# 17

# *Vibrio cholerae* and Cholera

CHOLERA is probably the best known and most feared of the diarrheal diseases discussed in this book. Although it is by no means the most important cause of diarrhea in terms of total morbidity or mortality, it has caused, and in some parts of the world continues to cause, dramatic outbreaks of acute disease accompanied by considerable loss of life. In other areas cholera is a part of the overall spectrum of endemic diarrhea, and in these situations it often occurs with a regular seasonal periodicity. Cholera has a long history of scientific investigation, with some features of its epidemiology being clarified in London (England) by John Snow in the 1850s; the first full accounts of its clinical, bacteriological, and epidemiological aspects were published in the 1880s as a result of work done in Egypt (Koch 1884).

## Description of Pathogens and Disease

Despite the long history of study referred to above, cholera is attracting renewed scientific interest, and some traditional understandings are being considerably modified. New information is being gained not only on the mechanisms of pathogenesis and immunity but also on certain aspects of epidemiology and transmission. The information summarized in this chapter must therefore be considered as somewhat provisional.

### *Identification*

Cholera is caused by bacterial infection of the small intestine. The causative organism, *Vibrio cholerae*, exists in two biotypes—classical and El Tor. Both can cause an acute intestinal disease characterized by profuse rectal loss of water and electrolytes. The disease begins with sudden painless evacuation from the bowel; as it progresses, (acidotic) vomiting may start, together with muscle cramps due to lowered blood potassium levels (hypokalemia). If untreated, some patients become rapidly dehydrated, pass into shock, and die. Other patients experience much milder diarrheal illness. Sixty percent or more of untreated classical chloera cases die, whereas El Tor is generally regarded as a milder infection with a lower fatality rate and a higher proportion of asymptomatic infections. Recent evidence from Bangladesh suggests, however, that El Tor virulence may be increasing (Khan and Shahidullah 1980). It is not possible to distinguish classical from El Tor cholera clinically by reference to any particular case.

The effects of cholera are due to the action of an exotoxin, produced by the vibrios, which affects the epithelial cells of the gastrointestinal mucosa and leads to massive secretion of water into the lumen of the gut. Diagnosis is by isolation of the bacteria either from stool samples early in the clinical phase of watery diarrhea or by rectal swab from convalescents. It is usual to attempt direct plating on selective media as well as enrichment in alkaline peptone water before plating. To confirm suspected isolates, agglutination tests with anticholera O-group 1 serum are carried out together with microscopic investigation for vibrio morphology and biochemical charactrerization for isolates failing to agglutinate. The El Tor biotype differs from the classical vibrio in very few of its laboratory properties.

Fatality rates can be reduced to under 1 percent in well-managed treatment centers. The treatment of cholera primarily consists of preventing the patient from dying from loss of salts and water. The infection is then self-limited, but its duration is shortened by appropriate antibiotic therapy. Rehydration may be by mouth in patients that are not vomiting and is by giving clean water containing appropriate quantities of salt, potassium chloride, alkali such as sodium bicarbonate, and glucose to promote the absorption of the electrolytes. Patients, particularly children, in a state of shock or vomiting require appropriate intravenous

fluids rapidly. Normal hydration and acid-base balance should be achieved for adults within 2 hours of admission to a treatment center but is achieved more slowly for children weighing less than 20 kilograms.

*Occurrence*

The classical cholera vibrio is the historic cause of cholera. From its homeland in Bengal and the Ganges Valley, six classical cholera pandemics have spread. The El Tor biotype, first identified in Sinai in 1905, has only comparatively recently been accepted as *V. cholerae*. A focus of El Tor cholera was known to exist in the Indonesian island of Sulawesi in the 1930s. In 1961 this focus exploded and began to spread, thereby initiating the seventh known pandemic of cholera. It spread eastward to the Philippines, northward to Taiwan and Korea, and westward into India, where it replaced the classical biotype, and then on to Pakistan, the Middle East, and Europe. It also spread into East and West Africa and the Pacific islands (figure 17-1).

Where it is endemic, cholera develops a regular periodicity, and epidemic waves occur at one or two seasons of the year. These seasonal patterns are not the same in various places, and there is no good explanation of how the cholera infection cycle correlates with climatic conditions.

Endemic cholera prior to 1960/1961 was confined to India, especially the Ganges system, Bangladesh, and Sulawesi. Since then it has invaded many parts of the world and is, at the time of writing, considered to be endemic in several areas of Africa and Asia. Many national health authorities are very reluctant to admit or report endemic cholera because of the possible effect on tourism and international travel (for instance, the pilgrimage to Mecca). For this reason endemic El Tor cholera exists in a number of countries that officially deny it. The present pandemic has not yet spread to the

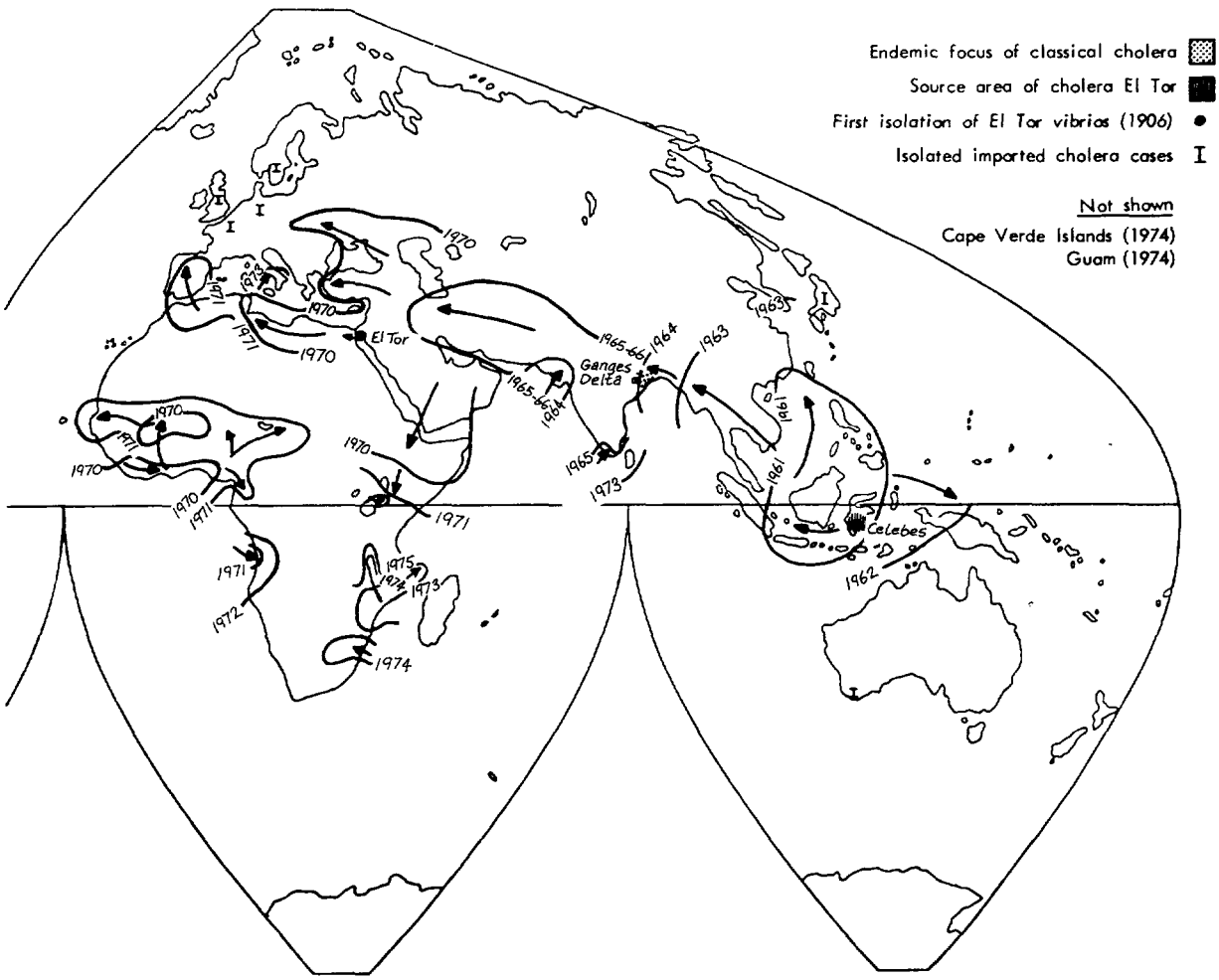


Figure 17-1. *The global spread of cholera, 1961–75*

Americas, although the risk of its introduction is very great.

*Infectious agents*

The family Vibrionaceae includes several human enteric pathogens of the genus *Vibrio*, and the taxonomic status of some of them remains uncertain and controversial. They are all Gram-negative, motile rods (0.5 by 1.5–3 micrometers) usually having a curved or comma shape. They are nonsporulating, noncapsulated, facultative anaerobes and possess a single polar flagellum (figure 17-2). The terminology for the various pathogenic and closely related vibrios used here is the one most commonly used at the present time, although it is not ideal and may be revised (WHO Scientific Working Group 1980).

Of greatest public health importance, and the main topic of this chapter, are organisms that have traditionally been called *Vibrio cholerae* or cholera vibrio, but which are now strictly known as *V. cholerae* O-group 1 or O1. They will be called *V. cholera* in this chapter. *V. cholerae* is the cause of epidemic cholera and exists in two biotypes (classical and El Tor) and three serotypes (Inaba, Ogawa, and the much less common Hikojima). *V. cholerae* produces an enterotoxin that has been extensively studied and is similar to *Escherichia coli* heat-labile enterotoxin (see chapter 13). Adherence to the intestinal mucosa is also an important virulence factor but is poorly understood.

A second group of *V. cholerae*, which agglutinate O1 antiserum but which do not produce enterotoxin, have been recently recognized. These are known as atypical *V. cholerae* O1 (in this chapter atypical *V. cholerae*), and some of them have biochemical properties that differ from those of *V. cholerae*. Atypical *V. cholerae* have been isolated from water both in areas where endemic clinical cholera is known to occur and in areas—such as Brazil, England, and the USA—where it does not occur. Atypical *V. cholerae* are thought not to be enteric pathogens.

The third group of *V. cholerae* strains are those which do not agglutinate O1 antisera but which are biochemically and genetically similar to *V. cholerae* O1. These are now called non-O1 *V. cholerae*, but until very recently were called non-agglutinating vibrios (NAGs) or non-cholera vibrios (NCVs). They are currently classified into seventy-two O-group serotypes, but this typing scheme is tentative and provisional. Non-O1 *V. cholerae* have been associated with many individual cases of cholera-like diarrhea and with some small outbreaks. Some non-O1 *V. cholerae* produce a cholera-like enterotoxin.

Finally, there are other potentially pathogenic vibrios that are clearly not *V. cholerae*. *V. parahaemolyticus* is a halophilic marine organism responsible for numerous outbreaks and attacks of food poisoning associated with seafood. It has a marine rather than an enteric reservoir and so is not considered in this chapter, although it is briefly discussed in chapter 7. The Group F (or Group EF6) vibrios (often mistakenly identified as *Aeromonas*) have been isolated from the stools of patients with diarrhea in many countries, but it is uncertain whether they are toxin-producing or pathogenic. Other vibrio species


Figure 17-2. V ibrio cholerae *under scanning electronmicroscopy*. The single polar flagellum of the organism is prominent. Scale bar = 1 micrometer. (Photo: J. Gallut, Institut Pasteur, Paris, France. Reproduced by courtesy of *Bulletin of the World Health Organization*)

occasionally isolated from man—*V. alginolyticus*, *V. metschnikovii*, *V. vulnificus*, and L+ *Vibrio*—are not believed to cause diarrhea.

*Reservoir*

The primary source of infection that has been clearly documented is the human case or carrier. There is speculation over the role of environmental isolates of atypical *V. cholerae* and non-O1 *V. cholerae* in cholera epidemiology and the possibility of an environmental reservoir (see below, the section "Occurrence and Survival in the Environment"). There is also speculation about the role of animal reservoirs, especially for non-O1 *V. cholerae* or for *V. cholerae* were isolated interepidemic periods. Sanyal and others (1974) examined 1,287 fecal samples from 195 domestic animals following an outbreak of cholera in Varanasi (India) during 1972. The proportions of animals from which *V. cholerae* or non-O1 *V. cholerae* were isolated were: dogs, 27 percent; chickens, 18 percent; cows and goats, each 11 percent. There were no isolations from buffalo, donkeys, or horses. Out of a total of fifty-four strains of *V. cholerae* isolated, eight were *V. cholerae* O1 (El Tor, Ogawa). Neither this nor other studies have clearly shown that animal infections with *V. cholerae* or non-O1 *V. cholerae* play any role in the epidemiology of human infection and disease.

*Transmission*

Cholera is transmitted by the fecal-oral route from person to person, and transmission is encouraged by inadequate water supply and excreta disposal facilities and, more generally, by poverty and overcrowding. Convalescent and asymptomatic individuals may excrete $10^2$–$10^5$ *V. cholerae* per gram of feces, whereas an active case excretes $10^6$–$10^9$ per milliliter of rice-water stool (Dizon and others 1967; Greig 1914; Smith, Freter and Sweeney 1961).

Infective doses are high in healthy adult males. Hornick and others (1971) required $10^8$ classical *V. cholerae* in water to produce diarrhea in 50 percent of adult volunteers (the median infective dose, or $ID_{50}$), and $10^{11}$ organisms to produce cholera-like diarrhea. With the prior administration of 2 grams of sodium bicarbonate, the $ID_{50}$ was lowered to $10^4$ for diarrhea and $10^8$ for cholera-like diarrhea. No diarrhea or infection was produced by $<10^8$ organisms without $NaHCO_3$ or by $<10^3$ organisms with $NaHCO_3$ (see also Cash and others 1974).

Gastric acidity is an important barrier to cholera infection, and those with lowered acidity (hypochlorhydria) may be infected by lower doses than others. More recent volunteer studies with El Tor strains have shown that infective doses are lower when the organisms are administered in food than in small volumes of water (WHO Scientific Working Group 1980). This could be due to more rapid gastric emptying, neutralization of gastric acid by food, or protection of vibrios that are adsorbed to, or embedded within, food particles. Nothing is known about the dose needed to cause acute diarrhea in 1 percent of malnourished children, but it may be $10^2$ or even less.

If it is assumed that the environmental reservoirs of *V. cholerae* described below are epidemiologically unimportant, then cholera transmission must take place by direct person-to-person contact or by the fecal contamination of water or food. Waterborne and foodborne transmission have both been clearly demonstrated on specific occasions. Cholera has classically been regarded as a waterborne disease, and there are some experts who believe that this is its dominant and normal mode of transmission. Others maintain that this may be true in Bangladesh but not elsewhere, while a third opinion holds that cholera transmission among poor people in developing countries is primarily nonwaterborne. This subject has attracted recent debate (for instance Feachem 1976; Levine and Nalin 1976) and is of considerable importance in designing control strategies. The topic has been comprehensively reviewed by Feachem (1981, 1982).

*Incubation period*

The incubation period is generally short and clinical symptoms occur within 0.5 to 5 days (usually 1–3 days) of ingesting the bacteria. Incubation periods may be inversely related to the dose of organisms ingested.

*Period of communicability*

Convalescents generally excrete *V. cholerae* intermittently and only for short periods. Thus, 50 percent of cholera cases will be found to excrete the pathogen for up to 5 days, 30 percent continue to excrete for up to 15 days, and 10 percent for up to 25 days. By 1 month usually less than 5 percent of cases are still excreting *V. cholerae*, and it is very uncommon to find carriage persisting beyond 2 months. The truly chronic carrier—such as Cholera Dolores from the Philippines (Azurin and others 1967)—is a very rare phenomenon. Asymptomatic infection is common, and the El Tor biotype produces a higher infection to case ratio than classical cholera.

*Resistance*

In endemic areas, it appears that repeated reinfection by *V. cholerae* leads to a gradual build-up of immunity with increasing age (Gangarosa and Mosley 1974). This may be one reason why the attack rates in children in endemic areas are considerably higher than in adults, whereas in epidemic situations where cholera has been recently introduced the reverse is often true. However, among those infected overt disease is more common in adults than in children.

A previous attack of cholera diarrhea confers solid immunity against reinfection with the same serotype of *V. cholerae* for about 1 year. An investigation in Bahrain showed that infants who were principally bottle-fed had a significantly higher risk of cholera than infants who were breast-fed, although it was not clear whether this arose from contaminated milk and bottles or from protective ingredients in maternal milk (Gunn and others 1979).

Cholera is a disease of the lower socioeconomic groups. Fishermen and boatmen, living along polluted water courses, are specially at risk. So also are people with hypochlorhydria, either due to malnutrition or other natural causes, or following gastric surgery (Sack and others 1972). Although the El Tor biotype may be less virulent than the classical, causing more mild cases of cholera, the host is probably equally susceptible to colonization by either.

*Epidemiology*

Studies on *V. cholerae* El Tor infection, in both epidemic and endemic situations, have repeatedly emphasized that the severe cases that reach the attention of treatment centers and physicians are the tip of an iceberg of widespread asymptomatic and mild clinical infection in the community. Estimates of a case to infection ratio of 1:30, or less, are commonly quoted. The asymptomatic infections are generally short lived but can be of crucial epidemiological importance in transmitting and geographically spreading cholera. Attempts to reconstruct the modes of transmission and spread of cholera that concentrate on known clinical cases are unlikely to be successful. To understand cholera epidemiology, it is necessary to take full account of the transient carrier, and to document the occurrence of transient carriage it may be necessary to undertake multiple fecal examinations and use serological techniques to determine whether an asymptomatic individual has been infected. These difficulties are one reason why so many investigations of cholera outbreaks are inconclusive or fall back on plausible but usually unproven explanations of waterborne transmission.

One of the most characteristic features of endemic cholera is its very pronounced seasonal pattern. For instance, in Dacca (Bangladesh) cholera used to peak dramatically during November–January, whereas 200 kilometers away in Calcutta (India) the peak was April–June. Recently these peaks have shifted and now occur during September–November in both areas. The reasons for these and other seasonal patterns of cholera remain entirely unexplained.

Non-O1 *V. cholerae* has been isolated from stools of persons with diarrhea in many countries in Asia, Africa, Europe, and, significantly, North and South America. Large epidemics have not been reported. In the USA most infections occur during the warmer summer months, while in Bangladesh there appears to be a peak in spring and summer before the annual cholera peak. Small foodborne outbreaks are common in the developed countries, but little is known of transmission and epidemiology in developing countries.

The epidemiology of cholera remains in many ways uncertain and controversial. The importance of waterborne transmission, the maintenance of cholera during interepidemic months of the year, the explanation of seasonality, the failure of tubewells in Bangladesh to reduce incidence, and the role of a possible aquatic reservoir for *V. cholerae* are all topics of current debate. Space does not permit a full review of these issues here. For a conventional account of cholera epidemiology, the reader should consult Gangarosa and Mosley (1974); Feachem (1981, 1982) provides a review of the more recent literature and debates.

## Control Measures

The most cost-effective control measures to deal with either endemic or epidemic cholera remain uncertain. Understanding of control will increase as more information is gathered on the epidemiological issues discussed above. Cholera control among people who are poor has so far proved to be extremely difficult. The course of a cholera epidemic is often dramatic and short-lived, and by the time control measures are applied the epidemic may be waning naturally. This can give a false impression of the efficacy of the control measures and lead to unjustified claims—as was the case when John Snow removed the handle from the Broad Street pump in London (England) in 1855.

*Individual*

Prophylactic antibiotics have been used to control some cholera outbreaks and to limit their spread. There is no evidence that this practice is effective, and there is mounting concern over the rising prevalence of antibiotic-resistant strains of *V. cholerae* in some countries. Large amounts of tetracycline (1,788 kilograms in the first 6 months) were used therapeutically and prophylactically following the outbreak of cholera in Tanzania in October 1977. Initially, all strains of *V. cholerae* tested were fully sensitive to tetracycline, but after 6 months 76 percent of isolates were resistant (Mhalu, Mmari and Ijumba 1979). Subsequent work showed that this antibiotic resistance was mediated by transferable plasmids that confer multiple antibiotic resistance (Towner and others 1980; Towner, Pearson and O'Grady 1979). Multiple antibiotic resistance has also been reported from 5–36 percent of *V. cholerae* isolates from Bangladesh (Threlfall, Rowe and Huq 1980).

Immunological prevention by vaccines is at present disappointing. Killed vaccines do afford a measure of protection but are usually less than 70 percent effective, and such immunity as is produced does not last at reasonable levels for more than about 4 months. A study in Bangladesh showed that mass vaccination was costly and ineffective (Sommer and Mosley 1973). Current research is directed at further characterizing pathogenesis and virulence factors and at developing and testing a variety of alternate vaccines based on live mutant strains or nonviable antigens such as the B subunit of the cholera enterotoxin.

Rigorous personal cleanliness and care in eating and drinking habits are probably the surest ways by which an individual can reduce the risk of cholera in an endemic or epidemic situation.

*Environmental*

There is no doubt that some combination of improved water supplies, excreta disposal facilities, better housing, and all the various improvements in daily life that come with increased wealth and education have been responsible for the elimination of cholera from the developed countries and from many middle-class communities in developing countries. Cholera was and remains a disease of poverty and the living conditions that are associated with poverty. Countries that experience the problem of endemic or epidemic cholera today are faced with the question of how to control the disease among poor communities in the short-term while poverty persists. Many claims have been made for the efficacy of various environmental control methods, but few of these have been justified, and most programs have been unsuccessful. Indeed, the experience with environmental control among the rural and urban poor has been so bad that some experts feel that the priority allocation of resources should be toward the establishment of networks of treatment centers for providing simple but highly effective rehydration therapy to reduce mortality (Greenough 1979).

The impact of water supply and sanitation schemes on endemic or epidemic cholera in poor communities is uncertain. Six studies in Bangladesh showed no impact (Briscoe 1978; Feachem 1982), whereas a study in the Philippines showed a very considerable impact (Azurin and Alvero 1974). The interpretation of these findings is controversial and has been recently reviewed in detail by Feachem (1982).

In some outbreaks—for instance, in Tanzania from 1977 to 1980—the geographical spread of cholera was due to the movement of infected individuals and gave rise to the characteristic pattern of spread along major railway and road routes. In such circumstances the limitation of movement of people in or out of areas known to be affected may reduce the risk of spreading the disease. Travel restrictions are difficult to enforce, however, and may seriously disrupt the movement of foodstuffs. If travel restrictions are combined with issuing prophylactic tetracycline to those who must travel, as was done in Tanzania, the problems of increased antibiotic resistance described above may occur.

Cvjetanović (1979) and Cvjetanović, Grab and Uemura (1978) used a mathematical model to compute the relative economic merits of sanitation, chemoprophylaxis, and immunization as methods of cholera control. Unfortunately, the cost of sanitation was set far too low (US$0.15 per capita at 1971 prices), and the effectiveness of sanitation was overestimated. Not surprisingly, this analysis showed sanitation to be highly cost-beneficial (with benefits taken only as the medical treatment costs saved), whereas immunization was shown to have costs far exceeding benefits because the currently available vaccine would have to have been given annually to have had any major impact on disease. Nonetheless, the analysis highlighted the benefits of sanitation as a measure having potential effects on a range of enteric and other diseases, as compared with vaccination, which, even if a more protective vaccine were available, is difficult to administer to most children, probably requires repeated readministration, and only protects against a single pathogen.

*Carrier surveillance and international regulations*

Since the chronic carrier is extremely rare, surveillance to identify carriers is not of significance in the control of this disease. This is in marked contrast with typhoid. The principal types of cholera carriage are incubatory, convalescent, and contact.

Up to December 31, 1970 International Sanitary Regulations were in force. They stipulated a 5-day quarantine period for travelers from areas where cholera was established. The regulations were abandoned when it was recognized that they were not preventing the spread of the current pandemic. Among the reasons for this failure were the concealment or denial of the existence of the disease in a country, together with the unknown importation of cases across unpatrolled borders. Current surveillance at national and international levels has been ineffective in preventing the spread of cholera into receptive countries—those with poor sanitation, hygiene, and health services. Nonetheless, surveillance to identify clinical cases (and, hence, the geographical advance of the disease) provides valuable epidemiological information and allows the organization of treatment in the absence of effective control measures.

## Occurrence and Survival in the Environment

The study of *V. cholerae*, atypical *V. cholerae*, and non-O1 *V cholerae* in the environment is attracting increasing attention at the present time. The conventional view that *V. cholerae* is an organism only found in the environment in close association with human cases or infections, and only surviving for a few days at most, is now being revised.

*In water*

The relationship between *V. cholerae* and water has been the focus of many investigations and is crucial to an understanding of the epidemiology of cholera. The traditional view of this subject—as stated by Felsenfeld (1974):

> some authors claimed that cholera vibrios may survive in water, particularly, seawater, for as long as 2 months. This is, however, scarcely possible under natural conditions if reinfection of the water does not take place

—is now known to be incorrect.

Data on the occurrence of *V. cholerae* in water are of two types. First, there are the numerous reports of *V. cholerae* isolations from rivers, tanks, ponds, wells, and household water jars in or near communities where cholera cases or infections are known to be occurring. Some of these reports are reviewed in a separate publication (Feachem 1981). Second, there are the more recent findings of *V. cholerae*, especially but not exclusively atypical O1 and non-O1 strains, in water and wastewater at sites distant from any known human *V. cholerae* infection. These findings are reviewed below in the section on possible aquatic reservoirs.

The reason that the view expressed by Felsenfeld was so strongly held for nearly 100 years is, first, that researchers had failed to find *V. cholerae* in the aquatic environment except in close association with human infection (due to a combination of not looking, looking in the wrong manner and looking in the wrong place), and, second, that survival experiments conducted in the laboratory had shown *V. cholerae* to be an organism with only limited survival ability in certain aquatic environments.

Some of the considerable accumulation of data on *V. cholerae* survival in water is summarized in tables 17-1 to 17-5. In clean water (for instance, dechlorinated tap water), survival times are up to 1 month at 4°C and 2–14 days at 20–30°C. In raw well water, survival times are over a month at 4°C and generally between 1 and 20 days at 20–30°C, although reports from India and Tanzania suggest survival of the El Tor biotype in raw well water of up to 55 days. A single report of *V. cholerae* survival in refrigerated raw surface water gives a survival time of 48 days, while survival at 20–30°C is generally 1–6 days, with occasional reportings of longer survival and one exceptional report from Tanzania of 48 days. As would be expected, survival in seawater is prolonged, with durations of 2 months at 4°C and 6–60 days at 20–30°C. Finally, a single report from the USSR (table 17-4) and epidemiological evidence from Portugal (Blake and others 1977) suggest the ability of *V. cholerae* to survive for prolonged periods in certain mineral waters.

It is clear from the tables that survival can be greatly prolonged in nutrient-rich waters and seawaters that have been boiled or autoclaved prior to contamination with *V. cholerae*, thus eliminating competing microorganisms and possibly also making the chemical composition of the water more favorable for *V. cholerae* survival. Although the nature and extent of *V. cholerae* inhibition by a mixed microflora in a natural surface water are not known, one study showed a failure of *E. coli*, *Pseudomonas* spp., and *Aerobacter* spp. to suppress *V. cholerae* El Tor survival in artificial sterile well water (Pandit and others 1967). Sunlight considerably curtails *V. cholerae* survival.

Table 17-1. *Survival of* Vibrio cholerae *in surface waters*

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Survival*[a] |
|---|---|---|---|---|
| Cheng (1963) | El Tor $1.5 \times 10^5$ | River water | 21–31°C | 3 days |
| | | Drain water | | 2 days |
| | | Pond water (all taken in or near Taipei) | | 6 hours |
| Gohar and Makkawi (1948) | Classical | Nile water | Room temp. (Egypt) | |
| | from feces | | | 5 days |
| | from culture | | | 10 days |
| Khan and Agarwal (1929) | Classical (clinical isolate) | Jumna and Ganges river waters | Room temp. (Allahabad) | |
| | | Raw | | 8 days |
| | | Filtered | | 18 days |
| | | Boiled | | 29 days |
| | | Boiled & filtered | | 14 days |
| | Non-O1 (water isolate) | Raw | | 20 days |
| | | Filtered | | 20 days |
| | | Boiled | | 18 days |
| | | Boiled & filtered | | 20 days |
| Konchady and others (1969) | Classical $10^4$ | Calcutta | 25°C | |
| | | River Hooghly | | 6 days |
| | | Canal water | | 6 days |
| | | Pond water | | 6 days |
| Lahiri, Das and Malik (1939) | Classical (Inaba) $10^6$ | Spring water | Room temp. (Calcutta) | |
| | | Raw | | 1 hour |
| | | Autoclaved | | 18 hours |
| | | River Hooghly (Calcutta) | | |
| | | Raw | | 18 hours |
| | | Autoclaved | | 3 days |
| | | Filtered | | 2 days |
| | | Autoclaved & filtered | | 2 days |
| | | Tank waters (Calcutta) | | |
| | | Raw | | 2–3 days |
| | | Autoclaved | | 3–12 days |
| | | Filtered | | 7 days |
| | | Autoclaved & filtered | | 15–18 days |
| Lema, Ogwa and Mhalu (1979) | El Tor $10^5$ | Swamp water in Dar es Salaam | 4°C | 48 days |
| | | | 30°C | 48 days |
| | | | 32°C in sunlight | 3 days |
| Mukerjee, Rudra and Roy (1961) | Classical $2 \times 10^6$ | River Hooghly (Calcutta) | Room temp. (Calcutta) | |
| | | Raw | | 1–6 days |
| | | Autoclaved | | 4–22 days |
| | | Filtered | | 3–12 days |

Table 17-1 (*continued*)

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Survival*[a] |
|---|---|---|---|---|
| | | Tank water (Calcutta) | | |
| | | Raw | | 1–6 days |
| | | Autoclaved | | 4–23 days |
| | | Filtered | | 3–7 days |
| | El Tor (clinical isolate) $2 \times 10^6$ | River Hooghly (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 11 days |
| | | Tank water (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 13 days |
| | El Tor (water isolate) $2 \times 10^6$ | River Hooghly (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 11 days |
| | | Tank water (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 16 days |
| | Non-O1 (clinical isolate) $2 \times 10^6$ | River Hooghly (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 9 days |
| | | Tank water (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 12 days |
| | Non-O1 (water isolate) $2 \times 10^6$ | River Hooghly (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 11 days |
| | | Tank water (Calcutta) | | |
| | | Raw | | 2 days |
| | | Autoclaved | | 13 days |
| Neogy (1965) | Classical | Pond water | Room temp. (India) | 1–2 days |
| | El Tor | | | 8 days |
| Read and others (1939) | Classical | Autoclaved tank waters (Calcutta) | Room temp. (Calcutta) | >30 days |

*Note:* Older literature is reviewed by Pollitzer (1959).

a. Times given, for instance, as 22 days are durations at which viable organisms could no longer be detected. Times given as >30 days indicate that organisms were still viable at that time but that sampling was discontinued.

Some experiments have included direct comparisons of the survival of classical and El Tor biotypes, and occasionally also non-O1 strains (tables 17-1, 17-2 and 17-4). Two studies showed markedly longer survival of El Tor than classical *V. cholerae* (Felsenfeld 1965; Neogy 1965); one study showed similar survival between the two biotypes (Sayamov and Zaidenov 1978); one study showed non-O1 *V. cholerae* surviving for longer than classical *V. cholerae* O1 (Khan and Agarwal 1929); and one study showed no difference in survival between classical O1, El Tor O1 and non-O1 *V. cholerae* (Mukerjee, Rudra and Roy 1961). It would appear from this literature review that the widely held belief that El Tor *V. cholerae* survives for considerably longer periods in water than the classical biotype is not firmly based. This is especially true in view of the major

Table 17-2. *Survival of* V. cholerae *in well water*

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Survival*[a] |
|---|---|---|---|---|
| Cheng (1963) | El Tor $1.5 \times 10^5$ | Well water (village near Taipei) | 21–31°C | 1 day |
| Felsenfeld (1965) | Classical | Shallow well water | ? | 8 days |
| | El Tor | | ? | 19 days |
| Khan and Agarwal (1929) | Classical (clinical isolate) | Well water (Allahabad) | Room temp. (Allahabad) | |
| | | Raw | | 1 day |
| | | Filtered | | 6 days |
| | | Boiled | | 9 days |
| | | Boiled & filtered | | 8 days |
| | Non-O1 (water isolate) | Raw | | 12 days |
| | | Filtered | | 6 days |
| | | Boiled | | 18 days |
| | | Boiled & filtered | | 26 days |
| Konchady and others (1969) | Classical $10^4$ | Well water (Calcutta slum) | 25°C | 6 days |
| Lema, Ogwa and Mhalu (1979) | El Tor $10^5$ | Well water (Tanzania) | 4°C | 55 days |
| | | | 30°C | 55 days |
| | | | 32°C in sunlight | 1 day |
| McFeters and others (1974) | ? $10^5$ | Sterile well water | 9.5–12.5°C | >2 days ($t_{90}$ = 1.3 days)[b] |
| Pandit and others (1967) | El Tor (Ogawa) $10^3$ | Well water (Punjab) | 21°C | 18 days |
| | | | 37°C | 4 days |
| | | Well water (Uttar Pradesh) | 21°C | 51 days |
| | | | 25°C | Fourfold growth after 1 day Survival for >7 days |
| | | | 37°C | 4 days |
| | | Experiments with well water simulating actual removal and replacement of water in well following single contamination with $10^3$ *V. cholerae* per milliliter | 25°C | 10–12 days |
| Pesigan, Plantilla and Rolda (1967) | El Tor $10^6$ | Deep well water (Manila) | | |
| | | Raw | 5–10°C | 18 days |
| | | | 30–32°C | 13 days |
| | | | Sunlight | 4 days |
| | | Autoclaved | 5–10°C | 42 days |
| | | | 30–32°C | 17 days |
| | | | Sunlight | 8 days |

Table 17-2 (*continued*)

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Survival*[a] |
|---|---|---|---|---|
| | | Raw well water stored in clay jar | 30–32°C ambient, but jar storage may have cooled water | 32 days |
| Shrewsbury and Barson (1957) | Classical | Sterile, synthetic well water of same composition (pH = 5.6) as Hagar's Well (Mecca, Saudi Arabia) during the cholera epidemic of 1883 | 5°C | 1 day |
| | | | 21°C | 1 day |
| | | | 25°C | 1 day |
| | | Same water with: | | |
| | | pH 7 | 5°C | 3 days |
| | | | 21°C | 3 days |
| | | pH 8 | 5°C | 3 days |
| | | | 21°C | 77 days |
| | | pH 9 | 5°C | 3 days |
| | | | 21°C | 3 days |

*Note:* Older literature is reviewed by Pollitzer (1959).

a. Times given, for instance, as, 18 days are durations at which viable organisms could no longer be detected. Times given as > 7 days indicate that organisms were still viable at that time but that sampling was discontinued.

b. $t_{90}$ Time for 90 percent reduction.

probable strain-by-strain differences within each biotype and the differences between laboratory cultures, fresh clinical isolates, and water isolates. On the basis of the literature reviewed here it remains unproven than El Tor is a more persistent organism in water than the classical biotype, and the true interbiotypic and intrabiotypic variabilities in survival remain to be documented. It follows that explanations of the differences in epidemiology between El Tor and classical cholera—for instance, the greater "endemic tendency" of the former—cannot, at the present time, make use of putative differences in environmental persistence between the two biotypes.

Laboratory experiments on *V. cholerae* survival in water may accurately reflect conditions in manmade containers of clean water (such as reservoirs, cisterns, jars, and glasses), but they cannot replicate conditions in natural water bodies such as rivers, ponds, or even open wells. In these latter waters there may be abundant flora and fauna, and many varied surfaces, not reproduced or simulated in the laboratory experiments. There is increasing evidence (reviewed below) that *V. cholerae* in natural waters are frequently in close association with bottom sediments, chitinous fauna, and plant surfaces; therefore, laboratory data must be interpreted with extreme caution.

### *In feces and night soil*

Except for the atypical *V cholerae* and non-O1 *V. cholerae* which may maintain an environmental reservoir, the primary source of *V. cholerae* in the environment is the feces of man. Persons infected by *V. cholerae*, though not sick, may excrete $10^2$–$10^5$ per gram of feces, while those with active and severe disease may excrete $10^6$–$10^9$ per milliliter of rice-water stool (Dizon and others 1967; Greig 1914; Smith, Freter and Sweeney 1961). Unlike most other enteric bacterial infections, the prevalence of excretion of *V. cholerae* by the general healthy population is very low—typically well under 1 percent, even in endemic areas.

In areas of endemic cholera, or during a cholera outbreak, it is to be expected that *V. cholerae* will occur in the night soil produced by the affected communities. Forbes, Lockhart and Bowman (1967) and van de Linde and Forbes (1965) reported numerous isolations of *V. cholerae* from night soil in Hong Kong, both when cholera cases were and were not occurring in the city.

Table 17-3 *Survival of* V. cholerae *in tap water*

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Chlorine residual milligrams per liter* | *Survival*[a] |
|---|---|---|---|---|---|
| Cheng (1963) | El Tor $1.5 \times 10^5$ | Taipei tap water | 21–31°C | 0.5 | 2 hours |
| Konchady and others (1969) | Classical $10^4$ | Tap water from deep tubewell (Calcutta) | 25°C | 0 | 6 days |
| Lahiri, Das and Malik (1939) | Classical (Inaba) $10^6$ | Calcutta tap water | Room temp. (Calcutta) | ? | |
| | | Raw | | | 18 hours |
| | | Autoclaved | | | 24 hours |
| | | Filtered | | | 2 days |
| | | Filtered & autoclaved | | | 12 days |
| Lema, Ogwa and Mhalu (1979) | El Tor $10^5$ | Dar es Salaam tap water | 4°C | Chlorinated at treatment works but probably no residual chlorine remaining at tap | 34 days |
| | | | 30°C | | 14 days |
| | | | 32°C in sunlight | | 3 days |
| Mukerjee, Rudra and Roy (1961) | Classical $2 \times 10^6$ | Calcutta tap water | Room temp. (Calcutta) | ? | |
| | | Raw | | | 2–8 days |
| | | Autoclaved | | | 4–18 days |
| | | Filtered | | | 2–6 days |
| Pandit and others (1967) | El Tor (Ogawa) $10^3$ | Delhi tap water | 21°C | De-chlorinated | 12 days |
| | | | 37°C | | 1 day |
| Pesigan, Plantilla and Rolda (1967) | El Tor $10^b$ | Manilla tap water | | | |
| | | Raw | 5–10°C | 0.6 | 1 hour |
| | | Raw | 30–32°C | 0.6 | 1 hour |
| | | Raw | Sunlight | 0.6 | 1 hour |
| | | Autoclaved | 5–10°C | 0 | 10 days |
| | | Autoclaved | 30–32°C | 0 | 1.6 days |
| | | Autoclaved | Sunlight | 0 | 12 hours |

*Note:* Older literature is reviewed by Pollitzer (1959).

a. Times given are durations at which viable organisms could no longer be detected.

During a 10-month sampling period, 46 percent (200 of 433) of bucket latrines in the slums of eastern Calcutta (India) were positive for *V. cholerae* on one or more occasions (Sinha and others 1967). *V. cholerae* isolations from latrines were obtained during months when no cholera cases were reported. In contrast, during 1968 in Dacca and Chittagong (Bangladesh) a total of 72,494 night soil samples yielded only 56 isolations of *V. cholerae*. all of which occurred at times when cholera cases were being reported (Bart, Khan and Mosley 1970).

Some reported data on *V. cholerae* survival in feces are summarized in table 17-6. Clearly survival is inversely related to temperature. Cheng (1963) and

Table 17-4. *Survival of* V. cholerae *in mineral water*

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Survival*[a] |
|---|---|---|---|---|
| Sayamov and Zaidenov (1978) | Classical | Spring water from spa (Matsesta, USSR) | | |
| | $9 \times 10^5$ | Raw | 20–24°C | 22 days |
| | $1.5 \times 10^3$ | Diluted | | 15–65 days |
| | $9.5 \times 10^5$ | Boiled | | >1429 days |
| | $1.6 \times 10^3$ | Diluted | 37°C | >289 days |
| | El Tor | | | |
| | $1.2 \times 10^6$ | Raw | 20–24°C | 22 days |
| | $10^3$ | Diluted | | 18–39 days |
| | $9 \times 10^5$ | Boiled | | >1429 days |
| | $1.6 \times 10^3$ | Diluted | 37°C | >413 days |

*Note:* Further evidence of prolonged survival of *V. cholerae* in mineral water is provided by the investigation of the cholera outbreak in Portugal in 1974 (Blake and others 1977).

a. Times given, for instance, as 22 days are durations at which viable organisms could no longer be detected. Times given as >289 days indicate that organisms were still viable at that time but that sampling was discontinued.

Shoda, Koreyeda and Otomo (1934) found that survival was longer in liquid stools than in soft or solid stools. In summary, at ambient temperatures in tropical and subtropical countries, *V. cholerae* is unlikely to survive beyond 5 days in feces.

### *In sewage*

There are very few reports of *V. cholerae* in sewage. This is primarily because, in most developing countries, the section of the population that experiences the highest attack rates of cholera produces no sewage because their houses do not have flush toilets. Instead, they produce night soil (where *V. cholerae* has been found) or they defecate beside or into open water bodies (where *V. cholerae* has also been found).

Kott and Betzer (1972) reported estimates that Jerusalem sewage contained between 10 and $10^4$ *V. cholerae* per 100 milliliters during the 1970 cholera epidemic in Israel. Daniel and Lloyd (1980*a*) reported geometric mean concentrations of 2,600 and 160 non-O1 *V. cholerae* per 100 milliliters of very strong sewage (suspended solids 17,000 and 7,400 milligrams per liter, respectively) in two refugee camps near Dacca (Bangladesh). Isaacson and others (1974) reported the use of Moore pads to detect *V. cholerae* in sewage at mines in the Transvaal (South Africa) during 1973–74, when the spread of cholera from Malawi, Mozambique, and Angola was feared. *V. cholerae* (El Tor, Inaba) was isolated from the sewage prior to and during cholera outbreaks at the mines and acted as an effective early warning system for the outbreaks.

Survival of *V. cholerae* in sewage is summarized in table 17-7. Three studies (Altukhov and others 1975; Daniel and Lloyd 1980*b*; Zaidenov and others 1976) suggested that some sewages provide a permanent culture medium for some strains of classical, El Tor, and non-O1 *V. cholerae*. The other studies found that survival times were 1–24 days in sewage at 20–30°C. Survival times are shorter at warmer temperatures and longer in sterilized sewage than in raw sewage.

Direct comparisons of different biotypes and serotypes showed no differences in survival among classical O1, El Tor O1, and non-O1 strains (Mukerjee, Rudra and Roy 1961). Altukhov and others (1975) found an El Tor, Ogawa strain better able to multiply in bath house sewage at 37°C than a classical, Ogawa strain, although even the classical strain had not fallen below its initial concentration after 10 days. Daniel and Lloyd (1980*b*) found a sewage-derived non-01 strain better able to multiply in sewage than a laboratory reference strain of El Tor O1, although even the El Tor strain showed no reduction in concentration between 6 hours and 48 hours at 22–25°C. As with water, therefore, there is little evidence at present to suggest that the El Tor biotype is necessarily better able to survive in sewage than the classical biotype.

### *Summary of survival in water and wastewater*

In some survival studies the initial concentration of organisms present was reported, and it is therefore possible to estimate a death rate expressed as a $t_{90}$ value—the time in hours for a 90 percent or 1 log unit decline in concentration. In only a few studies were death curves plotted from which accurate $t_{90}$ values might be taken. For other studies the $t_{90}$ value can only be estimated from the initial concentration and the

Table 17-5. *Survival of* V. cholerae *in seawater*

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Survival*[a] |
|---|---|---|---|---|
| Cheng (1963) | El Tor $1.5 \times 10^5$ | Coastal water near a fresh-water source | 21–31°C | 6 days |
| Jamieson, Madri and Claus (1976) | El Tor $1.5 \times 10^7$ | Sterilized seawater with adjusted salinity (percent) | | |
| | | 0.5 | 4°C | 5 days |
| | | | 25°C | 3 days |
| | | | 37°C | 2 days |
| | | 2.0 | 4°C | 4 days |
| | | | 25°C | 3 days |
| | | | 37°C | 1 day |
| | | 3.5 | 4°C | 4 days |
| | | | 25°C | 1 day |
| | | | 37°C | 1 day |
| Lema, Ogwa and Mhalu (1979) | El Tor $10^5$ | Seawater (Dar es Salaam) | 4°C | >58 days |
| | | | 30°C | >58 days |
| | | | 32°C in sunlight | 5 days |
| Pesigan, Plantilla and Rolda (1967) | El Tor $10^6$ | Seawater (Manilla) | 5–10°C | 58–60 days |
| | | | 30–32°C | 10–13 days |
| | | | Sunlight | 10–11 days |
| Various studies between 1885 and 1920 reviewed by Pollitzer (1959) | Classical | Sterilized seawater (Marseilles) | ? | 81 days |
| | | Seawater (Copenhagen) | Summer | 7–17 days |
| | | | Winter | 47 days |
| | | Seawater (New York) | | |
| | | Raw | ? | 7–47 days |
| | | Sterilized | ? | >285 days |
| | | Seawater (Japan) | | |
| | | Raw | 4°C | 9–27 days |
| | | Raw | Room temp. | 7–41 days |
| | | Raw | 37°C | 3–12 days |
| | | Sterilized | 4°C | 53–230 days |
| | | Sterilized | Room temp. | 152–209 days |
| | | Sterilized | 37°C | 30–83 days |
| Yasukawa (1933) | Classical $3 \times 10^4$ | Artificial seawater Top of tank | 18°C | 23 days |
| | | Bottom of tank | 18°C | 30 days |
| | $3 \times 10^5$ | In sunlight | 19–40°C | 2 hours |

a. Times given, for instance, as 6 days are durations at which viable organisms could no longer be detected. Times given as >58 days indicate that organisms were still viable at that time but that sampling was discontinued.

Table 17-6. *Survival of* V. cholerae *in feces*

| *Source* | *Biotype and initial concentration per milliliter* | *Type of sample* | *Temperature* | *Survival*[a] |
|---|---|---|---|---|
| Abel and Claussen (1895); cited by Pollitzer (1959) | Classical | Naturally infected cholera stools | 13–16°C | 10 days for over half the samples with a maximum of 29 days |
| Cheng (1963) | El Tor | Naturally infected stools | 29–31°C | 1–4 days |
| | | Artificially infected stools | 29–31°C | 2–4 days |
| Gildemeister and Baerthlein (1915); cited by Pollitzer (1959) | Classical | Naturally infected stools | 12–21°C | 10 days for half the samples; with a maximum of 51 days |
| Greig (1914) | Classical $1.5 \times 10^8$–$2 \times 10^9$ | Naturally infected ricewater stools | 22°C | Min. 1–3 days<br>Max. 10–17 days<br>Av. 3–8 days |
| | | | 29°C | Min. 1 day<br>Max. 2–13 days<br>Av. 1–7 days |
| Shoda, Koreyeda and Otomo (1934) | Classical | Naturally and artificially infected stools | 4°C | 1–5 days |
| | | | Room temp. (Japan) | 0.5–2 days |
| | | | 37°C | 6 hours |

a. Times given are durations at which viable organisms could no longer be detected. Max. = maximum, Min. = minimum, Av. = average.

overall survival time, without knowing the shape of the intervening death curve or whether the number of organisms fell below detectable levels considerably prior to the stated survival time.

Bearing in mind these limitations, $t_{90}$ values have been derived where possible. The few studies that showed prolonged maintenance of concentrations equal to or greater than initial values have been excluded and are discussed separately in the next section. Derived $t_{90}$ values are presented in table 17-8. The mean figures in table 17-8 suggest maximum survival in well water and seawater. The mean figures for the El Tor biotype are greater than for the classical biotype, but this comparison is invalid since each experiment used very different techniques and a wide variety of strains of various origins. It remains uncertain whether the interbiotypic variability of survival is greater than the intrabiotypic variability.

These $t_{90}$ values may be compared with typical $t_{90}$ values for coliforms of 20 to 115 hours (median 60 hours) in surface waters and with 0.6 to 8 hours (mean 2 hours) in seawater (chapter 13). For shigellae, in surface waters at temperatures of over 20°C, $t_{90}$ values generally fall well below 60 hours (chapter 16). Thus, even discounting the prolonged survival findings reviewed below, the $t_{90}$ values for *V. cholerae* are not greatly lower than those reported for coliforms and may be similar to those reported for other bacterial enteric pathogens. In a direct comparison of various bacteria in sterile well water, McFeters and others (1974) found the following $t_{50}$ values: shigellae, 22–27 hours; coliforms, 17 hours; salmonellae, 2–19 hours;