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CHAPTER 10

Cholera

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1. Introduction

Cholera is an acute epidemic dehydrating diarrhea, usually sudden in onset and, if not properly treated, fatal in as many as half those affected. While a similar clinical entity can be caused by other agents, the term is usually reserved for all diarrheal illnesses caused by *Vibrio cholerae*, and expanded to include those with only a few loose bowel movements.

Cholera is the most dramatic of diseases. Periodically, it has poured out of its homeland in Asia as devastating pandemics with high fatality rates. A healthy individual could be dead within a few hours, so that to wish cholera on a person was a most serious curse in eastern Europe. The illness is equally dramatic in its response to treatment; literally within minutes after treatment with fluid and electrolytes is initiated, a moribund individual can become essentially well, other than a continuing diarrhea.

Governments have fallen and the political map of Europe has been shaped by outbreaks of cholera. More than any other disease, it forced the intrusion of society (government) into the privacy of one's castle to eliminate conditions presumed to cause the disease, leading ultimately to the establishment of boards of health with legal authority to limit a citizen's activities. The desire to prevent country-to-country spread led to the establishment of the International Quarantine Commission. The study of its spread laid an early foundation for development of modern methods of infectious-disease epidemiology.

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2. Historical Background

There is every reason to believe that cholera had existed on the Indian subcontinent for many centuries before the Europeans first arrived. At the beginning of the 16th century, the Portugese clearly described the picture of classic cholera. As India became better known to the Europeans, catastrophic outbreaks were reported, especially related to military movements and the aggregation of large numbers of pilgrims attending religious fairs. There is no evidence in any available language that the disease had spread to adjacent countries such as Burma, Ceylon, and Java.

However, on seven occasions since 1817, the disease poured out of Bengal, extending far beyond its normal habitat, and attacked large populations in many countries. The First Pandemic lasted from 1817 to 1823, reaching China and Japan to the East; to the west, it spread to the shores of the Mediterranean and Zanzibar on the east coast of Africa. The pandemic ended when the disease completely disappeared from any region outside India.

The Second Pandemic began in 1829. The disease spread to Persia, into Russia, Poland, Austria, Prus-

sia, England, Ireland, and France. It came to the New World in 1832 on ships coming to Quebec. It reached New York on June 23, 1832, Philadelphia on July 5, and spread through the rest of the country until 1835. Outbreaks of cholera occurred in Mexico, Cuba, Nicaragua, and Guatemala until 1837. The impact of the disease in Europe is evidenced by several historical episodes. In Newburn, England, on January 15, 1832, 50 people, almost 1 in 10 of the entire population, were attacked between nightfall and noon the next day; by the end of the epidemic, which lasted 1 month, 1 person of every 2 had had the disease and 1 person of every 8 had died.[59,81a]

In 1840, cholera was reintroduced into China by British troops transferred from India; the disease was carried westward in 1842 from China, reentering western Asia and Europe. It reentered the United States in 1848 through both New York and New Orleans, and during 1849 and 1850 rampaged through the country as what was called "American's greatest scourge."[24] In 1849, it was carried from New Orleans to Panama and thence to San Francisco. By 1852, the disease had generally disappeared, but still smoldered in the region of Russia and Poland. It was in this pandemic that John Snow appreciated the relationship between water and cholera and was motivated to write his classic report, *On the Mode of Communication of Cholera*,[81a] in 1849.

The Third Pandemic is stated by Pollitzer[75] to have begun in 1852, extending to Persia and Mesopotamia and to Europe. It covered northern Europe as a whole, but especially affected England, permitting Snow to complete his studies of the relationship of the disease to the water supply and describe the "most terrible outbreak of cholera which ever occurred in this kingdom," which was centered on the now-famous Broad Street pump.[81a] In 1854, cholera entered the United States, and again the disease spread eastward to China and Japan. By 1859, Europe was free of the disease.

The Fourth Pandemic began in 1863 and lasted until 1873 or 1875. The disease reached southern France and Italy, coming through Arabia and Egypt. From 1865 to 1867, cholera was again prevalent in the United States, complicated by the movement of military personnel in the aftermath of the Civil War. However, the mortality rate did not reach 5% in any community in 1866; in 1833, the death of 5–15% of the population of a locality had not been unusual; in 1849, the mortality rate had seldom reached 10%; and in 1856, the rate was low compared to the earlier experiences, but 50,000 are estimated to have died in the United States.[24] Disease continued in Europe, and in 1873, New Orleans and the Mississippi Basin were once again seriously invaded. Outbreaks continued until about 1877–1879.

The Fifth Pandemic, which started in 1881, provided Robert Koch the opportunity to study the disease in Egypt and Calcutta and to identify in 1883 the pathogen that produces the disease. A steamer with infected patients arrived in New York in October 1887; the application of newly learned laboratory methods was credited with the control of the secondary cases. In 1892, ships again arrived with cholera patients with no spread. However, South America was afflicted, as were Africa and eastern Asia.

The Sixth Pandemic began in 1899; disease persisted in eastern Europe until 1923.

The Seventh Pandemic, which is at present extant, originated in 1958 with endemic disease in Sulawesi (Celebes) in Indonesia. This disease was caused by a variant of *V. cholerae* that had been isolated in 1903 in the El Tor Quarantine Station, on the Sinai peninsula, from an individual not considered to have cholera, and the organism was originally considered to be nonpathogenic (for discussion, see Section 4). In early July 1961, an international regatta of dragon boats was held in Kuching, Sarawak, with representatives from Sulawesi. Cholera broke out with 270 cases and 61 deaths.[32a] Thence, the disease spread to Macao, Hong Kong, and the Philippines, and by 1965, cholera had spread westward into eastern Europe and Africa with incursions into Italy, Spain, and Portugal.

3. Methodology

3.1. Sources of Mortality Data

Cholera being one of the diseases dreaded by man, reporting of its occurrence has been required since records have been kept. Unfortunately, the accuracy of the reporting has reflected the competence of the health-care delivery system and its practitioners, often modified by political and economic motivations. The carefully collected statistical data contributed by the dedicated physicians of the In-

dian Medical Service are most valuable. However, the reporting was based on the cases seen in the treatment facilities; deaths in rural areas could well have been overlooked, and overreporting occurred, unless bacteriology was carried out on all cases, by inclusion of cases of acute dehydrating diarrhea from causes other than *V. cholerae*, such as the entity of "nonvibrio cholera."[58]

Cholera is reportable to the local health authority by the most rapid means, with rapid transmission to the World Health Organization (WHO). The information coming from infected areas is then disseminated to all health administrations. Unfortunately, this has often resulted in the imposition of restrictive measures, such as requirement of vaccination for all travelers coming from the infected area, prohibition of movement of food and even metal ore from the infected areas, restriction of the mail, and other irrelevant activities, often with economic implications. This, too, has led to concealment rather than reporting of the disease.

Complicating this, the appearance of cholera within a country is considered by some to indicate failure on the part of public-health officials. A misguided "scientific" approach may obscure the data. As one example, the health authorities of East Pakistan directed in 1958 that cholera and cholera deaths could be reported only if bacteriologically confirmed; because laboratory facilities were essentially not available, subsequent statistical data indicated a marked increase in mortality from "diarrhea" and "dysentery," but no cholera. A diagnostic complication was observed in reviewing a "Cholera Register" covering the period 1937–1964 in an East Bengal rural community. This record carefully preserved the identity, age, and outcome of every case reported as cholera to the district sanitary inspector. The entries on almost all children were lined out with the notation "Worms," deleting them from the final official report of cholera cases. In the experience of the Pakistan–SEATO Cholera Research Laboratory (CRL), the passage of a few ascarids either in the stools or in the vomitus of cholera patients was usual.

3.2. Sources of Morbidity Data

The problems associated with mortality data pertain even more to morbidity data. Added to this is the fact that the vast majority of illnesses caused by *V. cholerae* are manifested by a diarrheal bout no different from that frequently experienced in all parts of the world. Without bacteriological investigation of diarrheal attacks in endemic areas, it is impossible to assess accurately the morbidity caused by *V. cholerae*.

3.3. Surveys

Many surveys have been carried out, using bacteriological and serological methods, usually as components of research projects such as those carried out in East Pakistan–Bangladesh. They have included surveillance of the total population of a community,[63] serological surveys of a random sample of a large population sample,[70] and an organized surveillance system of large populations for diarrheal symptoms with bacteriological and/or serological identification of the etiological organisms.[13]

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. The cholera vibrio is one of the easiest bacteria to culture.[33] It grows readily on simple nutrient media at temperatures ranging from 22 to 40°C; maximal growth occurs at 37.5°C. The organisms are sensitive to acid and killed at a pH below 3; their tolerance to alkalinity is the basis of most selective media, which are needed only when the vibrios are relatively few in number, as in the stools of asymptomatic carriers. In the rice-water stool of the classic cholera patient, the vibrios are the predominant organisms and are easily isolated on simple nutrient agar, often as a pure culture. On gelatin agar (GA) (3% gelatin, 1% trypticase, and 1% NaCl in 1.7% agar), colonies of *V. cholerae* are surrounded by a halo formed by the gelatinase produced by the vibrio[81]; this becomes more obvious if the plates are refrigerated for 15 min before examination. Unfortunately, vibrios do not grow on the usual "enteric" media, such as eosin methylene blue agar (EMB) or *Salmonella–Shigella* (SS) agar; they may grow on MacConkey's agar and on some lots of deoxycholate agar.

In carriers and antibiotic-treated and convalescent patients, the use of selective media or enrichment before plating is necessary. Selective media, such as alkaline taurocholate–tellurite–gelatin agar

(TTGA)[67] or thiosulfate–citrate–bile salt–sucrose agar (TCBS),[62] permit use of heavy inocula. Incubation of the specimen overnight in alkaline (pH 8.5–8.6) peptone water or in other vibrio enrichment media before plating suppresses the growth of other organisms without inhibiting the growth of the vibrios. Vibrios survive well in transport media.[30] In some, such as the Cary–Blair modification of Stuart's medium, the organisms remain viable without significant multiplication; in others, such as alkaline taurocholate tellurite peptone media, multiplication of vibrios occurs. Unfortunately, when the specimen contains "noncholera" vibrios, these tend to overgrow and may obscure the presence of cholera vibrios after incubation for more than 24 hr.[68]

Barua and Gomez[7] have shown the feasibility of submitting a fecal specimen to the laboratory by wetting blotting paper (but not filter paper) with the liquid stool and placing this in a moisture-tight plastic bag.

Rapid diagnosis is made possible by the heavy bacterial load in the cholera stool; 82% of cases in Dacca had vibrio counts greater than 10^5/ml[68]; Huber[50] found counts greater than 10^7/ml in 12 of the 23 cases he studied in Manila. Direct examination of a wet mount of fecal material (a rectal swab immersed in 0.3–0.5 ml broth) by dark-field or phase-contrast microscopy reveals the rapid, darting motility characteristic of vibrios. Wet mounts can then be treated with preservative-free sera directed against the two serotypes, Ogawa and Inaba (see Section 4); the homologous vibrios are immediately immobilized, providing a specific diagnosis within 3–5 min in 50–70% of cases positive by standard culture. On reexamination of the broth after 6–18 hr at 37°C, 85–96% of positive cases were detected.[12,43] Sack and Barua[78] showed the effectiveness of the direct and the indirect fluorescent techniques in detecting infection when the cholera stool contained more than 10^6 and 10^7 vibrios/ml, respectively; the positive diagnosis was available in 1½ hr. Barua[6] found enough growth on nutrient agar plates after incubation for 4–5 hr at 37°C to recognize the typical vibrio colonies in the stereomicroscope and permit specific group-O1 serotype agglutination.

A series of 263 consecutive diarrhea cases at the Pakistan-SEATO CRL were followed in 1964 by daily rectal swabs plated directly, and after enrichment on TTGA and GA plates, and by antibody titrations on paired sera taken on admission and more than 6 days later. Vibrios were isolated on the day of admission from 93% of the 178 whose stools were positive at some time; a rise in bacterial agglutinins was found in 5 who were consistently negative on culture. In this series, positive cultures were found in 97% of the 183 with cholera; in 90% of these cases, vibrios were isolated from the admission specimen.[9a]

3.4.2. Serological Diagnostic Methods. Infection with *V. cholerae* elicits antibody responses directed against the antigens of the vibrios themselves and against the soluble exotoxin (enterotoxin) they elaborate. Antibodies to the specific vibrio antigens can result from natural infection or immunization; however, infection with organisms sharing common antigens with vibrios[76] may elicit antibodies, and the heat-labile enterotoxin (LT) of the geographically more widely distributed *Escherichia coli* is antigenically similar to the enterotoxin of *V. cholerae*.

Bacterial agglutinins directed against live vibrios are present in the convalescent patient, but shortly fall to low or undetectable levels.[41] A microtiter technique for bacterial-agglutinin testing was developed; of 364 bacteriologically positive cases with a second serum on the 6th or later day, a diagnostic rise in titer was seen in 90%. However, of those in the 0- to 4-year age group, only 78% showed a titer rise.[14]

The vibriocidal technique described by McIntyre and Feeley[64] was also modified to the microtiter system. This was more sensitive than agglutinin testing and no less specific. In a series of 370 bacteriologically positive cases, 4-fold or greater rises were observed in over 96%.[15] Among bacteriologically negative cases, a significant rise in titer was observed in 9 individuals; 6 of these were close relatives of a patient with bacteriologically confirmed cholera; 3 had also received cholera vaccine. Only in 3 others (0.8%) was a significant titer rise observed, but since exposure could not be documented, these could represent false-positive reactions. The vibriocidal test was positive in 7.3% of cases that did not exhibit a rise in agglutinins; in only 0.5% did the reverse obtain.

The microtiter techniques made possible the use of fingertip blood. This provides an ideal technique for field surveys; in a population that is needle-shy,

samples were obtained from every individual present in the community who had been preselected for bleeding.[70]

The antibody directed against the enterotoxin can be quantified by a variety of techniques: neutralization,[16] passive hemagglutination,[34] or enzyme-linked immunosorbent assay (ELISA). Since licensed cholera vaccines have not contained cholera toxoid, any antitoxin detected in the serum is a consequence of natural infection and not from vaccine. However, as noted above, the enterotoxin of *V. cholera* and the LT of *E. coli* are antigenically similar, so that an antitoxin rise alone cannot be taken as proof of cholera.

4. Biological Characteristics of the Organism

Vibrio cholerae is a gram-negative, slightly curved rod about 1.5 μm long and 0.4 μm wide. A single polar flagellum imparts to the organism a rapid darting motion that caused Robert Koch to compare the appearance of the organism in a hanging drop to the swarming of gnats on a summer evening. The range of temperature over which the vibrio grows is wide and includes the ambient temperature of tropical areas. The organisms grow well in alkaline media, but they are very sensitive to acidity. They are differentiated from related organisms by being oxidase-, gelatin-, indol-, and mannitol-positive; inositol-negative; lysine- and ornithine-decarboxylases-positive; and arginine-dihydrolase-negative. They form no gas from glucose and have a guanosine–cytosine base ratio of 42–47%.

Gardner and Venkatraman[38] divided vibrios serologically; those that were agglutinated by O-subgroup 1 (often shortened to O1) antiserum included the organisms isolated from patients with typical Asiatic cholera. These organisms were further differentiated into the Inaba and Ogawa serotypes by the use of sera absorbed to remove the common group 1 antigen. There is suppressed formation of the Inaba antigen in the Ogawa organisms; this becomes manifest when the organisms are grown at 20°C,[53] so that the organisms now are agglutinated by both specific antisera, the characteristic of the Hikojima serotype.

In 1906, Gotschlich[42] reported at the El Tor Quarantine Station on the Sinai peninsula the recovery of vibrios that were agglutinated by anticholera sera but differed from the classic organisms by elaborating a soluble hemolysin. They were isolated from the intestinal contents of cases dying of illnesses that were not considered to be cholera clinically or anatomically, and therefore the organisms were considered to constitute a separate El Tor biotype that did not cause cholera. They were later accepted to be the etiological agent of outbreaks of localized choleralike disease that occurred in Sulawesi (Celebes), called "El Tor disease" or "paracholera." As noted in Section 2, the present pandemic of cholera began with the spread of this disease out of Sulawesi into Hong Kong and the Philippines in 1961, but not until 1962 was the disease caused by the El Tor vibrio considered to be cholera from the point of view of the application of international sanitary regulations.[29] Paradoxically, the El Tor vibrios involved in the current pandemic have lost their hemolytic potency, but do differ from the classic strains in that fresh isolates from agar plates agglutinate chicken erythrocytes in a direct slide test (classic strains do not), give a positive Voges–Proskauer test, are sensitive to polymyxin B, and are resistant to Mukerjee's group IV cholera phage.[37]

The clinical disease produced by the classic and El Tor biotypes do not differ.[85] However, the different biological properties of the two biotypes do influence their epidemic behavior. The classic vibrios persist in water for only short periods of time,[17] and isolations from sewage have been rare. The El Tor vibrio, on the other hand, survives and in some circumstances actually persists in natural water supplies. The growth rate of the El Tor strains is more rapid, and they overgrow classic organisms in mixed cultures.[9] In culturing nightsoil, Bart *et al.*[5] recovered classic Inaba vibrios very rarely while disease was occurring in the community; when both classic and El Tor biotypes were concurrently causing disease, El Tor strains were isolated 10 times more frequently from the nightsoil. Comparing the incidence of infection and disease among family contacts of patients in the latter community, an infection/case ratio of 36:1 was found among El Tor Ogawa contacts, compared to 4:1 among contacts of cases with classic Inaba disease.[4]

These differences in infection/case ratios have been taken to indicate that the El Tor strains are less

virulent. That this is not necessarily so is suggested by the findings in the Dacca area in 1973.[55] Secondary infections occurred in 31% of the family contacts of cases hospitalized at the Cholera Research Laboratory for cholera caused by El Tor organisms; 78.6% of these were symptomatic, and 20% of the infected individuals required hospitalization. In this series, the infection/symptomatic case ratio was 1.3:1; the infection/hospitalized case ratio was 4.7:1.

These data can be interpreted to indicate that the greater ability of the El Tor biotype to survive in the environment results in a greater likelihood of dissemination. This can result in more frequent ingestion of the organisms, but at lower doses, resulting in a greater likelihood of asymptomatic or mild infections. Given comparable exposures, the susceptible individuals may develop comparable disease. The answer can be obtained only by human volunteer studies.

Vibrios that do not agglutinate in O group-1 sera have been called "NCV" (noncholera) or "NAG" (nonagglutinable) vibrios. Because of similarity in numerical taxonomy, DNA base composition, isozymic analysis, and DNA–DNA hybridization analyses, these organisms have been combined with the classic cholera strains into the taxospecies and genospecies of *V. cholerae*, but constituting differing serotypes. This results in confusion and apprehension in the public mind when the recovery of ubiquitous "NCVs" from local waters is reported as contamination with cholera vibrios.[52] While these are most frequently nonpathogenic environmental contaminants, some strains have been shown to produce an enterotoxin neutralized by cholera antitoxin[33] and have been incriminated as the causal agent of acute choleralike diarrhea.[65] In 1968, an outbreak of gastroenteritis caused by these organisms occurred in the Sudan, producing 544 cases with at least 31 deaths.[51]

The enterotoxin (choleragen) elaborated by cholera vibrios has a molecular weight of approximately 84,000. It is composed of two subunits, A and B, joined by sulfhydryl bonds. The B subunit (choleragenoid) is nontoxic but antigenic and has a total molecular weight of approximately 56,000; the A subunit has a molecular weight of 28,000. Strains of *V. cholerae* vary in their production of enterotoxin, which is controlled by a chromosomal gene. Enterotoxin production by *E. coli*, on the other hand, is mediated by a plasmid; the toxins are immunologically cross-reactive.[84]

Heating destroys the A subunit, and only the nontoxic B subunit remains. The B subunit is a potent antigen, and antitoxin in the convalescent cholera patient is specifically directed against this portion of the toxin.

In addition to enterotoxin, cholera vibrios also elaborate mucinase and neuraminidase, which contribute to the pathogenesis of the organism, as well as a host of other enzymes.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

The incidence of cholera disease is determined by a multiplicity of environmental and host factors. These include the degree of crowding, the level of sanitation of the community, and the presence in the population of this organism as well as other less well defined host factors. The high frequency of subclinical infections further increases the inaccuracy of any data. Studies in Dacca have shown that wide variations of rates occur within a geographic area; for example, over a 2-year period in Dacca Municipality, the rates were 55/100,000 and 33/100,000; within individual political units of Dacca City, however, the rates varied from 0 to 220.[60] In the Philippines in 1961, the incidence rate was 123/100,000[31]; in Taiwan in 1962, the rate was 3.4/100,000.[94] These data are based on those who came for medical treatment. Close surveillance of the vaccinees in the Matlab vaccine trials of 1963 and 1964 disclosed diarrheal rates of 470/100,000 and 560/100,000, respectively. The incidence of infection by *V. cholerae* can be monitored by serological follow-up and regular stool cultures. In an endemic area, an infection rate of 2740/100,000 was found in a community over a 10-week period with not a single case of diarrheal disease suggestive of cholera; 5 mild diarrheas were seen from which *V. cholerae* were isolated.[63]

5.2. Epidemic Behavior and Contagiousness

There have been seven major pandemics of cholera, of which the Seventh, still prevailing, began in 1961 (see Section 2). The spread of cholera vibrios

through insanitary areas is easily understood on a strictly fecal–oral basis. What is not clear is why each of the pandemics has been followed by an interval during which there is no evidence that *V. cholerae* persisted in any areas outside the Indian subcontinent. Cholera had existed in the Philippines until 1937; from that time until its reintroduction in 1961, despite the practice of good bacteriology, not a single isolation of *V. cholerae* was made.

The persistence of cholera in the Indian subcontinent suggests some special characteristics; the contagiousness of cholera varies with the physical state of the subject and the dose of organisms ingested. There may be a very much greater susceptibility among those who live on the Indian subcontinent than in other parts of the world, or there may be environmental or cultural practices that increase the probability of exposure to large doses of vibrios. There is no evidence that a racial factor is involved.

As a general hypothesis, an epidemic of cholera can be considered to be a group of common-source outbreaks,[60] usually from foods or from contaminated water. When the level of contamination of water is low, water still constitutes an important hazard if it is used for food preparation without being boiled. This was observed in the preparation of the Bengali dish of *panta bhat*, in which leftover rice is covered with water, held at ambient temperatures, and ingested on the following day. When this rice-handling was duplicated in the laboratory using water with minimal vibrio contamination, the vibrio count increased by several orders of magnitude when a small amount of sodium chloride was present.[17] The water source of many households is easily contaminated once the organism is introduced into a household, especially if there are small children and the water is obtained from dug wells within the household area that are exposed to surface contamination.

The importance of dose was clearly demonstrated by Hornick *et al.*,[49] who found that 10^3 vibrios, ingested by human volunteers after 2 g sodium bicarbonate had been taken, produced asymptomatic excretion of vibrios; a dose of 10^4–10^6 produced simple diarrhea in about 60% of the volunteers. Clinical cholera requiring intravenous treatment occurred in 1 of 8 who received 10^5 organisms, 6 of 23 (26%) of those who received 10^6, and 1 of 2 who received 10^8 vibrios.

5.3. Geographic Distribution

The homeland of epidemic cholera is in the Indian subcontinent, where each year cases of cholera have been recorded and out of which the pandemics have spread. The El Tor vibrio had been recognized as the cause of localized outbreaks of "paracholera" in Indonesia since 1937.[29] Since 1961, however, this biotype has spread out of Indonesia, as indicated in Fig. 1, first through eastern Asia and the Philippines, then westward to involve all of Asia, much of Europe, and most of Africa. Figure 1 covers the period until 1976. Intrusions into Europe have been temporary, while those in Africa have continued unabated. In 1977, cholera extended into Oceania with outbreaks in the Gilbert Islands[87] and in 1978 in Nauru.[89] During March to May 1978, a severe epidemic hit the Republic of Maldives, where no cholera had been known for the past 50 years. There was a total of 11,303 clinical cases for an overall attack rate of 7700/100,000 with 252 deaths and a case-fatality rate of 2.2%. On some islands, 30% of the population were sick within a span of 2–3 months. Deaths occurred within a few hours of onset, and as many as 4 cases came from one house. The outbreak was attributed to contamination of the water in the many superficial wells.[88] The interrelationships of health programs became apparent: chlorination of the wells to kill the vibrios also killed the larviparous fish that were effective in controlling breeding of the culicine vectors of dengue. Thus, cholera control increased the danger of dengue hemorrhagic fever.[24a]

Since 1873, the United States had been free of naturally acquired cholera. In August 1973, a case of typical cholera occurred in a 51-year-old man from the Gulf Coast town of Port Lavaca, Texas. He had not been out of the country since military service in the 1950s; no source case or secondary cases were found.[86] In August 1978, *V. cholerae* was isolated in southwest Louisiana along the Gulf Coast from a 44-year-old man with dehydrating diarrhea. An additional three individuals positive for vibrios had diarrhea, but were not hospitalized. Four more hospitalized cases of vibrio-positive diarrhea were found, and the organisms were recovered from three patient contacts, for a total of 11 infected persons. All had recently eaten boiled or steamed crabs, and vibrios were recovered from infected crabs

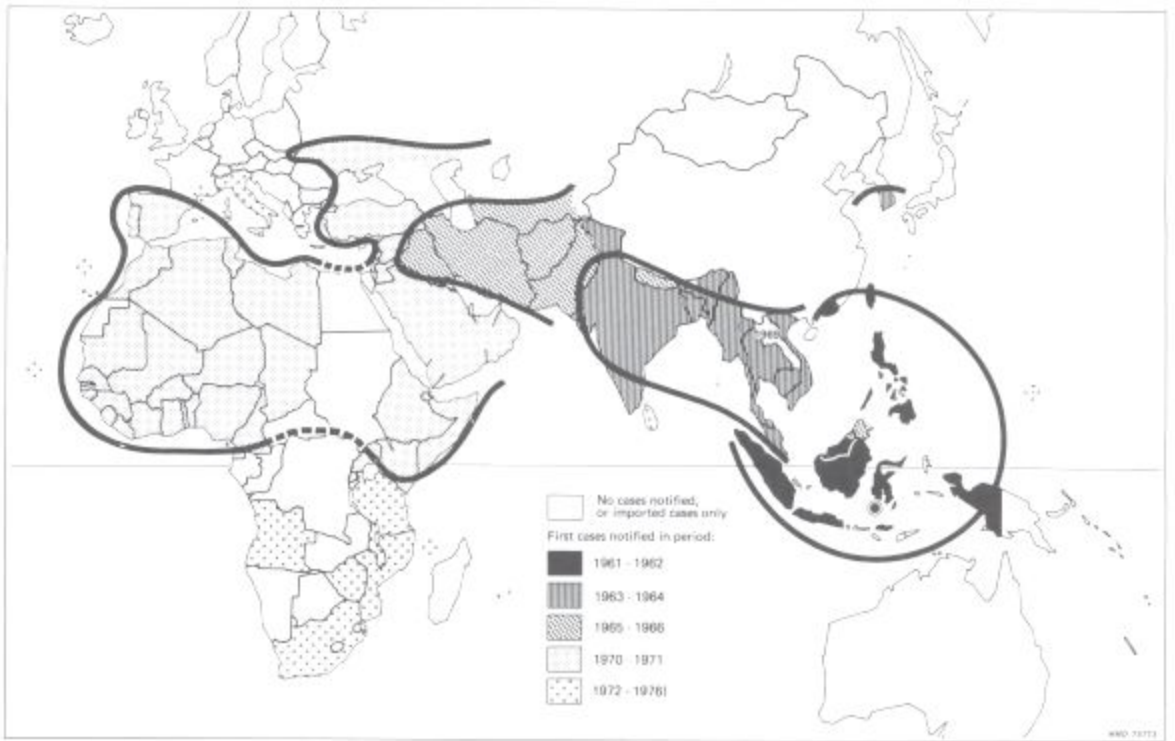

Fig. 1. Extensions of El Tor cholera in the Seventh Pandemic until 1976. In 1977, disease occurred in Japan and the Gilbert Islands; in 1978, in the Maldives, Burundi, Rwanda, Zaire, the United States, the Congo, Zambia, and Nauru; in 1979, in the Sudan and the Gabon. Courtesy of the World Health Organization.

boiled less than 8 min or steamed less than 25 min. Vibrios were also recovered from the sewage of six towns, three with no known infection. The isolates were hemolytic, in contrast to the nonhemolytic pandemic strains, and had bacteriophage sensitivities different from those of the pandemic strains; their characteristics were identical with those of the Port Lavaca strain. The geographic association of this area with that of the Lavaca case and the similar characteristics of the isolates suggest that there has been an ongoing focus of cholera in this portion of the United States since at least 1973.[18]

Cholera was present in Spain from July to November 1979, with 267 cases reported, including 141 cases in Malaga province, a popular resort area.[88a] In November 1979, a small outbreak occurred on the island of Sardinia with 10 cases, 6 of which were hospitalized, and the water and clams from a lagoon receiving raw sewage were infected.[92]

Cholera was reported in 1980 by 40 countries, with endemic disease in 14 African countries, 15 Asian, 1 American (Louisiana), 1 European (Spain), and Australia.[89a] In November 1980, explosive diarrhea, abdominal cramps, and vomiting were experienced by a 46-year-old woman who had not recently been outside western Florida. From her stools, a strain of *V. cholerae*, serotype Inaba, biotype El Tor, that had the same phage characteristics as the strains from Louisiana was isolated; the organisms, however, proved to be nontoxigenic, and the patient did not develop antibodies against the organism or toxin.[17a] She had ingested large numbers of raw oysters from an approved oyster bed in Florida. Nontoxigenic O-1 *V. cholerae* strains have been isolated from the water of the bay from which the oysters had been taken. In 1975, nontoxigenic *V. cholerae* organisms had been isolated from the gallbladder of a truck driver in Alabama who had been hospitalized for acute cholecystitis. He had not been out of the country, except for brief trips to Mexico over 30 years ago. He did eat large quantities of raw oysters, and gave a history of episodes of recurrent

diarrhea once a month for at least 14 years.[23] The Australian case was that of a 2½-year-old boy with diarrhea and dehydration in a town on the Albert/Logan river system of Queensland; toxigenic cholera vibrios have been intermittently isolated from this river system since 1977, when they had been recovered from a patient. Thus, it becomes evident that environmental persistence of *V. cholerae* O1 occurs; however, these organisms may be nontoxigenic and their presence in a diarrhea patient may be coincidental rather than causal.

During 1978, cholera occurred in Japan among those who ate lobsters imported from Southeast Asia; no secondary cases occurred. While there have been many importations of cholera into European countries, Australia, and Canada, secondary cases have rarely occurred. During 1980, nine imported cases occurred in the United States, most of them from Indochinese refugees, with no secondary spread. This did happen in Italy, in Spain, and in Portugal, but the outbreaks were of short duration. The 1973 outbreak in Naples lasted from August through October.[2] The 1974 outbreak in Portugal affected all but one district of the country; the first case occurred on April 24, and the country was declared free of disease on November 29.[19] The persistence of cholera is a reflection of the level of sanitation in the population group into which the organism is introduced; while the organisms may be endemic in some areas, such as Louisiana and Queensland, disease is extremely sporadic when sanitary standards are high.

5.4. Temporal Distribution

In endemic areas, cholera appears with a quite constant seasonal distribution, which varies from community to community. Thus, in Dacca, the annual peak occurs in November and December; in Matlab Bazar, 25 miles away, in January and February; while in Calcutta, 125 miles away, the outbreak usually peaks in May and June. A relationship in this area to the monsoon would be expected; at the beginning of the monsoon in Dacca in April or May, there are often outbreaks as the early rains wash contamination into water sources, but after this, cholera essentially disappears until the end of the monsoon season. In Calcutta, the peak occurs at the height of the monsoon; however, one must take into account the very different environmental conditions that pertain in the crowded bustee areas (native quarters) of Calcutta and the more open areas in East Bengal.

5.5. Age

The age distribution is a reflection of the endemicity of the disease. In the Dacca area, hospitalization rates were highest among those less than 10 years of age.[60] In the Matlab vaccine study area in 1963 and 1964, disease rates among those 0–4 years of age were 11.7 and 16.3 per thousand; among those over 15, the rates were 1.8 and 1.5 per thousand.[13] This is consistent with the observation that about 50% of the children in the 5–9 age group with no history of vaccination or disease have detectable vibriocidal antibody.[70] On the other hand, when cholera strikes a naïve population, the highest incidence of serious disease is usually found in the older age group. In Taiwan, those over 55 were particularly susceptible, with an incidence 2–3 times as great as that of those younger than 35.[94] In the Philippines, the incidence among those over 20 was twice as great as that of those under 20.[31] In later years, the incidence was greatest among the children.

5.6. Sex

The distribution of cases by sex reflects the degree of exposure. In Dacca, where males predominated in the population, there were more male than female cases. In the Philippines, in the first year of the epidemic, of those over 40 the incidence among men was twice that in women.[31] In Taiwan, there was an excess of females in age groups 20 to 29 and 40 to 49, which is consistent with the practice of the women in the household nursing the sick. In Dacca, when the index case was a male over 15, the secondary infection rate among family members was 6.3%; when the index case was a woman or a child under 15, the secondary infection rates were 44.4 and 31.9% respectively.[54]

5.7. Race

No specific racial predisposition has been evident that cannot be explained by socioeconomic factors. A racial or genetic characteristic is often proposed

to explain the country-to-country variation in severity of disease. In 1964, cholera in Saigon was caused by El Tor vibrios; the duration of purging was so short that antibiotic efficacy was not demonstrable. When the El Tor strain spread into India and East Pakistan, the pattern of full-blown disease was no less severe than that produced by the classic organism.[85] While this could be associated with genetic factors, one cannot exclude the differences in behavioral patterns. As an example, in Bengal, the traditional food item of *panta bhat*, as noted in Section 5.2, may ensure that the Bangali is exposed to a very large inoculum and therefore experiences more severe infections.[17] This may well be complicated by host factors produced by malnutrition, which may adversely affect gastric acidity and which has been shown to inhibit the mucosal immune response to intraluminal toxoid or toxin in rats.[3]

5.8. Occupation

Yen[94] was impressed by the numbers of patients who were farmers, fishermen, or laborers who had been exposed to considerable heat and sun with consequent severe thirst and consumption of large amounts of water; he attributed the higher incidence of disease in these groups to loss of salts through excessive sweating. It must be noted that the greater the amount of water one drinks in insanitary areas, the greater the likelihood of ingesting an infectious dose of vibrios.

5.9. Occurrence in Family and Other Settings

In Taiwan, Yen[94] reported a prevalence rate of bacteriological positivity of 9500/100,000 among those living in the household of a cholera patient. This contrasts with a rate of 340/100,000 among neighbors living within 500 m of the house of the index patient and 302/100,000 among residents of the rest of the village.

When an intensive study was made to uncover cases with milder diarrhea among family contacts of patients admitted to the Cholera Research Laboratory Hospital in Dacca City, a secondary attack rate of 11.1%, or 11,100/100,000, was found.[71] In the rural Matlab vaccine-trial population, infection with *V. cholerae* had occurred in 10.7% of family contacts, or 10,700/100,000[13]; 3.5% of the contacts (3500/100,000) had diarrhea. In the Philippines, 18.2% of family contacts were infected, but only 1.7% required hospitalization.[83]

Cholera has been a traditional affliction of those situations in which large numbers of people aggregate, especially if sanitary facilities are rudimentary. Most usually, this has been associated with religious festivals and fairs, but it is also a concomitant of refugee settlements in areas where the organisms are endemic, such as occurred among the East Pakistani refugees in India in 1971.

5.10. Socioeconomic Factors

Cholera is traditionally the disease of the poor. The failure of Pettenkofer to acquire cholera when he drank the culture fluid in his famous dispute with Robert Koch, and the illnesses of his students when they repeated the gesture,[32] have been taken to be indicative of the miserable salaries paid to laboratory technicians in those days; in fact, he was probably immune because of earlier exposures to cholera. The high prevalence of cholera in the poor segments of the community leads to the debate whether poverty, and the consequent nutritional deficiencies, evokes a greater host susceptibility to the disease, or whether the high incidence is due to the poor sanitary conditions under which the poor are forced to live.

5.11. Other Factors

A host factor of greatest importance is the gastric acidity. In the outbreak in Jerusalem in 1970, the disease afflicted many persons in the upper social classes. These patients were predominantly achlorhydric or had had a subtotal gastrectomy.[40] Studies carried out among volunteers showed that even among those who are not achlorhydric, there are a group whom Hornick *et al.*[49] designated "nonsecretors" because their gastric contents were still alkaline 30 min after ingestion of 2 g sodium bicarbonate. It was in this group that the greatest susceptibility to experimental infection was found; after a challenge of 10^6 vibrios given orally with bicarbonate, 7 of 11 "secretors" vs. 21 of 23 "nonsecretors" were infected.[49] The possibility that mal-

nutrition is associated with impaired gastric acidity provides a logical relationship between socioeconomic class and susceptibility to cholera.

6. Mechanisms and Routes of Transmission

Cholera is transmitted by the fecal–oral route. While the vomitus in the acute case may be heavily contaminated, this is essentially a reflux of intestinal contents. The nonoffensive odor-free cholera stool with a volume as great as 20 liters/day, containing approximately 10^7 vibrios/ml, is the primary source of potentially massive environmental pollution.

Infection occurs when these organisms enter the body in food or drink. In the classic study of John Snow, the water supplied by the Southwark and Vauxhall Company to several of the south districts of London was in essence diluted sewage; the identity of the water supplier could be recognized by adding silver nitrate and observing the heavy precipitate of silver chloride probably contributed by urinary contamination. The water of the Broad Street pump had its own special flavor, favored by some. In general, the usual vibrio burden of waters is not great. In the Matlab study area, the incidence of diarrhea associated with *V. cholerae* was not lower in those families that used tubewell water (generally bacteria-free) than in those that used surface waters for drinking.[26] However, that water can play an important part in the transmission of cholera was demonstrated by the 1974 outbreak in Portugal when a widely distributed bottled water was shown to have spread the disease.[20]

Foods can be contaminated by the classic transmitters of the enteric disease, e.g., fecal-feeding flies, foul fingers, or by adding water containing *V. cholerae* to foods that are not subsequently reheated. Given an infective dose and the absence or neutralization of gastric acidity, infection of the nonimmune individual will occur.

The reservoir of *V. cholerae* is man. Although some studies have succeeded in recovering O group-1 vibrios from animal sources[79] and *V. cholerae* are reported to have been recovered from Chesapeake Bay,[25] from surface waters in Kent, England,[8] and from sewage in Brazil,[90] the significance of these isolations is unclear. However, as noted in Section 5.3, there is very strong evidence for persistence of organisms infective for man along the gulf coast in Louisiana and Texas, and Queensland.

7. Pathogenesis and Immunity

7.1. Pathogenesis

The ingested vibrios must first survive the transit through the stomach, which can be achieved because of achlorhydria, by neutralization of the acidity by food, or by very rapid transit through the stomach in water. On reaching the alkaline small intestine, the vibrios multiply. The epithelial lining of the small intestine is protected by a mucous layer; the mucinase elaborated by the organisms provides a method for breaching this barrier.[80] Reaching the intestinal mucosa, the organisms lie in apposition to the brush-border surface of the cell by an adherence factor. The enterotoxin they produce attaches by the B subunit of the cholera toxin to the GM_1 ganglioside present in the brush border. Neuraminidase (or sialidase) is also elaborated by the vibrio, and this actually may convert other gangliosides to GM_1, providing more receptors for the toxin.[46] After a lag period of about 45 min, there begins an outpouring of fluid and salts into the lumen of the bowel. During the interval (which does not occur if the cell membrane is broken so that the toxin can enter directly toward its site of activity)[39], the sulfhydryl bonds between the A and B subunit are broken, and the A subunit penetrates through the cell wall into the interior of the cell to the locus of adenylate cyclase activity. This is now activated, resulting in an accumulation of cyclic AMP, which is associated with the increase in fluid transudate. This fluid has the same osmotic pressure as the serum and contains 45 mEq bicarbonate and 16 mEq potassium per liter of fluid.

These physiological changes occur with no morphological changes in the gut mucosa, other than some congestion, goblet-cell hyperplasia, and a mononuclear-cell inflammatory exudate.[36a] The availability of intestinal-biopsy techniques corrected the earlier belief that the diarrhea was due to a desquamated epithelium and showed this to be the result of postmortem autolysis.

This outpouring of fluid produces dehydration,

the loss of bicarbonate produces metabolic acidosis, and potassium loss results in hypokalemia. The severely dehydrated cholera patient presents with sunken eyes, wrinkled skin, rapid breathing, bizarre P waves and flat T waves on the electrocardiogram, and absent peripheral pulses and blood pressure. While cholera patients may be semicomatose, they can be roused and sometimes, despite absence of any detectable peripheral pulse, will stand and walk. Basal rales indicative of heart failure are often present. Studies by Harvey *et al.*[44] demonstrated that with acidosis, there is a shutdown of the peripheral circulation with central overload, so that in essence the patients go into cardiac failure. When Harvey and her group administered intravenous bicarbonate solution, raising the blood pH to 7.4, the circulatory abnormalities were corrected dramatically.

The loss of potassium is manifested by flat T waves on the electrocardiogram, but evidence of hypokalemia was rarely evident clinically. However, many anecdotal episodes were recounted in which convalescent cholera patients would suddenly drop dead on resuming normal activity; these were in cases that had been managed without potassium replacement. No such episodes were uncovered in the thousands of cholera cases treated by the Pakistan–SEATO Cholera Research Laboratory.

7.2. Immunity

The differences in age-specific disease incidence in endemic areas is ample evidence that immunity to cholera is real and important. However, second attacks have been documented 11–60 months after the initial illness[93]; the infection rates were significantly higher with the heterologous than with the homologous serotype. In volunteer studies, Cash *et al.*[22] rechallenged 27 men who had had diarrhea following their first challenge 4–12 months previously. None of 20 developed diarrhea after homologous challenge; 1 became stool-positive for vibrios but remained asymptomatic. Of 6 given a heterologous oral challenge, 4 developed diarrhea and 1 had an asymptomatic infection. Protection here was evidently antibacterial rather than antitoxic, since there is no difference in the enterotoxin elaborated by the different serotypes.

8. Patterns of Host Response

8.1. Clinical Features

The spectrum of disease elicited by infection by *V. cholerae* ranges from a completely asymptomatic infection, through an ordinary diarrhea, to a violent dehydrating diarrhea with an acute onset and death within as short a time as 4 hr if untreated. Volunteer studies suggest that the severity of the disease is related to the severity of the challenge, the interval between ingestion of the challenge dose and onset of symptoms being 36–53 hr, with extreme ranges from 14 to 143 hr; the severe cases had the shorter incubation periods.[21]

Classic cholera presents typically as a painless diarrhea; the stool initially is brown, but very shortly becomes rice-water in character, completely inoffensive, and essentially odorless, containing flecks of mucus. Vomiting occurs not infrequently, but appears several hours *after* the onset of purging and is apparently the consequence of acidosis or dehydration or both, since it ceases completely after proper treatment has been started. As body fluid is lost, the eyes become sunken, tissue turgor is lost, the blood pressure falls, the pulse becomes inapparent, and the patient becomes semicomatose and in shock. Replacement of the lost fluid and electrolytes[73] corrects the extraintestinal chemical imbalances, but purging persists for as long as 7 days. The diarrhea usually stops at the time that antibody (coproantibody) appears in the stool.[35a] When an antibiotic such as tetracycline is given, purging stops within 48 hr, and the bacteria disappear from the stool; in the untreated individual, the vibrios are shed for at least a week.

Cases with "ordinary" diarrhea cannot be differentiated by any clinical criteria from cases of diarrhea from any other cause; laboratory studies are required.

Women in the third trimester of pregnancy are severely affected by cholera. Dehydration is more severe than among nonpregnant women or those in the second trimester of pregnancy; 50% of the fetuses are stillborn. Fetal death is usual when the mother had an absent or thready radial pulse. With modern replacement therapy, maternal deaths no longer occur, but fetal death has usually occurred before the woman presents for treatment.[45] Chol-

era among children is in general similar to that in adults, but dehydration was less severe in those under 2 years of age; coma on admission was most frequent among those 2–10 years of age. Children tended to have prolonged drowsiness or semistupor lasting as much as 15 hr to 8 days after initial rehydration had been accomplished; sometimes this was related to hypokalemia. In Dacca, coma and convulsions due to hypoglycemia were observed in 1.4% of children in the 2- to 10-year range. While closer observation of intake and output was needed to keep the children from going into shock, the prognosis with modern therapy is excellent, with a case-fatality rate below 1%, even in a rural treatment center.[57]

8.2. Diagnosis

Cholera must be suspected when there is a sharp outbreak of severe dehydrating diarrhea appearing in a small geographic area or in a group of individuals associated with a common source of infection. Isolated cases are unusual, but a severe dehydrating diarrhea, with vomiting beginning several hours after the diarrhea, suggests cholera. The severe cholera case usually cannot be differentiated clinically on admission from cases of severe "nonvibrio cholera"; however, the purging in the latter rarely continues for more than 24–48 hr.[58] Many of these cases are due to enterotoxigenic *E. coli* or other pathogens. In children, a "white diarrhea" can be produced by rotavirus. Laboratory support is necessary to identify the etiological agent, with isolation of the organism or the demonstration of an antibody rise directed against *V. cholerae* confirming the case as one of cholera (see Section 3.4).

9. Control and Prevention

9.1. General Concepts

The ultimate control of cholera is based on high levels of sanitation and personal hygiene to assure that fecally shed organisms are not ingested in sufficient numbers to cause disease. This involves the availability of adequate facilities for sanitary disposal of feces, effective hand-washing after defecation, the availability of water supplies free of vibrios, and a level of culinary standards in the home and in the commercial food establishment sufficient to prevent fecal contamination of foods which may be held at room temperature (which should in any case be brief, as stressed in Chapter 4). Until this ideal is realized, control of cholera will depend on pragmatic measures.[10]

The cholera patient who excretes large quantities of fecal fluid containing 10^{7-8} vibrios/ml is capable of seriously contaminating the home environment, resulting in secondary infections within the household. This is particularly true of children, especially those who are not yet toilet-trained. Removal of cases to a treatment facility permits effective decontamination of the stool, as well as assures survival from this onetime dramatically fatal disease by proper intravenous or oral treatment, or both, with electrolytes and fluid. This allays the panic and hysteria that so often are associated with an outbreak of cholera. The chronic carrier sheds a relatively low number of organisms, and while carriers have been implicated in the introduction of vibrios into a new area,[94] they are not important within the epidemic itself. While shedding of vibrios for as long as 11 years has been documented in "Cholera Dolores,"[1] no secondary cases have been found that could be attributed to her over the years of close surveillance. Other studies have indicated the unimportance of the known long-term carrier in spreading disease.[36,54]

The frequency of asymptomatic infections in which organisms are shed for several days invalidates any possibility that strict isolation of cases would be beneficial in epidemic control. Even though it was cholera that induced the establishment of the International Quarantine Commission, quarantine has had a negative value. Quarantine posts are established along major routes of travel, which are avoided by migrants and the poor among whom the disease predominates. Worse yet, quarantine has too often been associated with restrictive measures; in the current pandemic, boatloads of food were dumped, shipments of commercial items like iron ore delayed, and mail service interrupted when they came from a country that had reported the presence of cholera. The predictable result was deliberate concealment of the presence of cholera in a country.

Reporting the presence of the disease in an area

is of the utmost importance. Because cholera is so often similar to "ordinary" diarrhea, routine bacteriological monitoring of diarrhea cases is important, using culture media that will detect the presence of *V. cholerae*, so that the earliest cases will be detected and reported and a possible epidemic anticipated. This will then permit health authorities to prepare necessary supplies and to alert the medical profession with regard to the proper management of the cholera case. This was well demonstrated when cholera entered the Philippines in 1961; as the disease appeared in individual communities, case-fatality rates as high as 50% were often seen, but only during the first few days of the attack.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

Tetracycline (and other broad-spectrum antibiotics as well as furazolidone) is an effective vibriocidal compound and rids the patient of the organisms within a very short period. These agents are valuable for the prophylaxis of members of the patients' hearth-group, those with whom he eats, among whom secondary cases are so frequent.[61] However, the use of such agents for mass prophylaxis results in the predictable emergence of organisms resistant to the antimicrobial agent used. In Tanzania, vibrio isolates in 1977 were sensitive to tetracycline; after 5 months of use of the drug therapeutically and in mass prophylaxis, 76% of isolates were resistant.[66] This resistance was attributed to one of two plasmids that were transferable among *V. cholerae* strains and also to other members of the enteric group of bacteria such as *E. coli*.[91] Tetracycline was an effective antibiotic in the East Pakistan–Bangladesh study area from 1963 to 1980 when some cholera patients failed to respond as expected to treatment and the isolated organisms were found to be resistant *in vitro* to tetracycline, ampicillin, kanamycin, streptomycin, and trimethoprim–sulfamethoxazole.[23a]

9.3. Immunization

Cholera vaccines over the years have consisted of a mixture of the Inaba and Ogawa serotypes, usually heat-killed and phenol-preserved. Carefully controlled studies carried out in East Pakistan and in the Philippines have shown beyond any doubt that these vaccines do indeed induce immunity against natural infection[11] and that lipopolysaccharide (LPS) itself was effective, that a monovalent Ogawa vaccine afforded protection against both Inaba and Ogawa infections, and that protection against infection with El Tor organisms was comparable whether the vaccine was prepared with El Tor or with classic organisms.

Vaccine was much more effective among adults than among children in the Matlab field studies; e.g., the Ogawa LPS was effective only among adults in protecting against Inaba disease. These studies have been carried out in endemic populations; the superior protection afforded to adults was clearly a secondary response. With some vaccines, two doses were necessary to produce a significant level of protection to children.[69]

While the protection afforded by bacterial cholera vaccine is real and significant, this protection is unfortunately transient. With all trials, except for the first, in which an unusually potent vaccine was used, protection was no longer present after 3–6 months. This means that an immunization program must be scheduled annually to be carried out not earlier than 6 months before the appearance of cholera is expected; such precise timing cannot be assured.

The mechanism of action of the antibacterial vaccine is not entirely clear. An inverse association between cholera attack rates and the level of vibriocidal antibodies in a population has been shown.[11] The administration of vaccine to children raises the mean antibody level to that of unimmunized adults; they then experience the attack rate found in the older group. That the effectiveness of a vaccine might differ significantly between indigenous and foreign populations is suggested by the report that a whole-cell cholera vaccine induced a marked rise in immunoglobulin A (IgA) against *V. cholerae* LPS in the breast milk of Pakistani women, but not in Swedish women.[82a] Freter[35] has shown that parenteral cholera vaccine produced detectable levels of coproantibodies, and that antibacterial antibody in intestinal loops of an experimental animal does not inhibit bacterial growth, but the vibrios are now found free in the lumen rather than attached to the mucosal surface. With individuals, however, volunteer studies have shown that infection occurred as frequently among those with as those without

vibriocidal antibody after oral challenge with 10^6 organisms.[22]

There would seem to be a greater hope for protection from an antitoxin directed against the enterotoxin. In the dog, Curlin and Carpenter,[27] using crossed circulations, have clearly shown that the presence of antitoxin in the blood supplying an isolated loop protected the loop against vibrio challenge. Pierce *et al.*[74] have demonstrated in the dog that a solid immunity followed the instillation of toxoid into the lumen of the bowel; to be effective, the oral antigen required a preliminary parenteral injection of toxoid.[3] Protection could be solid in the absence of demonstrable circulating antitoxin. This immunity depends on the local production of secretory IgA in mucosal plasma cells[72]; Barry and Pierce[3] have shown that protein deficiency impairs this response.

Field trials have been carried out in Bangladesh with a toxoid preparation free of LPS; overall protection of only 26% was seen against Inaba disease only in the 5–14 age group, and it was present only during the first 90 days.[28] Studies with toxoid are under way in volunteers; results have been disappointing, with no protection despite good levels of induced circulating antitoxin following oral ingestion of purified toxoid. Under the same test conditions, excellent homologous and heterologous protection was shown among those with earlier cholera diarrhea.[56]

The greatest hope for induced immunity might be by establishing stable mutants of *V. cholerae* that elaborate only B subunit (choleragenoid) and cannot revert to toxin production. A candidate strain has been described by Honda and Finkelstein[48]; since it retains the ability to adhere to the mucosal surface, it should provide effective antibacterial immunity after oral administration without producing disease. The duration of that immunity, and proof that the strain cannot produce disease in any people, need to be demonstrated.

As indicated above, no vaccines now available are effective for more than a very short period of time,[82] and immunization programs generally tend to miss those who are most likely to be exposed to cholera. More important, immunization has had a negative effect on cholera control because health authorities squander scarce resources carrying out immunizations rather than improving sanitary conditions. Emphasis has been placed on whether or not there was the proper entry on the yellow immunization certificate, on the assumption that this assured freedom from infection; unfortunately, vaccination, even when effective in preventing disease, did not prevent asymptomatic infection.[13] The elimination of vaccination as a requirement for international travel has been an advance in the control of cholera.

9.4. Prevention of Death

The single most important advance in the control and prevention of cholera, of course, has been the improvement in treating the disease, which has changed cholera from a rapidly lethal disease to one of short-term incapacity. The moribund, pulseless patient is restored to general health—with the exception of the small intestine—literally within minutes by the intravenous infusion of an alkaline isotonic solution containing potassium; the ability of the intestinal mucosal cells to be stimulated by glucose to absorb sodium ions has made it possible to effectively maintain the rehydrated state and to completely treat the moderately severely dehydrated cases with an oral solution. This extends the treatment capability to the village level rather than the sophisticated medical center. For intravenous treatment, the WHO Diarrhea Treatment Solution [containing 13 mEq K^+, 48 mEq HCO_3^- equivalence, prepared with 4 g NaCl, 6.5 g sodium acetate (or 5.4 g sodium lactate), 1 g KCl, and 8 g glucose/liter], Ringer's lactate, or a homemade 5:4:1 solution of 5 g NaCl, 4 g $NaHCO_3$ (or equivalent), and 1 g KCl/liter is effective. The oral solution, containing 3.5 g NaCl, 1.5 g KCl, 2.5 g $NaHCO_3$, and 20.0 g glucose/liter, can be prepared locally, or the dry salts can be obtained in packets for mixing with a liter of water, under national or UNICEF programs. Effective antibiotics play only a secondary role, serving only to shorten the period over which replacement fluids must be administered; the latter are the keys to survival.

10. Unresolved Problems

There would be value in having a vaccine that was generally acceptable and had a long duration of effectiveness and in which the cold chain was not

a critical element. An attenuated organism[48] must be shown to be stable in transportation under adverse conditions and to be innocuous when administered to immunodeficient recipients. Perhaps a subunit vaccine[47] or an oral toxoid preparation[74] will provide the long-lasting protection not now available.

The worldwide dissemination of cholera still needs definition. Are there other foci like the Gulf Coast of the United States in which vibrios persist and which become the source of isolated rare cases? Since *V. cholerae* strains are found in various waters in widely different geographic areas,[8,90] this becomes a more probable explanation than fecal droppings from airplanes.[77]

The therapy of cholera has been well developed; the problem has been that of achieving its implementation, particularly in underdeveloped countries in which the disease predominates. Still unresolved is the definition of the factors in host resistance that make this a disease of the poor; if a replaceable deficit in diet could be defined, appropriate food supplementation could be considered, such as the addition of iodine to table salt.

Progress in the understanding of cholera and its etiology, pathogenesis, epidemiology, and treatment has advanced by orders of magnitude in the past 20 years. Those who have been involved are proud of the accomplishments and look forward to the day when cholera is no longer considered a problem—a day that is well within sight.

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