MEDICAL REVIEW

Case Studies in Cholera: Lessons in Medical History and Science

Stephen M. Kavic\(^a\), Eric J. Frehm\(^b\), and Alan S. Segal\(^c\)

\(^{a}\)Department of Surgery, Yale University School of Medicine, New Haven, Connecticut; 
\(^{b}\)Department of Pediatrics, University of Pennsylvania, Philadelphia, Pennsylvania; and 
\(^{c}\)Departments of Medicine, Molecular Physiology and Biophysics, and Pharmacology, University of Vermont, Burlington, Vermont

Cholera, a prototypical secretory diarrheal disease, is an ancient scourge that has both wrought great suffering and taught many valuable lessons, from basic sanitation to molecular signal transduction. Victims experience the voluminous loss of bicarbonate-rich isotonic saline at a rate that may lead to hypovolemic shock, metabolic acidosis, and death within a few hours. Intravenous solution therapy as we know it was first developed in an attempt to provide life-saving volume replacement for cholera patients. Breakthroughs in epithelial membrane transport physiology, such as the discovery of sugar and salt cotransport, have paved the way for oral replacement therapy in areas of the world where intravenous replacement is not readily available. In addition, the discovery of the cholera toxin has yielded vital information about toxigenic infectious diseases, providing a framework in which to study fundamental elements of intracellular signal transduction pathways, such as G-proteins. Cholera may even shed light on the evolution and pathophysiology of cystic fibrosis, the most commonly inherited disease among Caucasians.

The goal of this paper is to review, using case studies, some of the lessons learned from cholera throughout the ages, acknowledging those pioneers whose seminal work led to our understanding of many basic concepts in medical epidemiology, microbiology, physiology, and therapeutics.

INTRODUCTION

Cholera is an infectious disease whose course can vary from a mild diarrheal syndrome to a rapidly fatal malady (cholera gravis) characterized by the sudden onset of profuse watery diarrhea with volume depletion, metabolic acidosis, and ultimately death from hypovolemic shock. The disease is caused by *Vibrio cholerae*, a short, curved, bacterium that produces an enterotoxin (cholera). Humans are the only known host for cholera vibrios [1], which can spread rapidly though a community primarily via fecal contamination of drinking water.

Throughout its history, cholera caused widespread panic because it can afflict an otherwise healthy person without warning and lead to death in as little as four to six
hours. Many victims have awoken with uncompromised health at the edge of the day and died gruesomely in their own dejecta before night’s return. More recently in several parts of the world, cholera pandemics have claimed thousands of lives in outbreaks in southern Africa and Latin America.

We will present three case scenarios based on actual patients illustrating many of the clinical manifestations of cholera. These will be used as a springboard to review 1) the colorful history of cholera including its impact on the origin of intravenous solution therapy, 2) the fundamental physiological principles that underlie the disease and its treatment, and 3) how aspects of cholera have intertwined with developments in modern science.

CHOLERA: SYMPTOMS, SIGNS, AND THE ORIGIN OF INTRAVENOUS THERAPY

Case Scenario 1:
Sunderland, Britain, 1831 [2]

A 25-year-old woman was found in grave distress. The patient was too lethargic to provide a history, but a friend states that she was in her usual state of good health until 24 hours previously when she developed the acute onset of profuse watery diarrhea, vomiting, and muscle cramps. The villagers had collected all of her diarrheal fluid in a canister kept near the patient, since they believed that part of the reason she was so ill was that her body had been depleted of vital substances contained in the excretions. No other history was available except that several other villagers had already died from this type of illness. The first death in Sunderland had been on Oct. 26, 1831.

On physical examination, the patient was extremely weak and lethargic. The silver-bluish color of her skin was reminiscent of lead. She was cool to the touch and had poor skin turgor. The wrinkled skin on her hands appeared as if it had been immersed in water for a prolonged time (“washerwomen’s hands”). Her facial features were flattened and her eyes were sunk deep into their sockets. A radial pulse was not palpable, but a heart rate of 160 per minute was assessed by listening over the chest. The patient’s breathing was labored, at a rate of 32 breaths per minute (hyperventilation in response to metabolic acidosis), but breath sounds over the lung fields were clear. The abdomen was soft. The extremities were cold and there was no peripheral edema.

Inspection of the canister showed approximately ten liters of nearly odorless watery fluid containing flecks of mucus (“rice-water” stools). Sensing the gravity of the patient’s condition, the apothecary boiled eight gallons (30 liters) of water and prepared two solutions:

Solution #1: 5 gallons (~19 liters) of a solution containing by weight 9 parts sodium chloride to 1000 parts water (0.9% isotonic NaCl).

Solution #2: 3 gallons (~11 liters) of a solution containing 12.6 parts soda of bicarbonate to 1000 parts water (isotonic bicarbonate solution).

As the patient was sinking towards death, and as the bewildered townspeople looked on, a hollow silver needle (designed by John Read [3]) was placed in the right femoral vein and attached to a breast pump syringe by a flexible tube fashioned from goose trachea. A quart of the NaCl solution was injected over fifteen minutes, followed by a pint of the NaHCO₃ solution over the next fifteen minutes. Three parts potassium chloride was also added to 1000 parts Solution #1 (KCl 40 meq/l) in a few quarts. After five cycles of this treatment, to the astonishment of all who had gathered, the patient became alert and animated. Her radial pulse returned at a rate of 112 per minute. The two solutions were continued, in
alternating fashion, until the patient stated that her bladder was full. By that time she had received eight quarts of the NaCl solution (four containing KCl) and eight pints of the NaHCO$_3$ solution, a total of over eleven liters.

The patient continued to pass “rice-water” stools and required sixteen liters of intravenous solution therapy the next day (Figure 1). By the fifth day, the diarrhea had significantly decreased, but the patient had not passed any urine since day three. Despite the valiant intravenous solution replacement therapy, the patient died of progressive renal failure on day twenty [4]. Nevertheless, this was the birth of intravenous solution therapy as we know it.

**CHOLERA IN THE UNITED STATES: EFFECTIVE THERAPY**

**Case Scenario 2:**
*Port Lavaca, Texas, 1973 [5]*

A 51-year-old man presented to the emergency department with a four-hour history of profuse watery, non-bloody diarrhea. The patient noted mild abdominal pain along with non-odorous diarrhea, and experienced one episode of emesis. The patient also complained of severe leg cramps.

On initial physical examination, no blood pressure could be obtained. The pulse was auscultated at 150 beats per minute, and Kussmaul-type respirations were present at a rate of thirty breaths per minute. The patient was afebrile, alert and oriented. The abdominal findings were minimal — soft, scaphoid abdomen, nontender to palpation, with active bowel sounds. His extremities were noted to have “splotchy” cyanosis, and no peripheral pulses were palpable.

Initial laboratory values included a white blood cell count of 13,400 per ml, hemoglobin of 18.1 g/dl, [Na] 136 meq/l, [K] 3.1 meq/l, [CO$_2$] 18 meq/l and BUN 40 mg/dl. Two hours after admission, a second total CO$_2$ level was only 5 meq/l. Central venous access was obtained and intravenous lactated Ringer’s solution infused. Supplemental potassium and bicarbonate was administered, along with intravenous gentamicin. After six hours, the patient’s condition improved significantly, with full return of peripheral pulses and intact mental status.

The attending physician reviewed the literature and found his patient’s symptoms were compatible with cholera, although no cases of domestically acquired cholera had been reported in the United States since 1911 [6]. The physician switched the antibiotic regimen to tetracycline and alerted the local health authorities. The diagnosis of cholera was confirmed by laboratory culture, which identified *Vibrio cholerae*, El Tor biotype, Inaba serotype. Further volume resuscitation ensued, and the patient was discharged home in good health one week from initial presentation.

**CHOLERA TODAY: VITAL ROLE OF ORAL REHYDRATION THERAPY**

**Case Scenario 3:**
*Dhaka, Bangladesh, 1999 [7]*

A 29-year-old man presented to the clinic with a ten-hour history of diarrhea and a seven-hour history of vomiting. The patient could provide no further coherent history. On examination, the patient was in mild distress, disoriented to place and time but talkative. The patient was afebrile, tachycardic, and had a blood pressure of 90/60. The patient had sunken eyes and poor skin turgor, but otherwise had an unremarkable exam.

Based on local disease patterns, the patient was treated with the presumptive diagnosis of cholera, which was subsequently confirmed with darkfield illumination of the stool. The patient was given six
hours of intravenous therapy with Dhaka solution [6]: [Na] 133 mmol/l, [K] 13 mmol/l, [Cl] 98 mmol/l, and [acetate] 48 mmol/l), for a total volume of six liters. This solution provides the volume, potassium, and bicarbonate (as acetate is converted to bicarbonate in the liver) required to offset the massive losses of these electrolytes in the diarrhea of cholera. Erythromycin therapy was also instituted, 500 mg every six hours for three days.

At the completion of the initial resuscitation, the patient was coherent and showed no signs of systemic infection. The intravenous fluids were discontinued. The patient was given World Health Organization Oral Rehydration Solution (WHO–OHS), containing [Na] 90 mmol/l, [K] 20 mmol/l, [Cl] 80 mmol/l, [citrate] 10 mmol/l (which is metabolized to bicarbonate), and [glucose] 111 mmol/l (to facilitate Na-glucose cotransport), for a total osmolarity of 311 mosm/l. The patient was given a volume of WHO-OHS equivalent to the total stool/emesis output, collected in graduated containers beneath a cholera cot. The patient was also given free access to food and water.

The total stool output in the first day was over 12 l, but the total output over the next two days was 4.2 l. The patient's diarrhea completely resolved 48 hours after presentation. The patient consumed a total of twenty-one liters of WHO-OHS and an additional two liters of free water. Final laboratory analysis confirmed infection with *Vibrio cholerae*, El Tor Ogawa.

THE HISTORY OF CHOLERA

Cholera has been endemic to the Indian subcontinent for millennia. Spread of cholera beyond the Indian subcontinent in 1817 marked the first worldwide pandemic
(see Table 1). However, compelling descriptions of severe diarrheal diseases consistent with cholera dating from the time of Hippocrates suggest the possibility of an even earlier emergence from Asia [8].

In 1959, Robert Pollitzer chronicled the first six pandemics [9]. Although reliable serotyping was not available prior to the fifth pandemic, one might presume that the first four pandemics were caused by the same agent (Table 1). The first case presented was based on cholera’s first arrival in Britain during the second pandemic. *Vibrio cholerae* O1 has two variants, the so-called classical biotype; and the El Tor biotype, the hardier but less virulent agent of the seventh pandemic. Since 1992, outbreaks of cholera caused by a new serogroup, *Vibrio cholerae* O139, have been reported in South Asia and China, raising the prospect of an Eighth Pandemic [10] (Table 1).

In 1854, John Snow linked a cholera outbreak in London to a single source: a contaminated water pump on Broad Street. This was the first convincing demonstration that cholera was a contagious and water-borne disease. The removal of the pump handle at Dr. Snow’s request stands as a landmark in the history of public health and preventive medicine. Another milestone in the understanding of cholera came in 1883 when Robert Koch, discoverer of the etiologic agent of tuberculosis, led an expedition to Egypt during which he identified a microbe he called *komma-bazillen* (comma bacilli) [11]. He subsequently detected kommabazillen in the stools of cholera patients in Calcutta. The name of this bacterium was later changed to *Vibrio cholerae*, in belated recognition of Filipo Pacini, who had bestowed the latter name on the bacillus he observed in the excreta of cholera victims in Italy thirty years earlier [9].

Fortunately, even before the etiologic agent was identified, there were those committed to the notion that the spread of cholera could be controlled “…not with prayer and fasting, but through disinfection and quarantine. [C]holera demonstrated forcefully that a disease that could not be cured must be prevented” [12]. From convictions such as these grew institutions like New York City’s Metropolitan Board of Health, credited with containing a cholera outbreak in 1866. The association of cholera with unsanitary water and crowded, squalid living conditions has always resulted in a disproportionate representation of the urban poor on its death rolls. Perhaps it was inevitable that so dreadful a scourge — one that generally spared the wealthy and refined while ravaging the

| Pandemic | Time Period | Serogroup | Biotype | Affected Areas<sup>b</sup> |
|----------|-------------|-----------|---------|---------------------------|
| First    | 1817 to 1823 | *Vibrio cholerae* 01 | Classical<sup>a</sup> | 1, 2, 3, 5 |
| Second   | 1829 to 1851 | *Vibrio cholerae* 01 | Classical<sup>a</sup> | 1, 2, 3, 4, 5, 6, 7 |
| Third    | 1852 to 1859 | *Vibrio cholerae* 01 | Classical<sup>a</sup> | 1, 2, 3, 4, 5, 6, 7, 8 |
| Fourth   | 1863 to 1879 | *Vibrio cholerae* 01 | Classical<sup>a</sup> | 1, 2, 3, 4, 5, 6, 7 |
| Fifth    | 1881 to 1896 | *Vibrio cholerae* 01 | Classical | 1, 2, 3, 4, 5, 6, 7 |
| Sixth    | 1899 to 1923 | *Vibrio cholerae* 01 | Classical | 1, 2, 3, 4, 5, 7 |
| Seventh  | 1961 to Present | *Vibrio cholerae* 01 | El Tor | 1, 2, 3, 4, 5, 8, 9 |
| Eighth (?)| 1992 to Present | *Vibrio cholerae* 0139 | Single biotype | 2 |

<sup>a</sup> Presumed.

<sup>b</sup> Affected areas: 1, Indian Subcontinent; 2, Southeast Asia; 3, Middle East; 4, Europe; 5, East Africa; 6, North Africa; 7, North American; 8, South America; 9, Indonesia.
impoverished and uneducated - would be seen as divine retribution for the sinful, profligate, and unworthy. Indeed, for proper members of the upper class, *Vibrio cholerae* could be both an intestinal pathogen and a character assassin — "to die of cholera was to die in suspicious circumstances" [13].

Over a century later, can we be sure that a parallel situation does not exist for patients with AIDS, who may face similar injustices as a consequence of societal ignorance? Long before this terrible disease challenged today's scientists exploring the intricacies of cellular signaling and toxin-mediated disease, cholera challenged the very idea of what a "disease" actually was, what it was not, and what thinking citizens might do about it.

**NORMAL INTESTINAL PHYSIOLOGY**

Under normal circumstances, the human gastrointestinal tract can absorb more than 90 percent of the water and ions presented to it. To appreciate fully the pathophysiology of cholera, a brief review of normal water and solute handling by the intestines is helpful.

**Water:** The healthy small intestine is able to absorb about 7 - 8.5 liters of the nine liters that enters it daily, leaving an ileocecal flow rate of 0.5-2 l/day. It is important to remember that in the small intestine, mature epithelial cells near the tips of the villi are engaged in net absorption, while the less mature cells in Lieberkühn's crypts function in the net secretion of water and electrolytes. Typically, only about 600 ml of fluid is presented to the colon per day, of which 500 ml are absorbed, leaving the 100 ml contained in the average daily fecal weight of 150 g. Therefore, one functional definition of diarrhea is an average daily fecal weight exceeding 200 g. Since the colon is capable of absorbing 4 to 6 liters of water per day, the 500 ml absorbed represents about 10 percent of the colon's absorptive capacity.

Fluid transport in the small intestine generally results from the passive movement of water across epithelial membranes driven by osmotic and hydrostatic pressures. In the absence of food, ions are the most important contributors to osmotic pressure in the lumen. Since the cells of the small intestine are relatively permeable (leaky), luminal fluid generally remains isotonic with plasma. Because most water absorption occurs in the absence of a transmucosal osmotic gradient, any increase in the osmotic content of the lumen will tend to reduce water absorption and lead to diarrhea. Since 60 to 90 percent of stool weight is water, diarrhea is mainly due to excess fecal water.

**Sodium:** Sodium is actively absorbed throughout the small intestine, the site most affected in cholera. The favorable electrochemical gradient (negative membrane potential and low intracellular [Na]) allows sodium to enter the cell across the apical brush border, and exit across the basolateral membrane via the Na, K ATPase pump. The net rate of Na⁺ absorption is highest in the jejunum, where it is enhanced by the presence of glucose, galactose, and neutral amino acids via Na⁺-sugar cotransport. Indeed, the identification of Na⁺-coupled cotransport systems stands as one of the most important medical discoveries of this century, since these processes form the basis of oral rehydration therapy used in many parts of the world. The net rate of Na⁺ absorption is smaller in the ileum due to a lower density of cotransporter proteins.

**Potassium:** The movement of potassium in the small intestine is usually in the direction of absorption. As water is absorbed luminal [K] rises, thereby increasing the driving force for absorption. It is important to note that since most K⁺ absorption results from its rising luminal
concentration as water is absorbed, significant K⁺ loss may occur in diarrhea, especially in the high-flow secretory diarrhea of cholera.

**Anions:** The jejunum absorbs large amounts of both Cl⁻ and HCO₃⁻ anions, and by the end of the jejunum most of the HCO₃⁻ from hepatic and pancreatic secretions has been absorbed. It follows that diseases affecting the small intestine can result in significant HCO₃⁻ losses.

**PATHOPHYSIOLOGY OF CHOLERA**

Cholera stimulates solute and water secretion in the small intestine. With continuous parenteral fluid replacement, the ileocecal flow rate may reach 15 to 20 liters per day. Such a secretory diarrheal process can lead to severe losses of volume and electrolytes. The initial stool output in cholera gravis can exceed a full liter per hour, resulting in death in as little as four to six hours. It has been said that cholera is a disease that begins where other diseases end — with death.

It is important to emphasize that since the fluid loss in cholera is essentially isotonic, “dehydration” is not the problem; rather the patient becomes severely volume-depleted. Conceptually, it is absolutely critical to distinguish hypovolemia (loss of salt in isotonic proportions) and dehydration (loss of solute-free water) [14]. Hypovolemia is assessed clinically by physical exam and does not require any laboratory tests, and the diagnosis of salt (volume) depletion implies the life-saving therapy of isotonic salt (volume) replacement. In contrast, “dehydration” implies solute-free water loss, or hypertonicity, which is a laboratory diagnosis.

**Chloride secretion**

The transport function of epithelia is inextricably linked to the polarized distribution of transport elements in the apical and basolateral membranes of epithelial cells (Figure 2) [15]. Secretion of fluid across the intestinal epithelium is primarily driven by the net transport of solute into the lumen and the secondary movement of water down the resultant osmotic gradient. Net transport in a preferred direction requires an asymmetric arrangement of transport elements; that is, cell polarity.

The transepithelial secretion of chloride is central to fluid secretion across

![Figure 2. Secretory epithelial cell model. The Na⁺, K-ATPase pump and triple ion transporter are shown on the basolateral side of the cell, with the CFTR chloride channel illustrated on the apical membrane. This arrangement allow for secretion of chloride across the cell.](image-url)
many secretory epithelia including the small intestine. In secretory epithelial cells such as those in the crypts of Lieberkühn, the asymmetry of Cl⁻ transport elements underlies Cl⁻ secretion. The basolateral Na,K-ATPase pump drives active Cl⁻ transport, and inhibitors of the pump such as ouabain abolish Cl⁻ secretion. The low intracellular [Na] maintained by the pump and the low intracellular [Cl⁻] favor the entry of Cl⁻ across the basolateral membrane via a furosemide- and bumetanide-sensitive Na:K:2Cl⁻ triple ion cotransporter. The pump also maintains a high intracellular [K⁺], and together with the large basolateral K⁺ conductance, causes the apical and basolateral membrane potentials to be cell-negative. This negative membrane potential provides a key driving force for conductive Cl⁻ exit when chloride channels localized in the apical membrane open.

Thus, the active secretion of Cl⁻ by polarized epithelia is mediated by an asymmetric arrangement of a basolateral Cl⁻ entry element (the electroneutral triple cotransporter) and an apical Cl⁻ exit element (a transmembrane channel). Simply put, transcellular transport requires a way in and a way out. Of great significance is that the major Cl⁻ conductance in the apical membrane of secretory epithelia (including the crypt cells) is the now famous Cystic Fibrosis Transmembrane conductance Regulator (CFTR), the protein that is defective in patients with cystic fibrosis (CF). CFTR is a multi-functional protein, but its characterization as a cAMP-activated Cl⁻ channel is well documented [16, 17]. In secretory epithelia, continued Cl⁻ secretion hinges on the basolateral membrane being selectively conductive to K⁺, while CFTR in the apical membrane is selectively conductive to Cl⁻. Basolateral K⁺ efflux hyperpolarizes the cell membrane potential, which increases the driving force for Cl⁻ secretion.

From such considerations, it follows that transcellular Cl⁻ transport is influenced by the electrochemical (Nernst) potential for K⁺. Assuming typical values for intracellular and extracellular [K] of 120 mM and 4.4 mM, respectively, the Nernst potential for potassium (Eₖ) would be -84 mV. However, for the hypokalemia existing in a patient ([K] = 3.2 mM) Eₖ increases to -92 mV, an 8 mV hyperpolarization. Under these conditions, as basolateral K⁺ channels open to allow K⁺ to recycle, the membrane potential hyperpolarizes even more than with normal potassium levels, which heightens the increase in the driving force for chloride secretion when apical CFTR Cl⁻ channels open.

Even if Eₖ were to remain constant, an increase in the basolateral conductance for K⁺ would result in hyperpolarization of the cell membrane potential. The presence of ATP-sensitive K⁺ (Kₐₚ₆) channels in the basolateral membrane could provide such a mechanism [18], since an increase in transport activity requires an increased turnover of the Na,K-ATPase pump, which tends to lower intracellular ATP levels.

Therefore, the development of hypokalemia enhances the secretion of Cl⁻ into the lumen. This addition of Cl⁻ to the lumen then provides both an electrical driving force for the paracellular movement of Na⁺ and an osmotic driving force for water to go into the lumen. The driving force for water flow into the lumen subsists when the luminal fluid becomes isosmotic, which explains why the secretory diarrhea of cholera is essentially isotonic. To make matters worse, the increasing water content of the lumen "dilutes" the luminal [K], reducing the major driving force for K⁺ reabsorption, promoting further K⁺ loss. The process then becomes a vicious cycle of isotonic Na⁺ (with Cl⁻ and HCO₃⁻) and K⁺ loss, resulting in progressive hypovolemia, hypokalemia, and metabolic acidosis. Without intervention, the
The precise mechanism of action is still under investigation, but several studies have yielded information on both its structure [22, 23] and action [24, 25, 26]. There seems to be a differential effect of the toxin on the enterocytes: it exerts a direct stimulatory effect on the secretory crypt cells, and an anti-absorptive effect on the villous cells, which both favor net secretion. Fortunately, the decrease in absorption is much less than the increase in secretion, making oral rehydration therapy (ORT) possible (vida infra). The colon is usually in a state of absorption since it is relatively insensitive to the toxin, but its absorptive capacity is quickly overcome in this "overflow" diarrhea.

The crystal structure of CTX was recently solved [22], and it closely resembles the heat-labile enterotoxin from Escherichia coli, LT [27]. Both are heterotrimeric complexes, comprised of a pentameric arrangement of five B subunits within which the wedge-shaped A subunit is loosely held [28]. Choleragenoid, the B subunit pentamer of cholera toxin, directs the enzymatic A subunit to its target by binding to the GM1 gangliosides exposed on the luminal surface of intestinal epithelial cells [29, 30, 31]. Interestingly, the two enterotoxins share approximately 80 percent sequence homology, but the other 20 percent may be the difference between a self-limited disease and death. The most significant difference between the two toxins is the carboxyl terminus of the A2 chain, which helps to tether the A subunit above the plane defined by the five B subunits. Apparently, it is differences such as these that lead to the production of much higher levels of cAMP with CTX than with LT.

The A subunit is the active component of the toxin [26]. It is thought that the entire A subunit (or a portion of it) gains entry to the cell through the ring of the B subunits on the apical membrane [32]. The A1 peptide, the active fragment of the A

patient dies of hypoperfusion acidosis and hypovolemic shock.

**Cholera toxins**

It is not an overstatement to say that the entire clinical syndrome of cholera is due to the action of the cholera toxin on intestinal epithelial cells. *Vibrio cholerae* is a non-invasive, aerobic, gram-negative bacillus that produces the choleragen, the cholera toxin (CTX). The organism attaches to the intestinal mucosa via various surface adhesion components, such as toxin coregulated pili (tcpA) [19]. The most sensitive regions are in the duodenum and upper jejunum; the ileum is less affected. The organism does not invade the mucosal surface, and bacteremia is virtually never seen. CTX is cytotoxic rather than cytopathic, as it disrupts intracellular processes but does not directly cause cell death.

Although Koch identified *Vibrio cholerae* as the etiologic agent of cholera, and first proposed that the disease was toxin-mediated in 1884 [10], CTX has only been characterized in the latter half of this century. In 1959, De showed that inoculation of ligated segments of rabbit small intestine with the sterile and bacteria-free filtrate from cultures of *Vibrio cholerae* caused marked distension due to the secretion of isotonic fluid [20]. No distension developed in the ligated segments from the same rabbits injected with control medium. This was the first convincing demonstration that *Vibrio cholerae* must be elaborating a soluble factor that itself is capable of mediating the disease.

The toxin satisfies all of "Koch's postulates," since the toxin: (1) is present in those affected and absent in healthy individuals, (2) can be isolated from infected patients (in this case, from their stool) and produced in culture, (3) is able to cause disease when reinoculated into susceptible hosts from pure culture, and (4) can be reisolated from the experimentally infected host [21].
subunit cleaved by proteolysis, binds to
NAD inside the cell [33, 34]. The toxin
subunit then catalyzes the addition of an
ADP-ribose from NAD to the G protein
that activates adenylyl cyclase, Gs [17, 35,
36]. The cAMP produced activates the
cAMP-dependent protein kinase (PKA),
whose catalytic subunit then phosphory-
lates apical membrane chloride channels.
There is some evidence that other proteins
are also ADP-ribosylated, but the signifi-
cance of these other additions has not yet
been determined [37].

**Normal physiology of G-proteins**

The signal transduction mechanism
involves heterotrimeric guanine nucleotide
binding (G) proteins comprised of an α
subunit, and a βγ complex (see reviews
[38, 39, 40]). Typically, a first messenger
(e.g., a hormone) binds to a membrane
receptor, activating a G protein within the
membrane. The α-subunit of the activated
G protein then exchanges GTP for GDP
and dissociates from the βγ complex. This
activated α-subunit directly interacts with
an effector molecule or enzyme such as
adenylyl cyclase.

One of the remarkable features of the
α-subunit is its intrinsic GTPase enzymat-
ic activity, providing an auto-negative
feedback mechanism for regulated deacti-
vation [41]. Once the GTP is hydrolyzed
to GDP, the α-subunit may re-associate
with the βγ complex, thereby turning off
the functional activity of the effector mol-
ecule.

**Cholera toxin and G Proteins**

Cholera toxin activates adenylyl
cyclase in at least three ways through the
ADP-ribosylation of the α-subunit [23,
42]. First, ADP-ribosylation reduces the
inherent GTPase activity of the α-subunit
by a factor of one hundred, resulting in a
tremendous increase in the production of
cAMP [43, 44]. Second, the addition of
ADP-ribose facilitates the physical disso-

ication of the βγ complex from the α-sub-
unit [45]. Finally, the ADP-ribosylation by
cholera toxin prevents the dissociation of
the α-subunit from the activated cyclase,
keeping the enzyme locked in its active
state [42, 46].

The ADP-ribosylation by cholera
toxin requires the presence of separate
ADP-ribosylation factors, or ARFs [47,
48]. ARFs are GTP-binding proteins, nor-
mally present in cells, that function in con-
junction with the cholera toxin to increase
the level of adenylyl cyclase activity [49].
ARFs appear to be allosteric activators of
the A1 peptide, interacting directly with
the toxin to stimulate its activity *in vivo*
[50, 51, 52]. Currently three highly con-
served classes of these factors have been
identified, and different ARFs may be have
optimal activity under different environ-
mental conditions [53, 54].

Thus, the result of the action of
cholera toxin is an elevation of cAMP to
pathologically high intracellular levels
through a G protein mediator. CTX-
induced elevations in cAMP lead to an
abnormally high apical membrane chlori-
de conductance, due to opening of the
CFTR chloride channels. Chloride can
then move down its concentration gradi-
ent, spilling from the cell in abnormally
high quantities. Due to the resultant elec-
tronegative lumen, sodium is secreted via
a paracellular pathway. This osmotic gradi-
ent drives water flow into the lumen,
with the net effect being the secretion of
massive amounts of isotonic fluid.

Cholera toxin exerts additional effects,
separate from the adenyl cyclase system.
For instance, CTX also triggers intestinal
enterochromaffin cells to release serotonin
[55]. Serotonin may contribute to height-
ened gastrointestinal secretion by serving
as an excitatory neurotransmitter of intra-
mural neurons within the enteric nervous
system [56, 57]. VIP-containing neurons
may serve as the effectors of vasodilata-

tion, increasing mucosal perfusion and, as a result, intestinal secretion [58, 59].

Furthermore, *Vibrio cholerae* produces other toxins in addition to CTX [60]. *Zonula occludens* toxin (Zot) increases intestinal permeability by weakening the tight junctions that maintain the integrity of the epithelial sheet [61]. Zot interacts with specific cell receptors, reversibly affecting the actin elements of the cytoskeleton, allowing increased loss of ions into the intestinal lumen [62]. Accessory cholera enterotoxin (Ace) also contributes to secretory diarrhea, possibly by opening channels and increasing the potential difference across the apical membrane [63]. Pathogenic strains of *Vibrio cholerae* may also produce a sodium channel inhibitor [64]. These additional toxins have a supplementary role to CTX in the pathogenesis of secretory diarrhea in cholera.

**CHOLERA AND THE HETEROZYGOTE ADVANTAGE HYPOTHESIS**

The impact of cholera goes well beyond the clinical syndrome of cholera gravis. To the present day, cholera continues to challenge definitions of disease and the underlying mechanisms that maintain diseases within the human population. On a fundamental level, the most important question is why certain diseases (like cystic fibrosis) exist at all, and to answer it, we are again confronted with the problem of cholera.

In select circumstances, there can be a theoretical advantage to heterozygosity for a damaging trait, when this combination of alleles protects the carrier from another severe disorder. In perhaps the best known example, heterozygotes for the abnormal protein Hemoglobin S do not have sickle cell anemia, but are protected to a certain degree against malaria [65]. Thus, there exists a “heterozygote advantage” that maintains the frequency of this otherwise deleterious recessive gene in the general population.

A similar advantage has been hypothesized for the diseases of cholera and cystic fibrosis (CF) [66, 67, 68]. In CF, the apical chloride channel (CFTR) is hyperactive or inactive, preventing the direct transport of chloride across cellular membranes [69, 70]. This homozygous recessive disorder occurs at a frequency of one in 2500 Caucasian live births, an alarmingly high rate given the severity of the disease [71]. This high rate is inadequately explained by the effects of genetic mutation or by a fertility difference of the carriers [72, 73].

Heterozygotes for CF have some abnormal channels due to the mutant gene, as well as normal channels due to the wild-type gene. In the absence of infection or severe stressors, the heterozygote would suffer few clinically evident abnormalities, due to the presence of the subset of normally functioning channels [74]. However, it is possible that when heterozygotes for CF become infected with *Vibrio cholerae*, the increase in apical chloride conductance is blunted, thereby attenuating the massive loss of salt associated with those cholera victims having a full complement of normal CFTR channels [75, 76]. A recent experiment confirmed this hypothesis using transgenic mice, showing that chloride secretion was directly correlated to the number of CFTR alleles [77]. However, other experiments have not provided as conclusive data [78].

Cholera has remained a scourge throughout the latter half of the last century. Considering the time scale necessary for adaptation, not enough time has passed to remove the allele from the gene pool [2]. Thus, it is possible that CF continues to exist in the human population because heterozygosity for this disorder is to some extent protective against cholera.
TREATMENT

Death from *cholera gravis* results from untreated hypovolemic shock with metabolic acidosis. The cornerstones of therapy are 1) to give the patient back what is lost — a lot of isotonic fluid — as the villagers believed; and 2) to stop the losses. To replete the lost substances in 1831, physicians attempted intravenous volume resuscitation. However, if they would have known about Na-coupled glucose transport, they could have made an oral rehydrating solution containing NaCl, KCl, and bicarbonate of soda of the same composition as what the patient was losing, along with glucose.

For this reason alone, the identification of sodium-coupled epithelial cotransport processes was a major medical breakthrough. Although Reid had provided experimental evidence supporting the transport of sugars against a concentration difference at the turn of the century [79], the dependence on luminal sodium was first observed by Riklis and Quastel in the late 1950s [80]. In the early 1960s, Crane and colleagues advanced these ideas and showed that sodium-dependent sugar cotransport occurred in the luminal brush border of small intestinal epithelial cells [81, 82]. The first of the family of sodium-glucose transporters was subsequently cloned from rabbit small intestine by Wright and coworkers in 1987 [83].

Normally the sodium-coupled transporters utilize the large electrochemical gradient of sodium to drive absorption of sugars and other nutrients (e.g., amino acids). Although cholera toxin biases the system strongly in the direction of secretion, reabsorptive processes are largely left intact. In fact, one study involving total intestinal perfusion with cholera toxin in human volunteers demonstrated a doubling of net sodium cotransport after exposure to CT [84]. Therapy with ORS takes advantage of cotransport, by using sugar to drive the reabsorption of salt. This attempt at restoring balance to a hyperactive secretory system can be lifesaving.

To stop the losses in cholera, the source of the infection must be eliminated. Fortunately, the *Vibrio* infection itself is self-limited; that is, bacteria do not remain active in the intestinal tract for extended periods of time. Although antibiotics may decrease the severity of the clinical symptoms or otherwise diminish the course of the illness, in most cases they are not essential to the treatment of the disorder. Therefore, in developing countries or in those in which medicine is in short supply, a regimen of ORS will permit the survival of the vast majority of those infected [85], as in case scenario [3].

In the present day, most physicians would begin treatment with intravenous isotonic saline, bicarbonate replacement, and antibiotics (tetracycline, doxycycline, or ciprofloxacin [86]). It should be stressed, however, that ORS would and does work. ORS is particularly valuable in countries where cholera is endemic and can be pandemic, as recently seen in Peru in 1991.

SUMMARY

Cholera has been a scourge of humankind, claiming millions of lives from antiquity to the present day. The history of cholera is also associated with the development of several medical breakthroughs (e.g., intravenous solution therapy, basic sanitation, oral rehydration therapy) that have saved millions of lives. Study of the cholera toxin continues to provide great insights into the cellular basis of diseases, the molecular mechanisms of signal transduction systems involving G-proteins, and our understanding of epithelial transport. Such progress has rendered cholera a curable disease. Despite this level of advancement, it is paradoxical that the fate of people with cholera in underdeveloped parts of the
world is no different from that of the unfortunate patient in 1831.

EPILOGUE

In 1831, intravenous therapy was not available. Indeed, it was during the cholera epidemic in Sunderland in 1831 that the idea of intravenous therapy was proposed by Dr. William Brooke O'Shaughnessy, a 22-year-old graduate of Edinburgh, in a paper on cholera to the Westminster Medical Society. This wasn’t attempted until 1832 when Dr. Thomas Latta “dissolved from two to three drachms of muriate of soda and two scruples of the subcarbonate of soda in six pints of water, and injected it at temperature 112° Fah.” (The Lancet of June 2, 1832). In modern units, this is about [Na] 58 meq/l, [Cl] 49 meq/l, and [HCO₃⁻] 9 meq/l [87]. Although they didn’t have antibiotics, they did have salts and sugars, and thus an oral rehydration solution would have worked.

ACKNOWLEDGEMENT: This article was based on work performed in Dr. Alan Segal’s section of the first year physiology course at the Yale University School of Medicine, where Drs. Kavic and Frehm were his dedicated, if not speedy, students.

REFERENCES

1. Rabbani, G.H., and Greenough III, W.B. Cholera. In: Lebenthal, E, and Duffey, M. eds. Textbook of Secretory Diarrhea. New York: Raven Press; 1990. p. 245.
2. Benyajati, C., Keoplug, M., Beisel, W.R., Gangrosa, E.J., Sprinz, H., and Sitprija, V. Acute renal failure in asiatic cholera: clinico-pathologic correlations with acute tubular necrosis and hypokalemic nephropathy. Ann. Intern. Med. 52:960-975, 1960.
3. Astrup, P., Bie, P., and Engell, H.C. In: Salt and Water in Culture and Medicine. Munksgaard Intl. Publishers, Copenhagen; 1993, p. 169.
4. In a desperate effort to save the patient, the woman’s abdomen was impaled with another segment of goose trachea to initiate peritoneal dialysis on day fifteen, but to no avail.
5. Weissman, J.B., DeWitt, W.E., Thompson, J., Muchnick, C.N., Portnoy, B.L., Feeley, J.C., and Gargarosa, E.J. A case of cholera in Texas, 1973. Am. J. Epidemiol. 100:487-498, 1974.
6. Blake, P.A., Allegra, D.T., Snyder, J.D., Barrett, T.J., McFarland, L., Caraway, C.T., Feeley, J.C., Craig, J.P., Lee, J.V., Puhr, N.D., and Feldman, R.A. Cholera – a possible endemic focus in the United States. N. Engl. J. Med. 302:305-309, 1980.
7. Alam, N.H., Majumder, R.N., Fuchs, G.J., and the CHOICE study group. Efficacy and safety of oral rehydration solution with reduced osmolarity in adults with cholera: a randomised double-blind clinical trial. Lancet 354:296-299, 1999.
8. Barua, D. History of Cholera. In: Barua, D. and Greenough III, W.B, eds. Cholera. New York: Plenum Medical Book Co.; 1992, pp. 1-24.
9. Pollitzer, R. Cholera. WHO, Geneva. 1959.
10. Kaper, J.B., Morris, Jr., J.G., and Levine, M.M. Cholera. Clin. Micro. Rev. 8:48-86, 1995.
11. Koch, R. An address on cholera and its bacillus. Brit. Med. J. 2:403-407, 1884.
12. Rosenberg, C.E. The Cholera Years: The United States in 1832, 1849, 1866. Chicago: University of Chicago Press; 1962, pp. 213-214.
13. Rosenberg, C.E. The Cholera Years: The United States in 1832, 1849, 1866. Chicago: University of Chicago Press; 1962, p. 42.
14. Segal, A.S. Salty language is confusing. Hosp. Pract. 31:81-84, 1996.
15. Segal, A.S., Boulpaep, E.L. and Maunsbach, A.B. A novel preparation of dissociated renal proximal tubule cells that maintain epithelial polarity in suspension. Am. J. Physiol. 270:C1843-C1863, 1996.
16. Welsh, M.J., Anderson, M. P., Rich, D.P. Berger, H.A., Denning, G.M., Ostedgaard, L.S., Sheppard, D.N., Cheng, S.H., Gregory, R.J., and Smith, A.E. Cystic fibrosis transmembrane conductance regulator: a chloride channel with novel regulation. Neuron 8:821-829, 1992.
17. Gadsby, D.C., Nagel, G., and Hwang, T.C. The CFTR chloride channel of mammalian heart. Ann. Rev. Physiol. 57:387-416, 1995.
18. Mauerer, U.R., Boulpaep, E.L. and Segal, A.S. Regulation of an inwardly rectifying ATP-sensitive K⁺ channel in the basolateral membrane of renal proximal tubule. J. Gen. Physiol. 111:161-180, 1998.
19. Taylor, R.K., Miller, V.L., Furlong, D.B., and Mikalanos, J.J. Use of phoA gene fusion to identify a pilus colonization factor coordinately regulated with cholera toxin. Proc. Natl. Acad. Sci. 84:2833-2837, 1987.
20. De, S.N. Enterotoxicity of Bacteria-free culture filtrate of Vibrio cholerae. Nature 183:1533-1534, 1959.
21. Joklik, W.K., Wilett, H.P., and Amos, D.B. Zinsser. Microbiology, 18th ed. Norwalk, Conn: Appleton-Century-Crofts, Norwalk, CT; 1984, p. 6.
22. Zhang, R.G., Scott, D.L., Westbrook, M.L., Nance, S., Spangler, B.D., Shipley, G.G., and Westbrook, E.M. The three-dimensional crystal structure of cholera toxin. J. Mol. Biol. 251:563-573, 1995.
23. Zhang, R.G., Westbrook, M.L., Westbrook, E.M., Scott, D.L., Otwinowski, Z., Mauilik, P.R., Reed, R.A., and Shipley, G.G. The 2.4 A crystal structure of cholera toxin B subunit pentamer: cholera-agenoid. J. Mol. Biol. 251:550-562, 1995.
24. Cassel, D. and Pfeuffer, T. Mechanisms of cholera toxin action: Covalent modification of the guanyl nucleotide-binding protein of the adenylyl cyclase system. Proc. Natl. Acad. Sci. USA 75:2669-2673, 1978.
25. Sharp, G.W.G., Hannah, C.M., Cohen, M., and Donowitz, M. Calcium and cyclic AMP-induced changes in intact cell phosphorylation of ileal microvillous membrane proteins. Fed. Proc. 45:742, 1986.
26. Ross, E.M. and Gilman, A.G. Reconstitution of catecholamine-sensitive adenylyl cyclase activity: Interaction of solubilized components with receptor replete membrane. Proc. Natl. Acad. Sci. USA 74:3715-3719, 1977.
27. Sixma, T.K., Pronk, S.E., Kalk, K.H., Wartna, E.S., van Zanten, B.A.M., Witholt, B., and Hol, W.G.J. Crystal structure of the cholera toxin-related heat-labile enterotoxin from E. coli. Nature 351:371-377, 1991.
28. Gill, D.M. The arrangement of subunits in cholera toxin. Biochem. 15:1242-1248, 1976.
29. van Heyningen, W.E., Carpenter, C.C.J., Pierce, N.F., and Greenough, III, W.B. Deactivation of cholera toxin by ganglioside. J. Inf. Dis. 124:415-418, 1971.
30. Mosser, G., Mallouh, V., and Brison, A. A 9 angstrom two-dimensional projected structure of cholera toxin B-subunit-Gm1 complexes determined by electron crystallography. J. Mol. Biol. 226:23-28, 1992.
31. Fishman, P.H. Role of membrane gangliosides in the binding and action of bacterial toxins. J. Membr. Biol. 69:89-97, 1982.
32. Bennett, V., O’Keefe, E., and Cuatrecasas, P. Mechanism of action of cholera toxin and the mobile receptor theory of hormone receptor-adenylate cyclase interactions. Proc. Natl. Acad. Sci. USA 72:33-37, 1975.
33. Gill, D.M. and Rappaport, R.S. Origin of the enzymatically active A1 fragment of cholera toxin. J. Infect. Dis. 139:674-680, 1979.
34. Spangler, B.D. Structure and function of the cholera toxin and the related Escherichia coli heat-labile enterotoxin. Microbiol. Rev. 56:622-647, 1992.
35. Moss, J. and Vaughan, M. Mechanism of activation of cholera toxin. Evidence for ADP-ribosyltransferase activity with arginine as a acceptor. J. Biol. Chem. 252:2455-2457, 1977.
36. Toyoshige, M., Okuya, S., and Rebois, R.V. Choleragen catalyzes ADP-ribosylation of the stimulatory G protein heterotrimer but not its free alpha-subunit. Biochem. 33:4865-4871, 1994.
37. Francis, M.L., Okazaki, I., Moss, J., Kurosky, A., Pecanha, L.M.T., and Mond, J.J. cAMP-independent effects of cholera toxin in B cell activation. III. Cholera toxin A subunit-mediated ADP-ribosylation acts synergistically with ionomycin or IL-4 to induce B cell proliferation. J. Immunol. 154:4956-4964, 1995.
38. Stryer, L. and Bourne, H.R. G proteins: a family of signal transducers. Ann. Rev. Cell Biol. 2:391-419, 1986.
39. Gilman, A.G. G proteins: transducers of receptor-generated signals. Ann. Rev. Biochem. 56:615-49, 1987.
40. Casey, P.J. and Gilman, A.G. Protein involvement in receptor-effector coupling. J. Biol. Chem. 263:2577-2580, 1988.
41. Brandt, D.R. and Ross, E.M. GTPase activity of the stimulatory GTP-binding regulatory protein of adenylyl cyclase, Gs. J. Biol. Chem. 260:266-272, 1985.
42. Moss, J. and Vaughan, M. Guanine nucleotide-binding proteins (G proteins) in the activation of adenyl cyclase: lessons learned from cholera and "travelers’ diarrhea." J. Lab. Clin. Med. 113:258-268, 1989.
43. Cassel, D. and Selinger, Z. Mechanism of adenylyl cyclase activation by cholera toxin: Inhibition of GTP hydrolysis at the regulatory site. Proc. Natl. Acad. Sci. USA 74:3307-3311, 1977.
44. Abood, M.E., Hurley, J.B., Pappone, M.C., Bourne, H.R., and Stryer, L. Functional homology between signal-coupling proteins. J. Biol. Chem. 257:10540-10543, 1982.
45. Kahn, R.A. and Gilman, A.G. ADP-ribosylation of Gs promotes the dissociation of its alpha and beta subunits. J. Biol. Chem. 259:6235-6240, 1984.
46. Burns, D.L., Moss, J., and Vaughan, M. Release of guanyl nucleotides from the regulatory subunit of adenylyl cyclase. J. Biol. Chem. 258:1116-1120, 1983.
47. Enomoto, K. and Gill, D.M. Cholera toxin activation of adenylate cyclase. J. Biol. Chem. 255:1252-1258, 1980.

48. Kahn, R.A. and Gilman, A.G. Purification of a protein cofactor required for ADP-ribosylation of the stimulatory regulatory component of adenylate cyclase by cholera toxin. J. Biol. Chem. 259:6228-6234, 1984.

49. Kahn, R.A., and Gilman, A.G. The protein cofactor necessary for ADP-ribosylation of Gs by cholera toxin is itself a GTP binding protein. J. Biol. Chem. 261:7908-7911, 1986.

50. Noda, M., Tsai, S-C., Adamik, R., Moss, J., and Vaughan, M. Mechanism of cholera toxin activation by a guanine nucleotide-dependent 19 kDa protein. Biochim. Biophys. Acta 1034:195-199, 1990.

51. Tsai, S-C., Noda, M., Adamik, R., Moss, J., and Vaughan, M. Enhancement of cholera-gen ADP-ribosyltransferase activities by guanyl nucleotides and a 19-kDa membrane protein. Proc. Natl. Acad. Sci. USA 84:5139-5142, 1987.

52. Murayama, T., Tsai, S-C., Adamik, R., Moss, J., and Vaughan, M. Effects of temperature on ADP-ribosylation factor stimulation of cholera toxin activity. Biochem. 32:561-566, 1993.

53. Tsuchiya, M., Price, S.R., Tsai, S-C., Moss, J., and Vaughan, M. Molecular identification of ADP-ribosylation factor mRNAs and their expression in mammalian cells. J. Biol. Chem. 266:2772-2777, 1991.

54. Price, S.R., Welsh, C.F., Haun, R.S., Stanley, S.J., Moss, J., and Vaughan, M. Effects of phospholipid and GTP on recombinant ADP-ribosylation factors (ARFs). J. Biol. Chem. 267:17766-17772, 1992.

55. Nilsson, O., Cassuto, J., Larsson, P.A., Jodal, M., Lidberg, P., Ahlman, H., Dahlstrom, A., and Lundgren, O. S-Hydroxytryptamine and cholera secretion: a histochemical and physiological study in cats. Gut 24:542-548, 1983.

56. Cassuto, J., Jodal, M., Tuttle, R., and Lundgren, O. On the role of intramural nerves in the pathogenesis of cholera toxin-induced intestinal secretion. Scand. J. Gastroenterol. 16:377-384, 1981.

57. Nocerino, A., Iafusco, M., and Guandalini, S. Cholera toxin-induced small intestinal secretion has a secretory effect on the colon of the rat. Gastroenterol. 108:34-39, 1995.

58. Cassuto, J., Fahrenkrug, J., Jodal, M., Tuttle, R., and Lundgren, O. Release of vasoactive intestinal polypeptide from the cat small intestine exposed to cholera toxin. Gut 22:958-963, 1981.

59. Jiang, M.M., Kirchgessner, A., Gershon, M.D., and Surprenant, A. Cholera toxin-sensitive neurons in guinea pig submucosal plexus. Am. J. Physiol. 264:G86-G94, 1993.

60. Kaper, J.B., Faasano, A., and Trucksis, M. Toxins of Vibrio cholerae. In: Wachsmuth, I.K., Blake, P.A., and Olsvik, O., eds. Vibrio cholerae and Cholera: Molecular to Global Perspectives. Washington, D.C.: ASM Press; 1994; pp. 145-176.

61. Faasano, A., Baudry, B., Pumplin, D.W., Wasserman, S.S., Tall, B.D., Kletky, J.M., and Kaper, J.B. Vibrio cholerae produces a second enterotoxin, which effects intestinal tight junctions. Proc. Natl. Acad. Sci. USA 88:5242-5246, 1991.

62. Faasano, A., Fiorentini, C., Donelli, G., Uzzau, S., Kaper, J.B., Margareten, K., Ding, X., Guandalini, S., Comstock, L., and Goldblum, S.E. Zonula occludens toxin modulates tight junctions through protein kinase C-dependent actin reorganization, in vitro. J. Clin. Invest. 96:710-720, 1995.

63. Trucksis, M., Galen, J.E., Michalski, J., Faasano, A., and Kaper, J.B. Accessory cholera enterotoxin (Ace), the third toxin of a Vibrio cholerae virulence cassette. Proc. Natl. Acad. Sci. USA 90:5267-5271, 1993.

64. Tamplin, M.L., Colwell, R.R., Hall, S., Kogure, K., and Strichatz, G.R. Sodium-channel inhibitors produced by enteropathogenic Vibrio cholerae and Aeromonas hydrophilia. [letter] Lancet 1:975, 1987.

65. Allison, A.C. Protection afforded by sickle-cell trait against subtertian malarial infection. Brit. Med. J. 1:290-294, 1954.

66. Quinton, P.M. In: Quinton, P.M., Martinez, R.J., and Hopfer, U., eds. Fluid and Electrolyte Abnormalities in Exocrine Glands in Cystic Fibrosis. San Francisco: San Francisco Press; 1982, pp. 53-76.

67. Rodman, D.M. and Zamudio, S. The cystic fibrosis heterozygote-advantage in surviving cholera? Med. Hypoth. 36:253-258, 1991.

68. Romeo, G., Devoto, M., and Galietta, L.J.V. Why is the cystic fibrosis gene so frequent? Hum. Genet. 84:1-5, 1989.

69. Hwang, T-C., Lu, L., Zeitlin, P.L., Gruentert, D.C., Huganir, R., and Guggino, W.B. CF channels in CF: lack of activation by protein kinase C and cAMP-dependent protein kinase. Science 244:1351-1353, 1989.

70. Li, M., McCann, J.D., Anderson, M.P., Clancy, J.P., Liedtke, C.M., Nairn, A.C., Greengard, P., and Welsh, M.J. Regulation of chloride channels by protein kinase C in normal and cystic fibrosis airway epithelia. Science 244:1353-1356, 1989.
71. Rotter, J.I. and Diamond, J.M. What maintains the frequencies of human genetic diseases? Nature 329:289-290, 1987.
72. Wright, S.W. and Morton, N.E. Genetic studies on cystic fibrosis in Hawaii. Am. J. Hum. Genet. 20:157-169, 1968.
73. Jorde, L.B. and Lanthrop, G.M. A test of the heterozygote advantage hypothesis in cystic fibrosis carriers. Am. J. Hum. Genet. 42:808-815, 1988.
74. Hallett, W.Y., Knudson Jr, A.G., and Massey, F.J., Jr. Absence of detrimental effect of the carrier state for the cystic fibrosis gene. Am. Rev. Respir. Dis. 92:714-724, 1965.
75. Hansson, G.C. CF and chloride-secreting diarrhea. Nature 333:711, 1988.
76. Baxter, P.S., Goldhill, J., Hardcastle, J., Hardcastle, P.T., and Taylor, C.J. Accounting for cystic fibrosis. Nature 335:211, 1988.
77. Gabriel, S.E., Brigman, K.N., Koller, B.H., Boucher, R.C., and Stutts, M.J. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. Science 266:107-109, 1994.
78. Cuthbert, A.W. An experimental approach to the genetic advantage hypothesis in cystic fibrosis heterozygotes. J. Gen. Physiol. 104:42a-43a, 1994.
79. Reid, E.W. On intestinal absorption, especially on the absorption of serum, peptone, and glucose. Phil. Trans. Roy. Soc. London Ser. B 192:211, 1900.
80. Riklis, E. and Quastel, J.H. Effects of cations on sugar absorption by isolated surviving guinea pig intestine. Can. J. Biochem. Physiol. 36:347-362, 1958.
81. Crane, R.K. Hypothesis for mechanism of intestinal active transport of sugars. Fed. Proc. 21:891-895, 1962.
82. Crane, R.K. Intestinal absorption of sugars. Physiol. Rev. 40:789-825, 1960.
83. Hediger, M.A., Coady, M.J., Ikeda, T.S., and Wright, E.M. Expression cloning and cDNA sequencing of the sodium/glucose cotransporter. Nature 330:379-381, 1987.
84. Schiller, L.R., Santa Ana, C.A., Porter, J., and Fordtran, J.S. Glucose-stimulated sodium transport by the human intestine during experimental cholera. Gastroenterol. 112:1529-1535, 1997.
85. Greenough, W.B., III. Oral rehydration therapy. [letter] Lancet 345:1568-1569, 1995.
86. Khan, W.A., Begum, M., Salam, M.A., Bardhan, P.K., Islam, M.R., and Mahalanabis, D. Comparative trial of five antimicrobial compounds in the treatment of cholera in adults. Trans. Roy. Soc. Trop. Med. Hyg. 89:103-106, 1995.
87. Cosnett, J.E. The origins of intravenous fluid therapy. Lancet 1:768-771, 1989.