Taking science to cholera in Bangladesh: the personal odyssey of Dr William B Greenough III and his colleagues

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ABSTRACT
Cholera is one of the most rapidly fatal infectious diseases known.1 It is caused by Vibrio cholerae (V. cholerae), a Gram-negative, comma-shaped, facultatively anaerobic and acid-labile bacteria, which lives in an aqueous environment. V. cholerae acts on the intestinal lining or mucosa via a secreted exotoxin which leads to an increase in cyclic adenosine monophosphate (cAMP), and causes purging diarrhoea of somewhat ‘fishy smelling’, voluminous ‘rice-water’ stools. If the volume is not restored quickly, it frequently results in death. The case fatality rate is 30–50%. Prompt, timely and adequate replacement of volume loss reduces the case fatality rate to less than 1%. A healthy individual may develop hypotension, vascular collapse, shock, confusion and lethargy, which can result in death in 18–24 hours if left untreated. In extreme cases, death can occur in less than 6–8 hours.2

V. cholerae is transmitted faecally–orally. Two patterns of transmission have been suggested for cholera: the environment-to-human pathway and the human-to-human pathway. Human-to-human transmission and household contacts are the basis of explosive outbreaks: the majority of cholera sufferers are poor and often victims of war or large-scale, socially disruptive migrations in crowded, unsanitary settings. Caregivers of infected patients are at high risk to become victims. Contacts are severely exposed through contaminated water and food, and outbreaks become explosive due to the significant amount of uncontrollable diarrhoea which is polluting the household, including drinking water, food preparation and bathing. Often, the rites of bathing the deceased in many cultures further expand and accelerate outbreaks of cholera. In essence, V. cholerae uses its victims to drastically increase and amplify its numbers, thus enhancing its spread.3–5 Any deficiency in water, sanitation and hygiene (WASH) services plays a key role in cholera outbreaks and larger epidemics. Populations that are poor and crowded with little access to sanitation or safe water supplies are the most vulnerable and are increasing due to wars, displacement of populations of refugees and famine.6

Historically, two major problems have been faced by those who care for patients with cholera: first, how to treat individual patients to prevent rapid death, and second, how to prevent the spread of this highly contagious disease in the immediate community and beyond.2 In this review, we will discuss how clinical scientists have approached these two issues with some personal experiences that Dr William B Greenough III encountered when he was addressing these problems and treating cholera victims at the Pakistan SEATO Cholera Research Laboratory in the early 1960s in Dhaka, East Pakistan.
and water replacement delivered promptly, as well as an immediate, affordable, safe and effective oral vaccine.

MY PERSONAL INVOLVEMENT (WILLIAM B GREENOUGH III, MD, FACP)

Care of patients with cholera

I completed my senior residency at the Peter Bent Brigham Hospital (now the Brigham and Women’s Hospital in Boston) in 1961, following a National Cancer Institute fellowship at the Mary Imogene Bassett Hospital in Cooperstown, New York, with Dr ED Thomas, where we accomplished the first successful bone marrow transplant for acute leukaemia in humans. I knew little about cholera before I was recruited by the US Public Health Services in July of 1962 and assigned to a team of clinical scientists led by Dr Robert S Gordon Jr. of the National Heart Institute; the team would join the Pakistan Southeast Asia Treaty Organization which had signed an agreement with the government of Pakistan in Dhaka, East Pakistan, in 1960. When I learnt of this assignment, I went to the adjacent Harvard Countway library to learn more about cholera. The first book I found had a bright yellow cover and was written by Dr SN De. In that book, he described decisive experiments proving that *V. cholerae* produced a potent exotoxin that caused massive fluid accumulation in rabbit ileal loops without damaging the epithelial lining of these loops.9

Dr SN De’s work was submitted to Western medical journals, which initially declined to publish his experiments because charismatic European pathologists and microbiologists, such as Rudolf Virchow, disagreed with his assertion that there was no histologic intestinal damage. Virchow had studied histologic samples from the intestines of patients with cholera who had died from the disease but had been in deep shock for an extended period. These had been sent to him from Kolkata (Calcutta), India. He had attributed the destruction of intestinal lining cells observed in these specimens directly to the action of *V. cholerae’s* invasion of the intestinal epithelium, rather than to the prolonged shock caused by volume depletion and vascular collapse. For nearly a century, medical students and physicians were taught authoritatively that *V. cholerae* was invasive and destroyed the intestinal epithelium, when actually what Virchow saw in his specimens were the results of prolonged bowel ischaemia and shock. He had concluded, erroneously, that *V. cholerae* had caused necrosis of victims’ intestines and represented, in essence, an ‘internal burn’ which explained the massive loss of circulatory volume.

However, in the late 1950s, clinical investigators from the US Naval Research Unit 2 in (NAMRU2) Taipei had taken a fresh look at this issue in the Philippines and Bangkok, Thailand. Intestinal biopsies, done with a ‘Crosby’ capsule, showed intact epithelium. Gordon clearly demonstrated that actively purging patients with cholera did not pass the large non-diffusible molecule 131 labelled polyvinyl pyrrolidine in their rice-water stools when it was injected intravenously. This effectively disproved the long-standing belief that cholera causes intestinal damage. Furthermore, intestinal biopsy specimens from active cholera victims had intact intestinal epithelium showing pathology consistent with tropical sprue. In the study, the electrolyte composition of purging cholera victims’ rice-water stools was measured, which established the electrolyte composition of cholera stools on which to base effective intravenous cholera replacement intravenous therapy solutions. Later, NAMRU2 physicians postulated that an oral replacement solution could be possible using the discovery of an intestinal sodium-glucose linked transporter type 1 (SGLT1), but they used a hyperosmotic formula which further aggravated intestinal losses.

I was posted in Dhaka (then East Pakistan) to the newly established PSCRL and arrived in July 1962. Dr Abraham Beneson was the director with the mission to set up a field-trial area to evaluate the effectiveness of the injectable cholera vaccine, which was at that time required worldwide for travellers by international health regulations. Professor KA Monsur, director of this Institute, set aside space for our team and taught us how to diagnose, culture and grow *V. cholerae* from the stools of cholera victims in the laboratory using his specific selective culture media.

In November of 1962, we began caring for cholera victims in transfer from the Mitford Hospital in Dhaka. Based on studies that defined the electrolyte losses in cholera by NAMRU2 investigators, we created an intravenous solution that mirrored the electrolyte composition of the voluminous rice-water stools of patients with cholera. We made the solution matching the stool loss composition of the rice-water stools of actively purging cholera victims from sterile reagent grade electrolytes: 5 g of sodium chloride, 4 g of sodium bicarbonate and 1 g of potassium chloride in a litre of sterile distilled water from a Barnstead distillation system (‘5/4/1’ solution).

At the end of December 1962, we were initially infusing saline through the femoral vein of the pulseless and often unconscious victims who began coming directly to the ‘Cholera Hospital’ in Mohakhali. Once circulation was restored, we switched to the 5/4/1 intravenous solution, restoring the electrolyte and volume losses. As word of mouth spread in the local communities that cholera victims could be resurrected even when nearly dead and in deep shock, increasing numbers of cholera victims came directly to the Dhaka Cholera Hospital located in Mohakhali.

Subsequently, as new victims of cholera were directly admitted to the Cholera Hospital, the mortality rate soon dropped below 1% indicating that our initial experiences with transferred victims of cholera from Mitford Hospital who had died after hydration in severe acidosis and pulmonary oedema were due to neglect of the acidosis. Due to the rapidly increasing admissions, Pakistan Health authorities delegated Bangladeshi physicians to us, and we trained local nurses and nursing assistants who soon became skilled in intravenous therapy, replacing volume...
losses calculated by placing the patients on cholera cots with calibrated buckets underneath and measuring the profuse watery diarrhoea which they captured; in this way intakes and outputs could be rapidly and visually seen by patients’ caregivers and all the treatment staff. By the 1963 winter epidemic, we had to put up tents and urgently trained more staff to accommodate the rush of patients.

During the 1963 winter cholera epidemic, the Dhaka Cholera Hospital began receiving many severely volume-depleted patients with cholera from a refugee camp in Narayanganj (a city which is located to the south of Dhaka). There had been communal disturbances between Bengali/Bihari and Hindu/Muslim communities at that time and many of those persecuted fled to the protection of a jute mill compound in Narayanganj. On a Sunday afternoon, Dr Robert S Gordon Jr and I gathered about 30 L of our precious, labour-intensive cholera replacement intravenous ‘5/4/1’ solution and travelled to the Laxmi Narayan jute mill to assess the situation there. On arrival, we found about 50 patients acutely purging rice-water stools and in shock, and twice that number in earlier stages of volume depletion with the typical rice-water stools. We triaged the limited intravenous solution to the sickest patients and instructed the community leaders to send all others to the Cholera Hospital in Dhaka at the earliest sign of diarrhoea. At that time we could only advise that patients who were purging, unconscious and in deep shock be separated from the healthy population and that their waste be isolated from others. I realised that afternoon that providing appropriate intravenous fluid therapy in the context of epidemic cholera in the deprived refugee settings where cholera usually appears globally was not going to be feasible. There had to be a way to replace purging losses of water and electrolytes orally.

In the same period of 1962–1970, there were two groups of scientists who simultaneously worked on cholera: a team of clinical researchers sent by Johns Hopkins to Kolkata (Calcutta) to study cholera and related diarrhoeal diseases, supported by a National Institutes of Health (NIH) grant, International Center for Medical Research and Training (ICMRT) and the physician investigators at Cholera Research Laboratory (CRL), Dhaka, funded by the National Heart Institute and the U.I. Public Health Service (Abraham Benenson, MD, Director). These two groups urgently sought to find a way to treat cholera victims with an oral replacement therapy (ORT) solution. Although competing with one another, they were in frequent communication and were working in parallel, sharing data and their unpublished findings.

Fortunately, by 1963, both teams were aware of the recently reported discovery of a sodium-linked glucose pathway (SGLT1) in rabbit ileum which had been demonstrated both in the Harvard and Air Force basic physiology laboratories (figure 1). The SGLT of the intestine needs to be distinguished from that of the kidney tubule. SGLT1 is located in the gut and SGLT2 is located in the kidney, and it may be now important to comment that ORT will not work at all if a person is on the increasingly popular SGLT1, blocking agent for diabetes mellitus. The challenge was to show that despite the massive volume of purging rice-water stools of active cholera victims, this sodium-glucose transport mechanism (SGLT1) might still be intact.

With accurate balance studies, direct measurements of electrical potentials of sodium ions and volume fluxes across the small intestine of cholera victims, the Dhaka and Kolkata (Calcutta) teams both showed that in cholera victims, the SGLT1 mechanism remained fully intact and was potentially available for testing glucose-sodium-based ORT. One of the earliest documentations of this fact was done by measuring the changes in electrical potential across the small intestine in patients with cholera. This observation recently received the Golden Goose Award (the award honours basic scientists who, despite their research being overlooked at the time it was conducted, went on to make significant advancements and impact society with their work). Previously, others had tried oral rehydration in patients with cholera but the composition of the fluids administered was either not correct or hyperosmotic due to the addition of too much glucose or electrolytes.

Following the findings that ORT was feasible and effective in a hospital research setting based on the new data from Dhaka CRL and the Hopkins Kolkata (Calcutta) ICMRT teams, the next step was to determine if it could efficiently substitute for intravenous hydration in the epidemic village and refugee settings. This was accomplished by a team led by David Nalin, showing that dehydrated purging patients with cholera could drink enough ORT to maintain their hydration and restore their urine outputs.

The ‘acid test’ for ORT came during the 1971 war in East Pakistan from which Bangladesh emerged as an independent country. During that war, eight to nine million refugees flooded out of East Pakistan into India, and large refugee camps were set up near Kolkata (Calcutta), India. Cholera broke out. Due to a lack of intravenous fluids and trained medical staff to administer the intravenous therapy, Dr Dilip Mahalanabis and the team led by Dr Thomas Simpson at the Johns Hopkins ICMRT in Kolkata (Calcutta) prepared packets of the ORT salts and glucose along with simple instructions on how affected families could prepare and administer the solution to cholera victims who were still conscious enough to drink (fortunately, vascular collapse makes victims extremely thirsty). They demonstrated that without access to intravenous fluids, in the case when the fluids were administered orally by family members without medical expertise, the mortality rate from cholera could be decreased from over 40% to approximately 3% (table 1).

This caught the attention of the WHO which initiated a global programme on diarrhoeal diseases under the leadership of Dr Dhima Barua, who had been a part of the Hopkins Kolkata (Calcutta) team. He also recruited Dr Michael Merson, an epidemiologist from the Dhaka Cholera Research Laboratory, who took over the project and went on to make significant advancements and impact society with their work.

![Figure 1](https://example.com/figure1.png)
Cholera Research Lab. Later, UNICEF incorporated ORT into its GOBI programme (Growth curves, ORT, Breastfeeding and Immunisations). Once implemented, these combined programmes have been credited with an 80%–90% reduction in the mortality rate of children under the age of 4 worldwide.18

**Cholera vaccine, setting up logistics and transportation**

In 1963, a field area for the cholera vaccine trials had been chosen (Matlab Thana). At that time, there was a government hospital with limited facilities in Matlab Bazar and there were no convenient roads to the area. All the villages were accessible only through the web of rivers and canals that came off the main Meghna River where Matlab Bazar was located. Access to the rural areas of East Pakistan relied on motor launches and country boats for supplies and travel. East Pakistan (now Bangladesh) is located on the Ganges River delta fed by three great rivers: the Ganges, Brahmaputra and Meghna (figure 2). In 1963, with the winter cholera season waning, I was deputised to work out water communications and supply lines to the Cholera Lab’s field station. I had grown up in the USA, in the state of Rhode Island, known as the ‘Ocean State’, and had the experience of working with small boats. Dr Gordon had procured a 12-foot aluminium outboard motorboat from scouring the secondhand unused stores at the NIH. I used that boat with a 5 hp outboard motor to work out the initial routes of communication with Matlab. Heavy supplies were sent by sail-driven country boats. Later the field trial area was served by a Dowty turbojet boat, contributed by the UK, that could make the trip from Dhaka to Matlab in a little over an hour, and later by Boston Whaler outboard motorboats, to bring cholera victims to the tent treatment centre in Matlab. Many field workers journeyed on foot and in small boats to do the demographic research for the 250,000 participants in

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**Table 1** Summary of mortality from cholera and cholera-like diarrhoeas at Bongaon Treatment Center, 24 June through 30 August 1971

<table>
<thead>
<tr>
<th>Patient population</th>
<th>Admissions</th>
<th>No of deaths†</th>
<th>Case fatality ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire centre</td>
<td>3703</td>
<td>135</td>
<td>3.6%</td>
</tr>
<tr>
<td>JH-CMRT demonstration unit only</td>
<td>1190</td>
<td>12</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

*Table reproduced with permission from Johns Hopkins University. Original source: Mahalanabis et al.17†Approximately half of the patients died before any rehydration therapy could be started.17 JH-CMRT, Johns Hopkins Center for Medical Research and Training.

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![Figure 1](http://egastroenterology.bmj.com) Oral rehydration therapy (ORT). Glucose is required for sodium (Na+) ions to be efficiently taken up by the intestinal epithelium (created with biorender.com). GLUT2, glucose transporter type 2; SGLT1, sodium glucose transporter type 1.
the subsequent vaccination trials. The initial task was to determine whether the cholera vaccine, internationally required at the time, was effective. Subsequently, trials were all done in the Matlab demographic area leading to the development of new, inexpensive and effective oral cholera vaccines. Dhaka urban trials were also done. The results of the test of the internationally required injected cholera vaccine showed little efficacy and significant side effects. These data emphasised the need to develop effective vaccines based on the knowledge of the mechanism of action of the cholera toxin.19 20

During the development of the Matlab field trial area, two anthropologists, Shirley and Robert Glasse, were located in a remote village in the Matlab area to gather information on how local practitioners were treating cholera victims. We taught them how to administer our sterile intravenous 5/4/1 in solution to save their patients’ lives. They gathered the first data on the customs in the clinical trial area and various attack rates of cholera.21

**Discovery of the mechanism of action of the cholera exotoxin and its receptors**

On my return to the USA, in 1965, I worked at the National Heart Institute for 2 years until I was recruited to join the Johns Hopkins Division of Infectious Diseases led by Dr CCJ Carpenter who had also led the Hopkins ICMRT Team in Kolkata (Calcutta), to continue research on cholera. Initially, I carried out studies in a dog model for cholera to determine the effect of splanchnic blood flow on the rate of small intestinal secretion: there was little if any relationship.22 In November of 1968, I was invited by Dr Irwin Rosenberg and Dr Norbert Hirschhorn to present the results of these studies in Boston. Dr Michael Field, a young assistant professor at Harvard, attended my talk. He had just shown that the rise of cAMP levels induced chloride secretion in an in vitro rabbit ileum preparation in an Ussing chamber. Over the next few weeks, in Boston and Baltimore, we demonstrated that a crude semipurified cholera toxin preparation made by Dr Richard Finkelstein also triggered chloride secretion in the rabbit ileal Ussing chambers.23 These decisive experiments were carried out by Dr Qais Al Awqati (a fellow in my laboratory at Hopkins). Subsequently, we obtained specimens from surgeons who were resecting intestinal tumours and showed that the cholera toxin also caused chloride secretion in humans.24 Soon after, Dr Field with his chief of gastroenterology, Daniel Kimberg, demonstrated that cAMP was indeed increased in ileal mucosa exposed to the crude cholera toxin.25 Now, years later, medical students are taught the mechanism of cholera and related diarrhoeal diseases in their basic physiology/microbiology courses (online supplemental video 1; [figure 3]). We discovered
that the specific receptor for the cholera toxin was the GM₁ ganglioside.²⁶

**Oral vaccine development**

The main rationale for establishing the PSCRL in Dhaka, East Pakistan, was to test rigorously the existing injected cholera vaccine required by international laws. The results of the Matlab field trial area showed both high rates of side effects and little if any protection against severe potentially fatal cholera.²⁰ Following this initial trial in the 1960s, the mechanism of action of the cholera exotoxin was delineated and a purified non-toxic component of the toxin (the B subunit) was tested in the succeeding trials, but the costs of the purification made the vaccine prohibitively expensive and impractical, although it was effective and free of side effects.²⁷ Dr John Clemens at the International Vaccine Institute in South Korea demonstrated that a less purified version of the B subunit combined with the B-subunit killed whole-cell (BS-WC) and killed whole-cell antigens were effective and inexpensive in field trial settings in Vietnam. This vaccine was then further tested and is now being disseminated globally.²⁸ The full impact of the oral cholera vaccine has yet to be assessed, but recent studies have confirmed its effectiveness in ‘real-world’ settings of poverty and poor sanitation that characterise the population at risk for epidemic cholera.

**CONCLUSIONS**

Two discoveries allowed global policy-makers to effectively address the issues of epidemic cholera and related diarrhoeal diseases, considering their high mortality rates and common sequelae of malnutrition, malabsorption and subsequent fatalities as a result of malnutrition: the discovery of ORT and the proof of the effectiveness of an oral cholera vaccine. These two breakthroughs implemented by the WHO UNICEF control programme for diarrhoeal diseases have been credited with an 80%–90% reduction in the global mortality rates of children under the age of 4 between the 1970s and the present time.²⁹–³¹

The first benefit of ‘taking basic science to where the diarrhoea is’ in the 1960s was to define the exact composition and volume of the fatal losses of salts and water in cholera and related diarrheas.³² That definition in turn provided the basis for creating correctly formulated intravenous replacement solutions. These intravenous solutions, if available, could virtually guarantee the survival of cholera victims. However, even with the correctly constituted intravenous solutions to replace the water and electrolyte losses in cholera, there were insurmountable logistical and cost barriers to implementing this curative technology in the desperately poor and chaotic situations where epidemic cholera usually occurred (poverty; remote villages in economically deprived situations around the globe; and warfare, famine and refugee situations). The discovery of ORT removed these barriers. It was founded on bedside physiological measurements in actively purging patients with cholera which proved that the glucose-sodium carrier–mediated transport system (SGLT₁) of the small intestine of actively purging cholera victims was fully intact, now even without the availability of intravenous therapy, the majority of cholera victims could be saved simply by having family members or other caregivers administering an appropriately constituted ORT solution to patients according to their thirst.³⁶ The caveat is that the ORT solution must be hypo-osmotic and given in sufficient amounts to replace initial deficits as well as continuing losses due to diarrhoea. The simplest observable parameters are at the bedside: ‘reasonable’ urine output every 4–6 hours and the patient’s reported or observed levels of thirst. The use of ORT by the Johns Hopkins group in Kolkata (Calcutta) during the refugee cholera outbreak in the war that liberated Bangladesh in 1971 was perhaps the most important proof of the power of ORT that led to its adoption in the WHO/UNICEF global programme for diarrhoeal diseases.¹⁷
In the 1970s, a further improvement of ORT was made: the utilisation of digestible starches from rice, corn or wheat that could deliver more glucose to the small intestine sodium-glucose cotransporter without increasing luminal osmolarity, which impedes absorption and raises the risk of hyperosmotic aggravation of diarrhoea and increased salt and water losses. Often the home-made ORT solutions were prepared with too much sugar and salt (this issue caused WHO and UNICEF to advise against home-prepared ORT in Africa), while utilisation of digestible starches served to demonstrate that ORT could be further improved and made more accessible in a variety of cultural contexts. Grain-based ORT is safer, culturally adapted and effective. Any residual undigested complex carbohydrate will facilitate further absorption of any residual diarrhoea in the large intestine. Availability for this purpose of the basic grain (complex carbohydrate) native to the affected country or area makes it easier for the mother and family members to make a safer and more effective ORT with home ingredients. In Bangladesh, mothers who had been taught how to prepare home-made ORT safely from local ingredients by the Bangladesh Rural Advancement Committee showed that diarrhoeal salt and water loss could be treated with home-made ingredients (Laban-gur) available in all village markets.

Now, globally, most of the related, high-volume diarrhoeas such as cholera can be managed at home in economically deprived settings. Ironically, in wealthy countries where emergency services are available at technologically advanced hospitals, intravenous fluid therapy is still the first choice, despite the high costs, risks of delayed use and the lack of properly constructed intravenous solutions to replace intestinal electrolyte losses. In the 21st century, we need to adapt the proven oral rehydration technologies to routine home practice before volume depletion occurs and patients have to be rushed to the emergency rooms of hospitals. Ironically, in the use of ORT, the practice in under-resourced settings is more rational and better implemented than in wealthy countries with their more expensive and risky treatments, due to delayed care in hospital emergency rooms and reliance on expensive, invasive technologies for volume depletion and shock. So far, there also has been a failure to replace the glucose-based ORT with digestible complex carbohydrate-based ORTs using the dietary staples of the countries involved, which can be home-made and are often more palatable.

Finally, since ORT can replace water and electrolyte losses, it can also be used to replace sweat losses and protect against heat casualties, and perhaps more importantly to replace the rapid salt and water losses in mass burn casualty settings.

The increasingly available inexpensive oral cholera vaccine provides grounds for hope that cholera can be anticipated and prevented in high-risk areas first and then globally, as supplies increase through national and international programmes and financial support.

POSTSCRIPT
Dr Greenough’s story of research on cholera and diarrhoeal diseases clearly demonstrates that progress in science and research involves many individuals. The importance of the contributions of the many scientists involved is perhaps best articulated by Howard Florey who won the Nobel Prize for Medicine for the discovery of penicillin with Alexander Fleming. This is how he described the process in a 1967 lecture to the National Press Club of Australia:

The great strides in understanding natural phenomena are the result of the labours of thousands of people, some of whom are good scientists and some not so good. Their combined labours might be likened to the pointillists who applied little dabs of colour to the canvas and built up a beautiful picture. Scientists can with luck, from time to time, put a nice dab of colour on the metaphorical canvas; but, for the elaboration of the finished work, they are dependent on the activities of thousands of colleagues.