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MEDICAL REVIEW

Case Studies in Cholera: Lessons in Medical History and Science

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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.f

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 (choleragen). 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

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[d] *Abbreviations:* Ace, accessory cholera enterotoxin; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CTX, cholera toxin; K_{ATP}, ATP-sensitive K^+; ORT, oral rehydration therapy; tcpA, toxin regulated pili, Zot, zonula occludens toxin.
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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 $NaHCO_3$ 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, non-tender 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

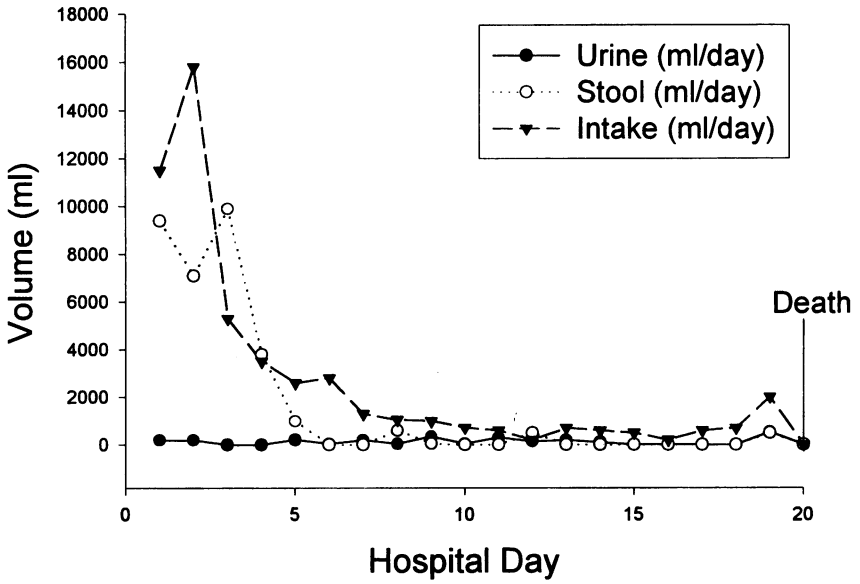

Figure 1. Chart of the total input, total output, and stool output for the patient described in case scenario one. Note that stool accounts for nearly all the output throughout the hospital course, and that initial stool output approached ten liters per day. The patient died of oliguric renal failure on hospital day 20.

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 *kommabazillen* (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

Table 1. The Cholera Pandemics (based on Pollitzer, 1959 [8]).

Pandemic	Time Period	Serogroup	Biotype	Affected Areas[b]
First	1817 to 1823	*Vibrio cholerae* 01	Classical[a]	1, 2, 3, 5
Second	1829 to 1851	*Vibrio cholerae* 01	Classical[a]	1, 2, 3, 4, 5, 6, 7
Third	1852 to 1859	*Vibrio cholerae* 01	Classical[a]	1, 2, 3, 4, 5, 6, 7, 8
Fourth	1863 to 1879	*Vibrio cholerae* 01	Classical[a]	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

[a] Presumed.
[b] 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 HCO_3^- anions, and by the end of the jejunum most of the HCO_3^- from hepatic and pancreatic secretions has been absorbed. It follows that diseases affecting the small intestine can result in significant HCO_3^- 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) repletion. 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

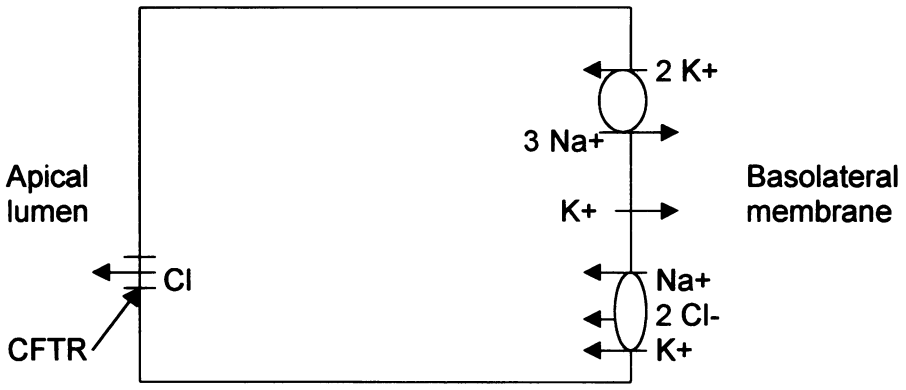

Figure 2. Secretory epithelial cell model. The Na, K-ATPase pump and triple ion transporter are shown on the basolateral side of the cell, with the CFTR chloride channel illustrated on the apical membrane. This arrangement allow for secretion of chloride across the cell.

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)[d], 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 (E_K) would be -84 mV. However, for the hypokalemia existing in a patient ([K] = 3.2 mM) E_K 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 E_K 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 (K_{ATP}) 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 isoosmotic, 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 HCO_3^-) and K^+ loss, resulting in progressive hypovolemia, hypokalemia, and metabolic acidosis. Without intervention, the

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 cytotonic rather than cytotoxic, 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].

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

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 phosphorylates apical membrane chloride channels. There is some evidence that other proteins are also ADP-ribosylated, but the significance 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 enzymatic activity, providing an auto-negative feedback mechanism for regulated deactivation [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 molecule.

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 dissociation of the βγ complex from the α-subunit [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, normally present in cells, that function in conjunction 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 conserved classes of these factors have been identified, and different ARFs may be have optimal activity under different environmental 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 chloride conductance, due to opening of the CFTR chloride channels. Chloride can then move down its concentration gradient, spilling from the cell in abnormally high quantities. Due to the resultant electronegative lumen, sodium is secreted via a paracellular pathway. This osmotic gradient 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 adenylyl cyclase system. For instance, CTX also triggers intestinal enterochromaffin cells to release serotonin [55]. Serotonin may contribute to heightened gastrointestinal secretion by serving as a excitatory neurotransmitter of intramural 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 [HCO_3^-] 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.

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