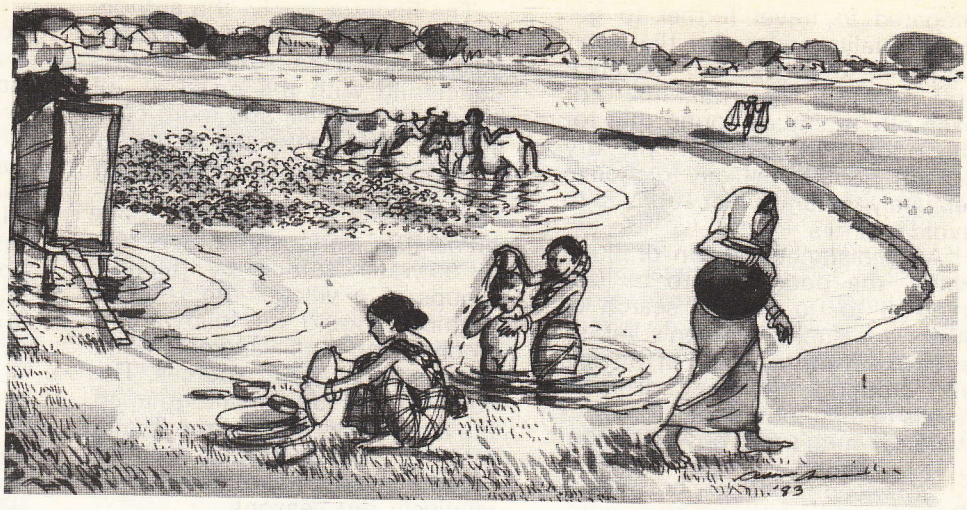
Finally, addressing the issue of "backlash" diarrhoeas, the scientists noted that it's important for a doctor to pinpoint a precise etiology for dysentery-type diarrhoeas, since oral rehydration therapy will not prevent the invasive dysenteric phase. Therefore, they said, models must be developed for simple etiologic diagnoses of dysenteries, in a field setting that can be used by minimally trained health workers.

Working from their preliminary data the researchers highlighted the most useful signs and symptoms for shigellosis diagnosis: complaints of stool with blood and abdominal pain, and the absence of watery diarrhoea and vomiting in patients over one-year-old. Finally, they concluded, the presence of visible blood, an observation readily made, correctly identified in their study 47% of patients with shigellosis. ■



In case of "invasive" diarrhoeas, it is important to pinpoint the causative organism and its resistance pattern, in order to prescribe the right drug. Therefore, it is important to develop models for simple etiologic diagnoses of dysenteries in field settings.

WHAT DETERMINES VILLAGERS' CHOICE OF WATER SOURCES ?



People will not necessarily use better quality water for drinking, cooking, etc., even if it's readily available and they're educated about its health benefits—unless the users *perceive* the water to be good.

So conclude ICDDR,B researchers, as a result of a study with major implications for changing water use patterns, and hence improving health in Bangladesh and other developing countries.

The findings challenge the widely–held belief that failure of efforts to induce poor, mostly illiterate rural populations to use improved water supplies are due to technical design deficiencies or lack of health awareness. Moreover, the study results question the wisdom of limiting water supply programs to installation of hand-pumped tubewells, which people often find hard to use and which may provide water considered unsuitable for cooking.

Finally, the results imply that the *distance* of water sources is not as important as is widely believed, but the *quarrels* over water ownership and access are more important to people's water use choices.

The solution, say the researchers, is not better engineering and health education, as well as narrowly–based localized water improvement schemes—but a more subtle understanding of existing water use patterns and preferences. This would enable planners to predict how a populace will respond to a particular water source, what they would use it for, and how such use would affect health.

This study was designed and data was collected when the authors–John Briscoe, Manisha Chakraborty and Shahana Ahmed–were employed by the Cholera Research Laboratory (now the International Centre for Diarrhoeal Disease Research, Bangladesh). **How Bengali Villagers Choose Sources of Domestic Water** was published in Water Supply and Management, Vol 5, pp. 165–181, Pergamon Press Ltd. 1981. The investigators conducted the study in Panipur, located in the deep-water flooding plain of the Meghna River in Bangladesh. Since knowledge of water use perceptions and patterns is very rudimentary, the most fruitful method in the primary stage of most economic and sociological** investigations–in-depth analysis of closely observed samples–was used. Out of the village's 365 families, 180 were selected for the in-depth study of about a year's duration, Village water sources constantly changed with seasonal variation, so that, every month, up-to-date water sources were marked on a map, and their distances from the study families also were updated.

**Lipton, M and Moore, M. (1972) The methodology of village studies in less-developed countries, Discussion Paper Number 10, Institute of Development Studies, Brighton, Sussex.

As the study results show, the three main variables affecting choice of water for diverse uses were, in order of importance, "quality" (taste and odor), "distance" and the possibility of "quarrels". The significance of these factors varied from rich to poor, from season to season, and depending on whether water was to be used for drinking or other purposes.

Drinking Water

Looking first at drinking water, the researchers found that, for rich and poor alike, the most powerful determinant affecting choice was *quality,* not *distance,* as often is assumed. For all peoples in all seasons, "perceived quality" has a greater effect on choice of water for drinking than for any other purpose.

As to distance, so important is *quality* that people often will travel relatively far to obtain what they perceive to be good quality drinking water. Moreover, for drinking water, during the relatively dry pre-and post-monsoon periods, the richer people are, the more willing they are to travel for good quality water. On the other hand, during such relatively water scarce periods, the poor, but not the rich, will travel to avoid quarrels in their search for good quality drinking water.

Thus, the researchers point out, of interest is not distance per se, but how distance interacts with other factors.

Non-Drinking Water

Turning to water for non-drinking purposes, the researchers found that, the year-round, the rich are ▶

prepared to travel further to get good quality water. For the poor, however, quarrels and distance are powerful determinants. For example, the study found that for the poor, particularly in the dry season, people are likely to choose relatively distant water sources, to avoid quarrels.

As for distance and non-drinking water, the poor tend to be less inclined to go afar in search of water for such "women's tasks" as cooking and washing of utensils and clothing. However, distance is less of a factor when it comes to water for men's and boy's bathing.

Conclusion

In summary, then, the study found that, for drinking water, *quality,* but not *distance,* is a powerful determinant of the attractiveness of a water source; and conflicts over access to water supplies adversely affect the poor's choices.

The results, the researchers maintain, have important implications for improving drinking water quality in rural Bangladesh. For to date, a joint UNICEF/ Bangladesh Government water supply program has assumed that the most important factor governing drinking water use is distance from the home.

The findings imply, the authors maintain, that water supply programs should be somewhat less concerned with the *distance* of improved sources, and more concerned with providing a *quality* of water *perceived* to be good by the users.

Furthermore, they say, since *ownership* seriously affects use of a water source by different groups in a village, the UNICEF example should be followed, in emphasizing public and communal ownership of water sources. This conclusion is further strengthened, the researchers add, by the finding that, for poorer groups, quarrels are the most important factor in relatively dry seasons.

Turning to other studies done on the effects of water use on cholera and other diarrhoeal diseases in rural Bangladesh, the authors of the current research point out that if people are induced only to *drink* better quality water, there will be little benefit to health*. While the reasons for this finding are unclear, they maintain that two other changes appear necessary if water supply programs are to improve health: people must use greater quantities of water for domestic purposes, and better quality water for non-drinking purposes, especially for cooking and bathing**.

These findings, the researchers, conclude, strongly argue against a water supply program, such as the one existing in rural Bangladesh—programs exclusively devoted to installation of hand-pumped tubewells. Because study-area tubewell water is high in iron and turns rice black when used for cooking, no study family used tubewell water for cooking, the researchers point out. Moreover, because pumping a tubewell is hard work, no one in the study area (including the investigators) used a tubewell, instead of a surface water source, for bathing.

It is essential, the researchers conclude, that the scope of water supply programs be broadened, to include technologies other than hand-pumped tubewells. ■

*Curlin GT, Aziz KMA, Khan MR. The influence of drinking tubewell water on diarrhoea rates in Matlab Thana, Bangladesh. Dacca : Cholera Research Laboratory, 1977.

Khan MU, Mosley WH, Chakraborty J, Sarder AM, Khan MR. Water sources and the incidence of cholera in rural Bangladesh. Dacca : Cholera Research Laboratory, 1978.

Levine RJ, Khan MR, D'Souza S, Nalin DR. Failure of the sanitary wells to protect against cholera and other diarrhoeas in Bangladesh. *Lancet* 1976;2:86–89.

**Briscoe J. The role of water supply in improving health in poor countries (with special reference to Bangladesh). *Am J Clin Nutr* 1978;31:2100–13.

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ABSTRACTS

Rahaman MM, Wahed MA. Direct nutrient loss and diarrhea. In : Chen LC, Scrimshaw NS eds. Diarrhea and malnutrition; interactions, mechanisms, and interventions. New York: Plenum, 1983:155-160

Renewed attention has been given to the infection-nutrition relationship. Diarrhoeas affect nutrition in various ways, one of the important mechanisms being the direct loss of nutrients in the gut.

This chapter describes a method whereby alpha–1 antitrypsin is used to measure the loss of protein in the gut, especially in the case of invasive diarrhoeas; and attempts to explain the disease process. Alpha–1 antitrypsin, an inhibitor of proteolytic enzymes found in measurable quantities in the serum, is resistant to further breakdown once secreted into the gut; and the quantity can be measured by the radial immunodiffusion technique. By measuring alpha–1 antitrypsin's concentration simultaneously in faeces and serum, and calculating the ratio between the two, quantitive loss of protein in the gut can be estimated.

In healthy subjects and in sicknesses not involving protein losing enteropathy, the ratio was found to be < 1. This method offers theoretical and practical advantages over the time-honoured ratio-isotope methods used to diagnose both protein-losing enteropathy and to measure protein loss during diarrhoea. The radioisotope method was complicated and costly, and did not offer the opportunity to observe day-to-day changes in the loss of serum protein. Above all, it was not found to be very accurate.

The alpha-1 antitrypsin method does not use any hazardous radioisotopes, and allows a more precise estimation of serum protein loss over a shorter period of time. Other distinctive advantages are its low cost, simplicity, and the ▶

chance to make day-to-day estimations heretofore impossible.

The researchers found that in *Shigella,* several factors may be responsible for the loss of serum protein. The two most important are through ulcerated and colonic muscosa; and due to endotoxemia, which is seen in severe shigellosis and which may interfere with hepatic functions of synthesizing the bulk of plasma protein, mainly albumin. Moreover, protein-losing enteropathy was detected in rotavirus, the main cause of diarrhoea in children aged six-months-to-two-years. Rotavirus cause patchy mucosal damage in the upper intestine, sometimes lasting weeks; and protein is lost in the gut, possibly through exudation and shedding of the mucosal epithelium. Protein loss is manifest only when secretion exceeds absorptive capacity, and partial destruction of the epithelial cells in rotavirus was seen to contribute to a great extent to protein loss.

Although the LT toxin of ETEC behaves like cholera, where protein loss in the stool is minimum, it was found that two-thirds of patients with ETEC demonstrated excessive protein loss in the gut. Since most ETEC infections in Bangladesh produce ST or ST/LT toxins, the loss of significantly high quantities of protein in ETEC diarrhoea may indicate epithelial damage, as in the case of rotavirus diarrhoea or with ST toxin. The fact that, even after two weeks of clinical recovery from ETEC diarrhoea, intestinal absorptive capacities are not fully restored, supports this speculation. Use of alpha–1 antitrypsin as a marker protein to calculate the loss of protein in the gut, combined with longitudinal studies of intraluminal and cellular enzymes in the gut, correlated with the histologic appearance of the gastrointestinal tract, should reveal the pathogenesis of malnutrition in a large proportion of patients with diarrhoeal disease.

Molla A, Molla AM, Sarker SA, Khatoon M, Rahaman MM. Effects of acute diarrhea on absorption of macronutrients during disease and after recovery. In: Chen LC, Scrimshaw NS eds. Diarrhea and malnutrition; interactions, mechanisms, and interventions. New York: Plenum, 1983:143-54

The aim of the present study was to determine the effect of diarrhoea caused by rotavirus, *Shigella,* and enterotoxigenic *Escherichia coli* (ETEC) on net absorption of fat, calories, nitrogen and carbohydrate during acute diarrhoea and 2 and 8 weeks after discharge. Twenty-nine male children aged under 5 years, with a history of acute, watery diarrhoea and moderate to severe dehydration without any obvious complication admitted to the hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh, were selected at random for the study. Of the 29 patients, 13 had rotavirus infection, 10 had *E. coli,* and 6 had *Shigella.* The patients, after intravenous rehydration and an overnight fast, were fed 5 g of D-xylose in 100 ml of water. Four properly weighed meals, with various combinations of rice, chicken, lentils, oil, egg, milk, butter, sugar, banana and bread, were offered daily *ad libitum* to each patient at regular intervals. Samples of all types of foods, breast milk from mothers, aliquots from stool specimens of patients and vomitus were analyzed for calories (with adiabetic bomb calorimeter), fat (by Van de Kamer procedure) and nitrogen (by micro-Kjeldahl procedure). Serum xylose estimation was done by method of Roe and Rice. Coefficients of absorption of nutrients both in the acute stage and at the recovery period were calculated by using the expression: (Intake–Output)×100/Intake. The results indicated several differences with regard to the absorption of nutrients during acute and recovery stages of diarrhoea, depending on aetiology. In the acute stage of rotavirus, absorption of all the nutrients was significantly lower compared to that in patients with ETEC diarrhoea. Absorption of nitrogen in rotavirus did not improve, even after 8 weeks of recovery, suggesting a period of prolonged malabsorption. The study demonstrated that 74% of ingested carbohydrate was absorbed in the acute stage of rotavirus infection, compared to 92% absorption in ETEC and 77% in *Shigella* diarrhoeas. During the acute stages of ETEC diarrhoea, absorption of all nutrients was better than in diarrhoea of other aetiologies, but failed to improve after 2 weeks of recovery. During the acute stage of *Shigella*-induced diarrhoea, absorption of nitrogen was minimum (41%) compared to the absorption of other nutrients (62 to 77%). While all these values became significantly higher after 2 weeks of recovery, xylose absorption was found to be much lower (below 20 mg% in rotavirus and ETEC diarrhoea, 37 mg% in *Shigella)* than the absorption of carbohydrates, which indicated that xylose absorption does not reflect the absorption of a natural carbohydrate. The study concluded that, despite reduced absorption of nutrients, feeding of children should be encouraged, because substantial absorption does take place even in acute diarrhoeal infection. This might help in the prevention of more seriously compromised nutritional status.

Molla AM, Molla A, Sarker SA, Rahaman MM. Food intake during and after recovery from diarrhea in children. In: Chen LC, Scrimshaw NS eds. Diarrhea and malnutrition; interactions, mechanisms, and interventions. New York: Plenum, 1983:113–23

The chapter reviews available data on intake of food during diarrhoea and the impact of diarrhoea on the growth of children; and presents the results of a study on the quantitative estimation of nutrient intake during acute diarrhoea due to various causes and after recovery, the pattern of intake of nutrients in the acute and convalescent stages of diarrhoea and after recovery, and the effect of food intake on stool volume during diarrhoea. Sixty-three children aged below 5 years were studied. *Vibrio cholerae* was isolated in 29, rotavirus in 15, enterotoxigenic *Escherichia coli* in 13 and *Shigella* in 6. The intake was ▶

measured during acute diarrhoea, and 2 and 8 weeks after recovery. Based on information available in the literature and on data from the present study, it was concluded that: (1) irrespective of aetiology there is about a 30% reduction in food intake during diarrhoea among hospitalized patients; (2) feeding, including breast feeding, should be continued even during the acute phase of diarrhoea; and (3) food has no apparent deleterious effect on the volume or duration of diarrhoea, but this requires further study.

Hossain MM, Glass RI, Khan MU, Huq F, Hierholzer JC. Outbreak of enterovirus 70 conjunctivitis in Bangladesh. Trans R Soc Trop Med Hyg 1983; 77(2):217-8.

An outbreak of acute haemorrhagic conjunctivitis (AHC) was observed in Dacca (now Dhaka), Bangladesh from April to July 1981. The high prevalence of this illness led to investigation of the epidemiological and clinical features and the causative organism. Patients with AHC visiting the ICDDR,B's staff clinic, and the Dhaka Medical College Hospital eye clinic were reviewed. Twenty-one percent of the ICDDR,B's 600 staff had eye symptoms clinically diagnosed as AHC. In 28 AHC patients clinical features were redness of the eyes (100%), discharge (96%), foreign body sensation (93%), blurred vision (11%) and subconjunctival haemorrhage (39%). Of the 135 family members of the 28 AHC patients 63% were also affected, with attack rates among different ages ranging from 40% to 72%. Conjunctival swabs from 19 patients aged 12–55 years were processed. The virus was recovered only from a 12–year–old boy, whose specimen had been taken 24 hours after onset of symptoms. The isolate was identified as enterovirus type 70 (EV 70), by serum neutralization tests using antiserum to prototype EV 70, done by the Centres for Disease Control (CDC), Atlanta. The study confirmed that EV 70 strains caused the AHC outbreaks. This is one of the few reports documenting isolation of EV 70 during the recent AHC pandemic. ■

Forthcoming Meetings and Conferences

London, U.K.
SEVENTEENTH TRIENNIAL CONFERENCE 3rd–10th. September, 1983. Organized by Institute of Medical Laboratory Sciences. Information from: Institute of Medical Laboratory Sciences, 12 Queen Anne Street, London WIM OAU, U.K.

Tokyo, Japan
CONFERENCE OF THE WORLD ASSOCIATION OF SOCIETIES OF PATHOLOGY 10th–14th. October, 1983. Organized by the World Association of Societies of Pathology. Information from: Dr. T. Kawai, Jichi Med. School, Minami-Kawachimachi, Techigi-Ken, 239–04, JAPAN

Jerusalem, Israel
FOURTH INTERNATIONAL CONGRESS OF THE WORLD FEDERATION OF PUBLIC HEALTH ASSOCIATIONS "Quest for Community Health: Experience in Primary Care" 19th-24th. February, 1984. Organized by the World Federation of Public Health Associations. Information from: WFPHA Secretariat, C/O APHA, 1015 15th. Street, N.W. Washington, D.C. 20005, U.S.A.

Auckland, New Zealand
NINTH INTERNATIONAL HEALTH RECORDS CONGRESS 13th–18th May, 1984. Organized by the International Federation of Health Records Organization (IFHRO). Information from: Mr. Gerald Wakely, 9th. International Health Records Congress, P.O. Box No. 7151m Auckland, NEW ZEALAND.

Surabaya, Indonesia
SECOND INTERNATIONAL CONGRESS ON TRADITIONAL ASIAN MEDICINE August, 1984. Organized by the International Association for the Study of Traditional Asian Medicine. Information from: Dr. Sutarjadi, School of Pharmacy, Arilangga University, 47 JL Dharmahusada, Surabaya, INDONESIA.

Alberta, Canada
ELEVENTH INTERNATIONAL CONGRESS FOR TROPICAL MEDICINE AND MALARIA 16th–22nd. September, 1984. Organized by the University of Calgary. Information from: Secretariat XI ICTMM, Conference Office, University of Calgary, Calgary, Alberta, CANADA T2N IN4.

Amsterdam, Netherlands
INTERNATIONAL SEMINAR ON WATER VIROLOGY & CONFERENCE OF INTERNATIONAL ASSOCIATION ON WATER POLLUTION RESEARCH AND CONTROL 17th–21st. September 1984. Organized by the IAWPRC Study Group on Water Virology. Information from: Dr. W.O.K. Grabow, National Institute for Water Research, CSIR, P.O. Box 395, Pretoria 00091, SOUTH AFRICA.

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