S320

INFECTIOUS DISEASE

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CHOLERA CASE STUDY

prepared for the Course Team by
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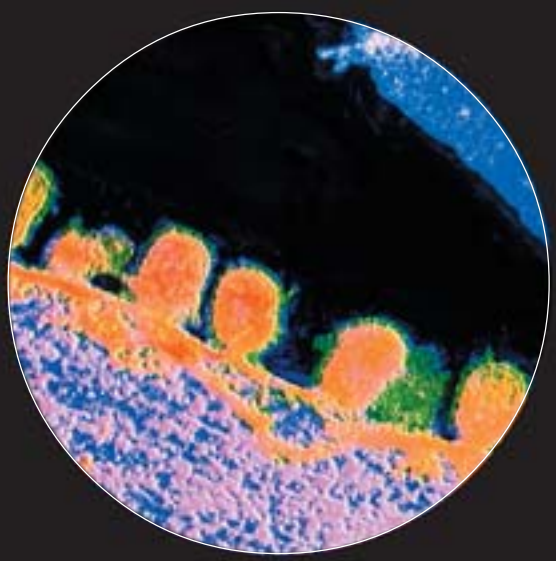

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Cover pictures

Top left: Schistosome parasites. Coloured scanning electron micrograph of adult female (upper, thinner) and male (lower, fatter) *Schistosoma mansonii* parasitic worms, cause of the disease bilharzia (schistosomiasis).

Top right: Scanning electron micrograph of *Staphylococcus* sp.

Lower left: Coloured electron transmission micrograph of *Mycobacterium tuberculosis*.

Lower right: False colour transmission electron micrograph of influenza viruses (orange) budding from the surface of an infected cell.

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CHOLERA CASE STUDY

You are advised to attempt this case study when you have finished your study of Book 2. However, there will be some material in Section 4 that may be clearer after you have studied Book 3; so if you find this material difficult, you might prefer to leave it for now and return to it later in the course. More detail is available in the papers cited in the text, which are listed at the end. You may wish to read some of these papers if you are particularly interested in this topic. There is also a lot of relevant information available on the World Health Organization website, should you wish to learn more. This case study should take you no longer than 3 hours.

1 The history of cholera

Cholera has been known in India for a long time, where it is endemic, but in 1816 it began to spread in an unprecedented manner. The British public first heard of 'Asiatic' cholera when the Marquis of Hastings' army succumbed to the disease, which had reached epidemic proportions in Bengal. The cholera swept on, affecting the whole of India, and spreading as far as Japan and the Philippines in the East, and Russia and Persia (now Iran) in the West. This first pandemic, as it was then called, petered out in 1826, ten years after it had begun.

However, that was not the end of the story, and throughout the 19th and early 20th centuries no fewer than five further cholera pandemics swept the world, with the disease retreating to the Bengal Basin in between assaults. The duration and frequency of these pandemics are shown in Table 1.1.

TABLE 1.1 The duration of cholera pandemics in the 19th and first half of the 20th centuries.

Pandemic	Duration/years	Period
first	9	1817–1826
second	8	1829–1837
third	16	1846–1862
fourth	11	1864–1875
fifth	13	1883–1896
sixth	61	1899–1960

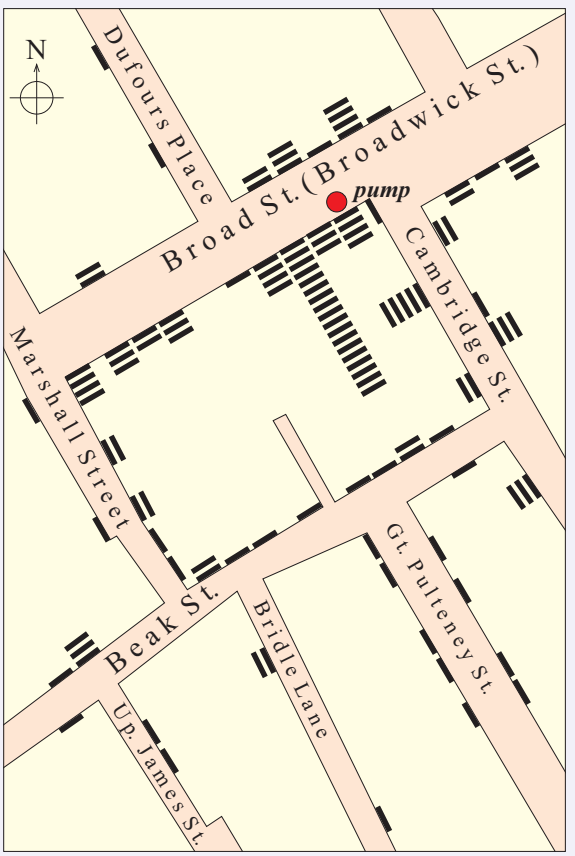
The second pandemic, which began in 1829, reached North Africa, Europe and the Americas. It could not have arrived at a worse time for the increasingly industrialized cities of Europe and North America. These cities were growing in size rapidly, as people flocked to them, eager to find work in the new factories. Once in the cities, they lived crowded together with no proper sewerage system and often no clean water. In 1836 the registration of births, marriage and deaths became compulsory in Britain and the first quantitative data on the nation's health became available. It was clear from these statistics that the poor suffered the most disease, but this observation led to the advancing of opinions that would be regarded as outrageous today. It was suggested that the poor were the *cause* of disease, and

brought it about by their unhygienic practices and immorality. These views have a certain logic when seen in the context of their time: disease was still thought to be caused by 'bad airs' or **miasmas**, and life was believed to be generated spontaneously from non-living matter.

However, the tide was starting to turn: Edwin Chadwick, Secretary to the Poor Law Commission, began to realize that poverty could be *caused by* disease, since the sick and disabled were unable to work. In 1842, he published his 'Report on the Sanitary Condition of the Labouring Population of Great Britain', which identified the main causes of disease among the poor as urban squalor, overcrowding and poor diet. Chadwick advocated a number of improvements in public health, such as the removal of rubbish from poor areas, the building of new sewers, and improved water supplies. Under the first British Public Health Act in 1848, some of these improvements were begun.

Improving the living conditions of the poor was given further impetus when cholera struck London during the third pandemic, in 1854. A London anaesthetist, John Snow, had already developed an interest in cholera during the second pandemic, in 1831. By 1849, he was suggesting that a water-borne agent that entered by the mouth, multiplied in the gut and left via the faeces, was the cause of cholera. This suggestion was in stark contrast to the established view that miasmas spread cholera. In the late summer of 1854, cholera killed around 500 people in the vicinity of London's Golden Square in just ten days. The ferocity of the outbreak was unusual, and Snow investigated the event while it was still in progress. He was already gathering data from an earlier cholera epidemic in 1848–1849, and his interest was centred on the source of the water drunk by the victims. Consequently, when he began his investigation of the 1854 Golden Square outbreak, he focused on the water used by households that were affected by the disease. Snow discovered that a disproportionate number of cholera victims had drunk water from a pump situated in nearby Broad Street (see Figure 1.1). When the handle of this pump was removed on 7 September, seven days after the outbreak began, the cholera subsided. Snow later presented his findings as maps, and this technique for investigating the geography of epidemics is still used in modern epidemiology (Brody *et al*., 2000; see also Book 6).

FIGURE 1.1
John Snow's map of cholera deaths in the Broad Street area of London. The small black bars indicate deaths from cholera.



Sadly, Snow's work did not convince everybody: Max von Pettenkofer, a German hygienist, developed a rival *soil theory*. He proposed that the cholera germ resided in the soil and that under particular environmental circumstances it would arise, form a miasma and produce a cholera epidemic. David Cunningham, who was a scientific assistant to the Sanitary Commissioner with the Government of India from 1869–1897, also espoused these views. The biggest killer of British troops in India was epidemic disease, and following the Indian mutiny of 1857, cholera was the leading cause of these deaths. Cunningham was recruited to study cholera, with a view to controlling it, following an epidemic in 1867 at a religious fair in Hardwar (in the state of Uttar Pradesh), which claimed the lives of over 100 000 people. Cunningham was a prominent worker on cholera for many years, but his

adherence to the miasmic theory of the cause of cholera meant that some preventative measures such as quarantines were not imposed, often with disastrous consequences (Isaacs, 1998).

The field of microbiology was now just beginning to open up, and in 1883, with the fifth cholera pandemic on its way to Europe, Robert Koch was despatched to Egypt. The German doctor was fresh from his triumph of discovering the causative agent of tuberculosis, and by working with cholera victims he was able to isolate the causative agent of cholera. Koch showed that cholera was caused by a comma-shaped bacterium, subsequently referred to as a 'vibrio' and named *Vibrio cholerae*. Koch isolated the bacterium again in India in 1884, and showed that the bacillus lived in the human gut and was spread by dirty water. Cunningham and von Pettenkofer remained unconvinced, but one must have some sympathy with the latter here. He asked Koch to send him some of his cholera vibrios, which he then drank. Von Pettenkofer remained in perfect health, which convinced him that his theory, that the cholera germ alone could not cause the disease, was vindicated! It seems that von Pettenkofer had a stomach acidic enough to kill *Vibrio cholerae*.

- What other reason might there have been for von Pettenkofer's continuing good health?

- Perhaps a very high dose of vibrios is required to cause disease – more than von Pettenkofer ingested. More prosaically, it is possible that the conditions under which the bacteria were transported were unable to maintain them in a viable condition.

As the 19th century drew to a close, the spectre of cholera had largely been vanquished. Improvements in public health in the developed world had finally achieved their goal, and many diseases, such as typhoid and tuberculosis as well as cholera, had declined in incidence as a result. The last cholera epidemic in the Americas occurred in 1895, and the disease was even absent from Africa for almost a century. Nevertheless, sporadic cases, as well as epidemics elsewhere, show that the disease is still a threat, as you will see below.

2 The disease

Cholera is only one of many types of diarrhoeal disease, but its global importance is underlined by its inclusion in the WHO Communicable Disease Surveillance and Response (CSR) list. Cholera is typically an acute disease, with an incubation period of 2–3 days, but asymptomatic infections are common. Over 90% of infections are, in fact, very mild or moderate and difficult to distinguish from other types of diarrhoea, such as that caused by enterotoxigenic *E. coli* (ETEC) (Book 2, Section 2.6.4). Fewer than 10% of infected individuals go on to develop the typical disease, with its sudden onset of profuse watery diarrhoea, effortless vomiting and in some cases fever.

- Recall the molecular mechanisms leading to the production of diarrhoea in cholera and in ETEC infection.

- Both cholera toxin and the heat-labile toxin of ETEC bind specific gangliosides in the membranes of gut epithelial cells, and activate membrane-bound adenylate cyclase. This increases cyclic AMP production, which causes efflux of Na^+ and

Cl^- ions across the membrane and into the gut lumen. The presence of these ions in high concentrations 'pulls' water out of the surrounding cells, and the result is copious amounts of water in the gut.

The main symptom of cholera is profuse, watery diarrhoea, which resembles the cloudy water left behind after rice has been boiled and so is often described as **rice-water stools** (see Figure 2.1). These stools are pale grey, flecked with mucus, and have a fishy smell. As much as 10–15 litres of diarrhoea may be produced during an infection, and fluid is also lost by vomiting and sweating. These symptoms can lead to severe dehydration (as demonstrated by Figure 2.2) and the loss of important electrolytes.

FIGURE 2.1
Rice-water stools typical of cholera.

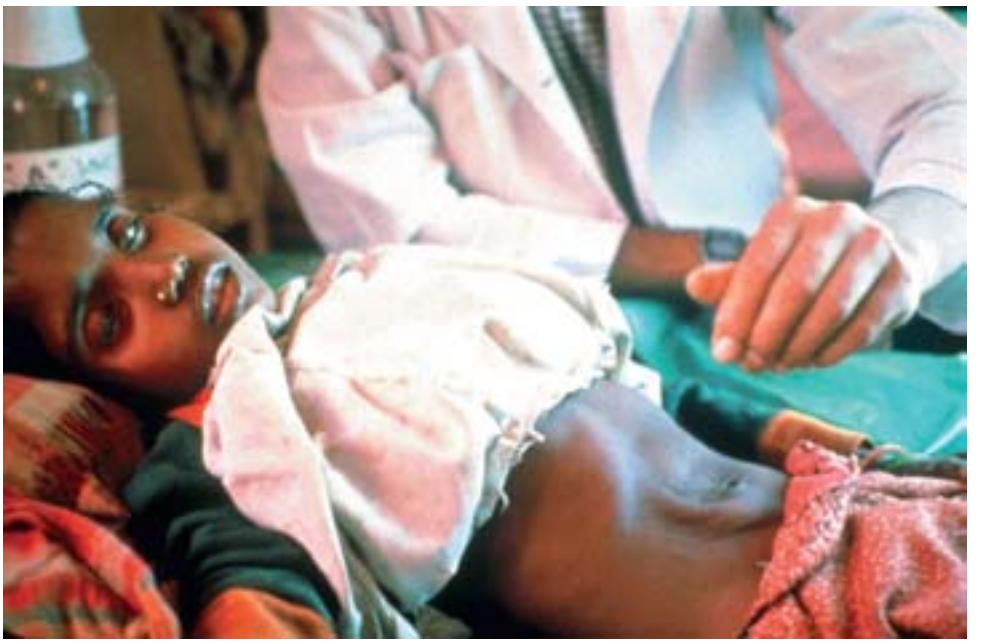


FIGURE 2.2
A child with severe dehydration. The main sign of this condition is a skin pinch test on the abdomen: after pinching, the skin of the abdominal wall takes more than two seconds to return to its normal position. The child also has sunken eyes, and a reduced level of consciousness.

- Name two important electrolytes that are lost during cholera.
- Na^+ and Cl^- ions, as mentioned above. Potassium (K^+) and bicarbonate (HCO_3^-) ions are also lost in large quantities.

The loss of K^+ ions results in cramps in the abdominal muscles, whereas a reduction in HCO_3^- ions can upset the pH balance of the body. The resulting severe dehydration causes the production of urine to cease, the skin to become wrinkled, and sometimes the eyes to appear sunken. The subsequent loss of fluid volume causes a drop in blood pressure and circulatory shock. If the patient remains untreated, they become progressively weaker, sometimes to the point of death, within 12–24 hours of the onset of symptoms. If the patient survives, then the infection usually lasts 1–5 days.

3 *Vibrio cholerae*, the causative agent of cholera

Cholera is caused by the Gram-negative, rod-shaped bacterium *Vibrio cholerae*, which on first isolation may appear curved, and is shown in Figure 3.1. The bacteria have a single, polar (at one end) flagellum, which renders them motile. Based on the properties of their O antigens (Book 2, Section 2.2), more than 130 groups have been identified, but only two of them, O1 and O139 ('O' for O antigen), have been known to cause epidemics of diarrhoeal disease. Until very recently, however, only O1 *Vibrio* strains were known to cause disease. These O1 strains fall into two **biotypes (**or **biovars**), distinguished by their metabolic activities – in this case, their different haemolytic activity, relative resistance to the antibiotic polymyxin B, and their different susceptibilities to bacteriophage. The two biotypes are called classical and El Tor. The classical biotype is further divided into two **serotypes** (or **serovars**), based on the antisera that recognize them, and named after the place where they were first isolated: Inaba and Ogawa. (You will learn more about the use of serum to identify microbes in Book 4.) Thus, any pathogenic strain of *Vibrio cholerae* has a name that reflects both the biotype and the serotype; for example, strain 569B has a classical biotype and the Inaba serotype.

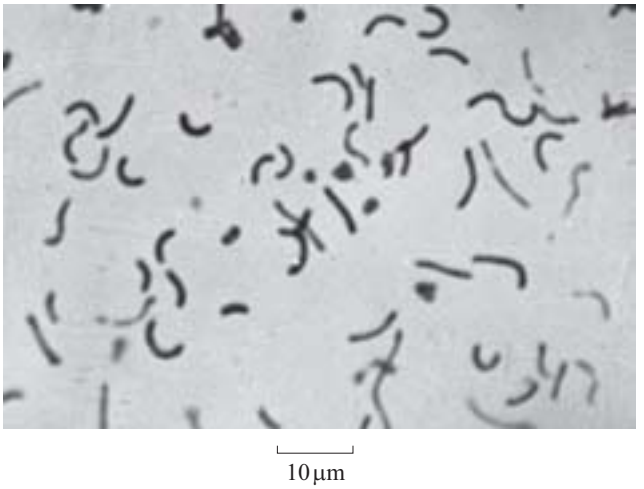


FIGURE 3.1 A high-power light micrograph of *Vibrio cholerae*, the causative agent of cholera. (The polar flagellum is not visible at this scale.)

Until recently, humans were thought to be the only host for *Vibrio cholerae*, especially as there are particular populations in which cholera is endemic. However, in general the vibrios are aquatic bacteria, and recent studies have shown that *V. cholerae* is also found naturally in aquatic environments, such as estuaries and brackish water.

3.1 *V. cholerae* pathobiology

Infection with the cholera bacterium occurs by the faecal–oral route, as a result of consuming food or water contaminated with *V. cholerae*. The infectious dose is high, requiring a minimum of 10^8 bacteria for classical *V. cholerae* in a healthy host (which may be why von Pettenkofer did not catch cholera – see above), but this figure falls when acid production by the stomach is impaired. The vibrios that survive passage through the stomach are able to multiply in the alkaline environment of the small intestine. In fact, the tolerance of cholera vibrios to alkaline conditions is one of their distinguishing features.

V. cholerae exerts its pathogenic effects in the small intestine, the structure of which is shown in Figure 3.2. Once in the small intestine, the vibrios must reach the epithelial cells, and they are probably aided in this task by their flagella, which help in propulsion, and by their ability to produce mucinase (an enzyme capable of degrading mucus) and other proteolytic enzymes. Actual attachment to the epithelial cells is mediated by a number of factors, the most important of which is a surface molecule called the toxin-coregulated pilus (TCP), discussed further below. A number of haemagglutinins, fimbriae and the O antigens of lipopolysaccharide have also been implicated in the attachment process.

The cholera vibrios do not penetrate the gut epithelium, but they release a potent enterotoxin called cholera toxin, CT (or choleragen), which is largely responsible for the symptoms of cholera. CT has an A–B structure, comprising five B subunits and one A subunit, which is an enzyme. The B subunits mediate attachment to the villi of the epithelial cells by binding to the ganglioside receptor GM1, and the CT is taken up by receptor-mediated endocytosis, as shown in Figure 3.3. The A subunit is then released and goes on to catalyse the transfer of an ADP-ribose from NAD to the α subunit of a G protein. The modified G protein is no longer able to switch off adenylate cyclase, a membrane-bound enzyme located on the basal and lateral membranes of the cell.

Adenylate cyclase produces cyclic adenosine monophosphate (cAMP) from ATP as shown in the equation below:

$$\text{ATP} \longrightarrow \text{cAMP} + \text{PP}_\text{i} + \text{H}^+$$

cAMP is a key regulatory molecule that carries information from extracellular signalling molecules such as hormones, to the cell's interior. For this reason it is called a second messenger.

- ☐ What would be called the first messenger?
- ■ The hormone.

The level of cAMP within a cell therefore influences its activity, but its precise role depends on the cell type. In a gut epithelial cell, cAMP level influences ion transport, whereas in a pancreatic cell, it regulates insulin secretion. As mentioned above, adenylate cyclase is regulated by a protein, known as a G protein, which is a

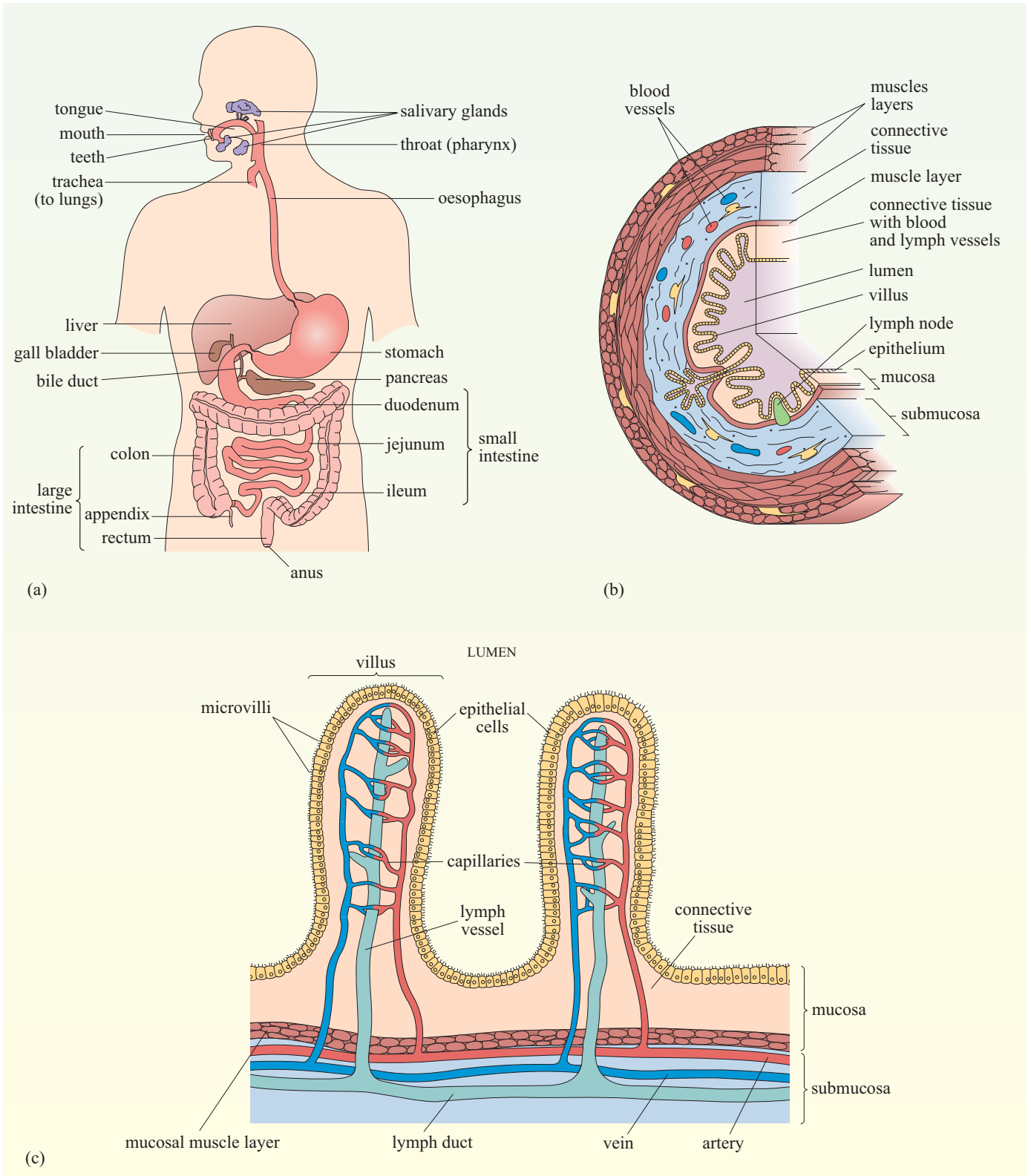


FIGURE 3.2 (a) Diagram of the human gut. (b) Diagram of a cross-section of the wall of the small intestine. (c) Larger-scale diagram of the innermost layer of the small intestine, showing the villi (singular: villus) and the epithelial cells covering them.

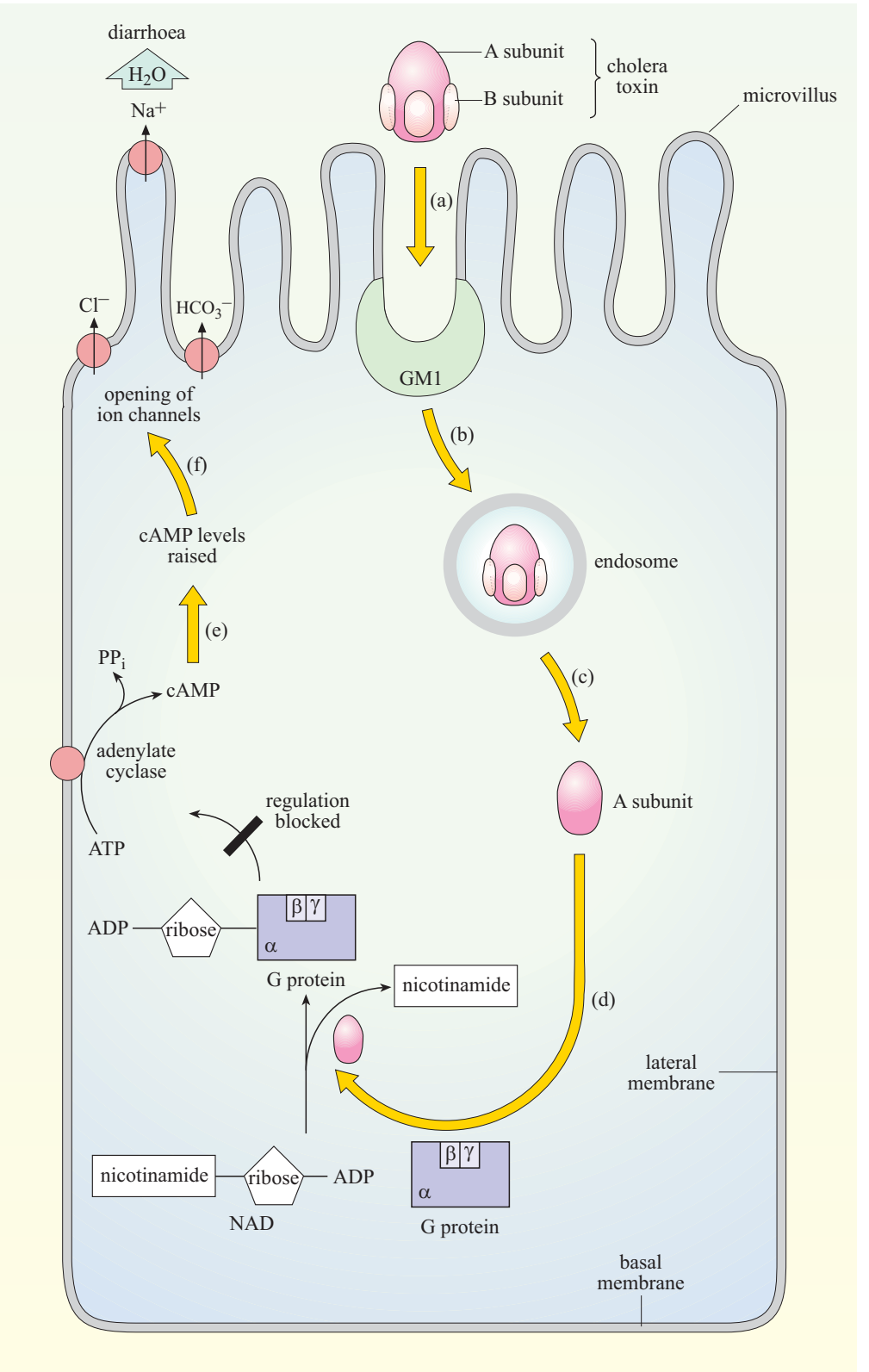


FIGURE 3.3
Mechanism of action of cholera toxin (CT). (a) The CT molecule binds to GM1 in the apical membrane of the gut epithelial cell. (b) The molecule is internalized in an endosome. (c) The A (enzyme) subunit is released, and catalyses the transfer of ADP-ribose from NAD to the α subunit of a G protein. (d) The G protein can no longer act to switch off adenylate cyclase, and the cyclic AMP level in the cell rises (e). This increase in cAMP causes ion channels in the apical membrane to open, allowing ions to escape from the cell (f).

complex of three different subunits, α, β and γ. The A subunit of the cholera toxin catalyses the transfer of ADP-ribose group from NAD to the α subunit of the G protein. This prevents the G protein from switching off the synthesis of cAMP, with the result that cAMP is produced constitutively (i.e. at a constant rate). The high levels of cAMP cause ion channels in the cell membrane of the crypt cells at the base of the villi in the small intestine to open, resulting in uncontrolled secretion of Cl^- and HCO_3^- ions into the lumen. The high cAMP levels also inhibit the uptake of Na^+ ions into the cells at the top of the villi, with the result that Na^+ and Cl^- ions accumulate in the lumen. Water moves out of the epithelial cells into the lumen by osmosis, and both water and electrolytes are lost from the body as the copious diarrhoea of cholera.

The virulence factors that are responsible for the pathogenesis of *Vibrio cholerae* are encoded by two lysogenic phages: CTXϕ (pronounced CTX fie) and VPIϕ. Phage CTXϕ encodes the cholera toxin (CT) and also two other toxins, accessory cholera toxin (Ace) and zona occludens toxin (Zot), whose roles in pathogenesis have not yet been clearly established. Zot is thought to contribute to the loss of water and electrolytes from the gut epithelial cells. Phage VPIϕ encodes the toxin-coregulated pilus (TCP, see above), which – curiously – is the receptor for the CTXϕ phage when it infects *Vibrio cholerae*.

4 Immunology of cholera

4.1 The immune response to cholera

The immune response to enteric pathogens is initiated in the gut-associated lymphoid tissue (GALT), known as Peyer's patches. The gastrointestinal tract is an important route of entry for pathogens, so this lymphoid tissue constantly 'samples' the gut contents for antigenic material. After appropriate processing and presentation of antigen (see Block 3), B cells are stimulated to differentiate into plasma cells that produce antibodies. The most important antibody classes in a cholera infection are secretory IgA and, to a lesser extent, secretory IgM, which are released from the gut mucosa.

What would you expect to happen to antibodies secreted into the gut?

Antibodies are protein molecules and so should be digested along with proteins in food.

In fact, the antibodies are thought to be protected from this degradation by their J chains, and secretory IgA has an additional polypeptide called a *secretory component* that is believed to serve a similar protective function. The IgA and IgM antibodies bind to the B subunit of cholera toxin, thereby inactivating it, and they also bind to the lipopolysaccharide molecules of the bacteria themselves. IgG antibodies are produced too in response to a cholera infection, but they are largely confined to the blood. However, some of them do end up in the gut after leaking from the bloodstream through the damaged epithelium, or following the migration of a B lymphocyte, and these antibodies are thought to be significant elements of the immune response to cholera (Qadri *et al.,* 1998).

4.2 Cholera vaccines

Recovery from a cholera infection brings about long-lasting natural immunity, but infection with cholera of the classical biotype may provide better protection than a similar infection with El Tor cholera. So far, the vaccines developed to combat cholera have been unable to produce the same results.

- What would be the best route for administration of a cholera vaccine?
- Since *Vibrio cholerae* is an enteric pathogen, an oral vaccine would have the most chance of provoking an immune response that resembled the response to a natural infection, and so result in a similar immunity to the organism.

Several vaccines against cholera have been licensed: killed whole-cell vaccine, killed whole-cell plus subunit vaccine, and live attenuated vaccines. We will look at each in turn.

A killed whole-cell (WC) vaccine, comprising a mixture of El Tor and classical biotypes and Inaba and Ogawa serotypes, has been tried. This vaccine was administered by injection and did not perform well. The WHO describes it as conveying 'incomplete, unreliable protection of short duration' and they do not recommend its use.

- Can you suggest why the vaccine performed poorly?
- Administration of the WC vaccine by injection would not bring the cholera vibrios into contact with the Peyer's patches and the most important part of the immune response against cholera would not be provoked.

A killed WC vaccine of a similar composition to the one above, but with the addition of the B subunit of the cholera toxin (BS–WC), underwent field trials in Bangladesh in the 1980s. This vaccine was given in two oral doses and stimulated a secretory IgA response equivalent to that seen in clinical cholera. High levels of immunity to cholera were obtained, but they were short-lived. This vaccination has been suggested as a preventative measure for refugee populations, but has not been found to provide increased cost-effectiveness in comparison with other prevention and control measures (Murray *et al.*, 1998).

Live attenuated cholera vaccines have also been developed; the strains used are altered so that they cannot cause cholera but still retain the ability to colonize the small intestine when given orally. The vaccine CVD103–HgR, derived from the classical Inaba strain 569B, was tested in Indonesia between 1993 and 1997. To produce CVD103–HgR from strain 569B, the *Tox A* gene, which encodes the A subunit of cholera toxin, was deleted. The vaccine induced an IgG as well as a secretory IgA response, but performed poorly overall. Since the current seventh pandemic is caused by El Tor cholera (see below), a vaccine containing El Tor *V. cholerae* might provide more protection than one based entirely on a classical strain. A cholera vaccine based on CVD103–HgR, but with the addition of an attenuated derivative of an El Tor strain lacking the genes encoding CT, Ace and Zot, has since been developed and was very promising when tested in the USA and Peru.

Research and development in cholera vaccination are still ongoing. Recent trials in Vietnam, which began in 1997, used a killed oral vaccine composed of *Vibrio cholerae* O1 and O139 (described as bivalent); the results are not yet (2003)

available. As yet, routine cholera vaccination is not cost-effective, because more than one dose is usually required, immunity does not last long and the disease is relatively rare. Nevertheless, pre-emptive vaccination of refugee populations seems to be effective in preventing large-scale epidemics. At present, no country demands a certificate of cholera vaccination as a condition of entry.

5 Treatment of cholera

With no cost-effective vaccine available, other measures such as treatment and prevention assume great importance. Prevention of cholera depends on interrupting the faecal–oral route, so that food and drink remain uncontaminated with faecal material. This can be achieved with proper sanitation, clean water and hygienic methods of food preparation, which are fairly simple practices, but sadly, well beyond the means of many people.

If a person contracts cholera, it is imperative that they are treated as quickly as possible, since untreated cholera can have a case fatality rate as high as 50%. Fortunately, effective treatment can reduce this rate to less than 1%. More importantly, for many countries, effective treatment is cheap, comprising oral rehydration to replace lost fluid and electrolytes.

Table 5.2 shows the composition of oral rehydration solution recommended by the WHO. The listed substances are dissolved in one litre of clean drinking water.

The case fatality rates for cholera have dropped dramatically since the 1950s, as shown in Figure 5.1.

TABLE 5.2
Recipe for an oral rehydration mixture. Taken from Greenwood *et al.* (2000).

Substance	Mass/g
sodium chloride	3.5
potassium chloride	1.5
sodium citrate	2.9
glucose	20.0

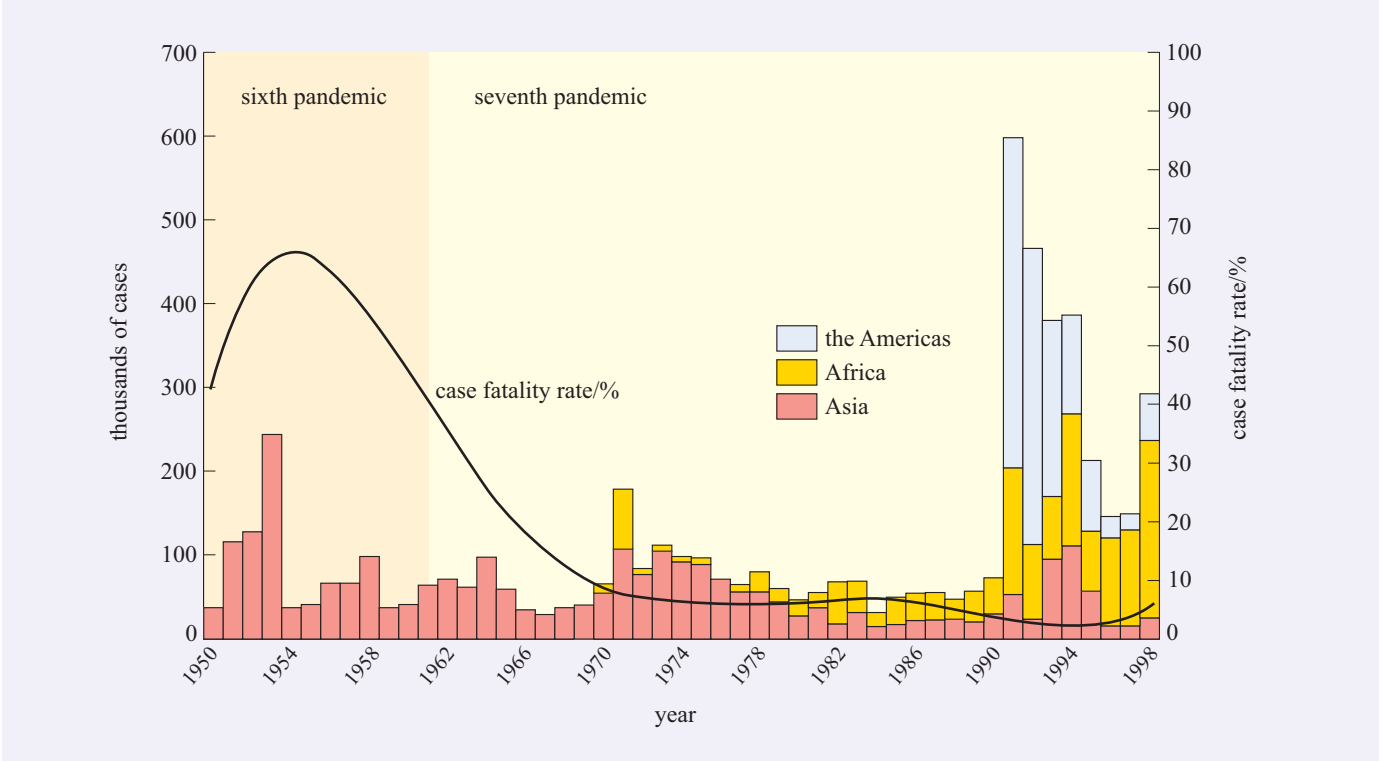


FIGURE 5.1 Reported global number of cases of cholera (bar chart) and the case fatality rate (solid line) between 1950 and 1998.

- What are the probable reasons for this fall?
- Case fatality rates are likely to have dropped partly because treatment has become more easily available, and, as you will learn below, partly because El Tor cholera is less virulent than classical cholera.

Oral rehydration therapy was first introduced in the early 1970s, and became widely available during the 1980s. In 80–90% of cases, oral rehydration alone is sufficient treatment, but when dehydration is severe, intravenous fluids are required. In these circumstances, antibiotics may be used to lessen the volume and duration of diarrhoea and reduce the carriage of vibrios in the faeces. Tetracycline is the drug of choice, but some *V. cholerae* are resistant to it and alternatives such as erythromycin, chloramphenicol, cotrimoxazole or others have to be used instead. The resistances exhibited by cholera vibrios can change rapidly; in their study of diarrhoeal stool samples, Kaur and Lal (1998) reported that the strains they isolated were sensitive to chloramphenicol until 1993, but resistant to this antibiotic from then onwards. Conversely, these strains were resistant to cotrimoxazole until 1996, but 100% sensitive to it in 1997.

Case fatality rates differ around the world, averaging around 1% for Asia, Europe and the Americas but rising to around 5% in Africa. This marked difference reflects disparities in access to treatment, rather than any variation in the virulence of *V. cholerae*.

6 The current cholera pandemic

The seventh and current cholera pandemic began in 1961, immediately after the ‘end’ of the sixth. It arose surprisingly not in India, but in Indonesia in Sulawesi (Celebes Islands). This time, the causative agent was not the usual, classical cholera, but a new biotype, El Tor. This biotype was first isolated among pilgrims in a quarantine station called El Tor, and belonged to the same group (O1) as classical *Vibrio cholerae*, but was a different serotype from either Inaba or Ogawa. The disease it caused was different too: the El Tor strain was less virulent, produced a less effective immune response and was better able to persist in the environment.

The new El Tor cholera began a relentless advance across the world that continues today. In 1963 it reached Bangladesh, in 1964 it got to India, and over the following years it went on to invade the former Soviet Union, Iran and Iraq. Africa was reached by 1970, but it took another 20 years for El Tor cholera to reach South America. In January 1991, a Chinese ship released bilge water infected with the cholera bacillus into the waters of Lima’s port city, Callao. It was summer time, and the locals were eating ceviche, a dish that contains raw shellfish. The shellfish quickly became contaminated with cholera bacteria, which soon infected the human population. The spread of the epidemic was made easier by Lima’s water supply, which was unchlorinated. In that year, cholera spread rapidly, causing 400 000 reported cases and 4000 deaths in 16 South American countries. Nowhere in the world had seen an epidemic of these proportions since 1969, the year that cholera was made reportable under International Health Regulations.

The question of exactly how *Vibrio cholerae* ended up in the bilge water of a ship is an interesting one. El Tor vibrios are known to persist longer in the environment than classical cholera bacteria. Research has shown that the El Tor bacillus is capable of parasitizing algae and even goes into a kind of reversible suspended

animation when suddenly placed in cold saltwater. Algae could therefore provide a reservoir of infection for epidemic *V. cholerae*. Recent studies have lent weight to this theory. An investigation into cholera in Bangladesh and the El Niño weather pattern of southern Asia found that the two were linked. This suggested that cholera patterns were related to temperature changes in the region (Pascual *et al*., 2000).

- How might temperature affect algal populations?
- Warmer water temperatures might encourage algal blooms to flourish.

Rita Colwell, a scientist who contributed to this investigation, has claimed for years that tracking the oceanic algal blooms that originate from Bangladesh and India, would allow the prediction of likely cholera outbreaks.

A group from Lima has also found evidence that the occurrence of cholera may be affected by environmental factors. Twelve environmental sites were sampled for cholera vibrios, each month, from November 1993 to March 1995. In the winter, no vibrios were found in the samples, but in summer, the bacteria were detectable before cases of cholera occurred in the local community. The researchers suggested that an increase in environmental vibrios is followed by the appearance of cases of cholera in the human population and that increasing temperatures might cause the increase in vibrio numbers (Franco *et al*., 1997). The idea that the cholera germ alone is not sufficient to cause epidemic cholera, but the correct environmental conditions are required as well, echoes von Pettenkofer's soil theory on the cause of cholera. Perhaps von Pettenkofer was right!

6.1 Emergent and resurgent cholera

While South America was reeling under the impact of the seventh cholera pandemic, scientists in Bangladesh and India were wondering if an eighth pandemic was just beginning. In 1992, in the southern coastal region of Bangladesh, a previously unknown strain of *Vibrio cholerae* was causing a cholera epidemic. This *emergent* group was the 139th to be distinguished by its O antigen, and so was labelled O139 and given the name 'Bengal'. In 1992 and 1993, Bengal cholera caused large epidemics in India and Bangladesh, which killed 5000 people; then, in 1994, cholera O139 suddenly disappeared. In Bangladesh it was displaced by a *resurgent* El Tor strain.

However, there was a resurgence of Bengal cholera itself during 1995 and 1996. The strain was identified in Dhaka and surrounding districts in Bangladesh (Faruque *et al*., 1997), and also in Calcutta in India (Mukhopadhyay *et al*., 1998). These events illustrate the rapid changes in cholera epidemiology that can occur.

Vibrio cholerae O139 had been reported in 11 Southeast Asian countries by 2000, but for the time being, remains confined to Asia. The origin of epidemic cholera strains is an area of fast-moving research. Bengal cholera lacks some of the genes that code for the O antigen of serogroup O1 cholera, and has instead a different DNA sequence that is unique to this strain. This finding has prompted the suggestion that Bengal cholera may have emerged from El Tor cholera by serotype-specific genetic changes (Faruque *et al*., 1997). A Dutch group (Mooi and Bik, 1997) has also suggested that horizontal transfer of genes encoding enzymes involved in cell-wall polysaccharide synthesis may have played a key role in the emergence of cholera O139.

7 Cholera at the beginning of the 21st century

In their recent 'Report on Global Surveillance of Epidemic-prone Infectious Diseases' the WHO concluded that:

> 'Cholera is a major public health problem that is becoming increasingly important as the number of countries affected continues to increase.'

Figure 7.1, taken from the above report, provides a clear picture of cholera in the closing years of the last century. The enormous impact of the seventh pandemic on the Americas can be seen, with the number of cases the highest recorded in a cholera outbreak in the last 30 years. The 1990s also saw large increases in cholera cases in Asia and Africa, and the trend globally is that of increasing numbers of people being affected. The WHO reports that the failure of effective epidemic control has led to an increasing number of areas becoming endemic for cholera. Africa, particularly, is bearing the brunt of this rise caused by the ongoing seventh pandemic – in 1998 African cholera cases represented 72% of the global total.

New major outbreaks of cholera continue to occur, and many of these are associated with climate changes such as El Niño, or the displacement of people into refugee camps. During 1994 and 1995, the Crimea and Southern Ukraine experienced a cholera epidemic that infected 1370 people, killing 32 of them. This epidemic was caused by *V. cholerae* O1, biotype El Tor, serotype Ogawa bacteria that were believed to have originated from the environment (Clarke *et al*., 1998). In April 1997, cholera broke out among 90 000 Rwandan refugees in the Democratic Republic of Congo (Morbidity and Mortality Weekly Report, 1998). It would seem that wherever there is upheaval, whether social or environmental, there is the risk of cholera. Furthermore, the threat of a new eighth pandemic caused by *V. cholerae* O139 Bengal cannot be dismissed.

8 Conclusion

It is clear from the above discussion that cholera is an economically and socially important disease that has been with us for centuries, and shows no signs of disappearing. The main strategies for combating *V. cholerae* appear to be socio-political: outbreaks occur when there is environmental stress to humans, such as poverty, displacement or climate change, and where there is no infrastructure available to support effective sewage disposal and the provision of safe water. Ironically, cholera treatment is cheap and straightforward, and can be made easily available almost everywhere. The search for an effective vaccine continues, but meanwhile new bacterial strains emerge to perpetuate this most distressing disease.

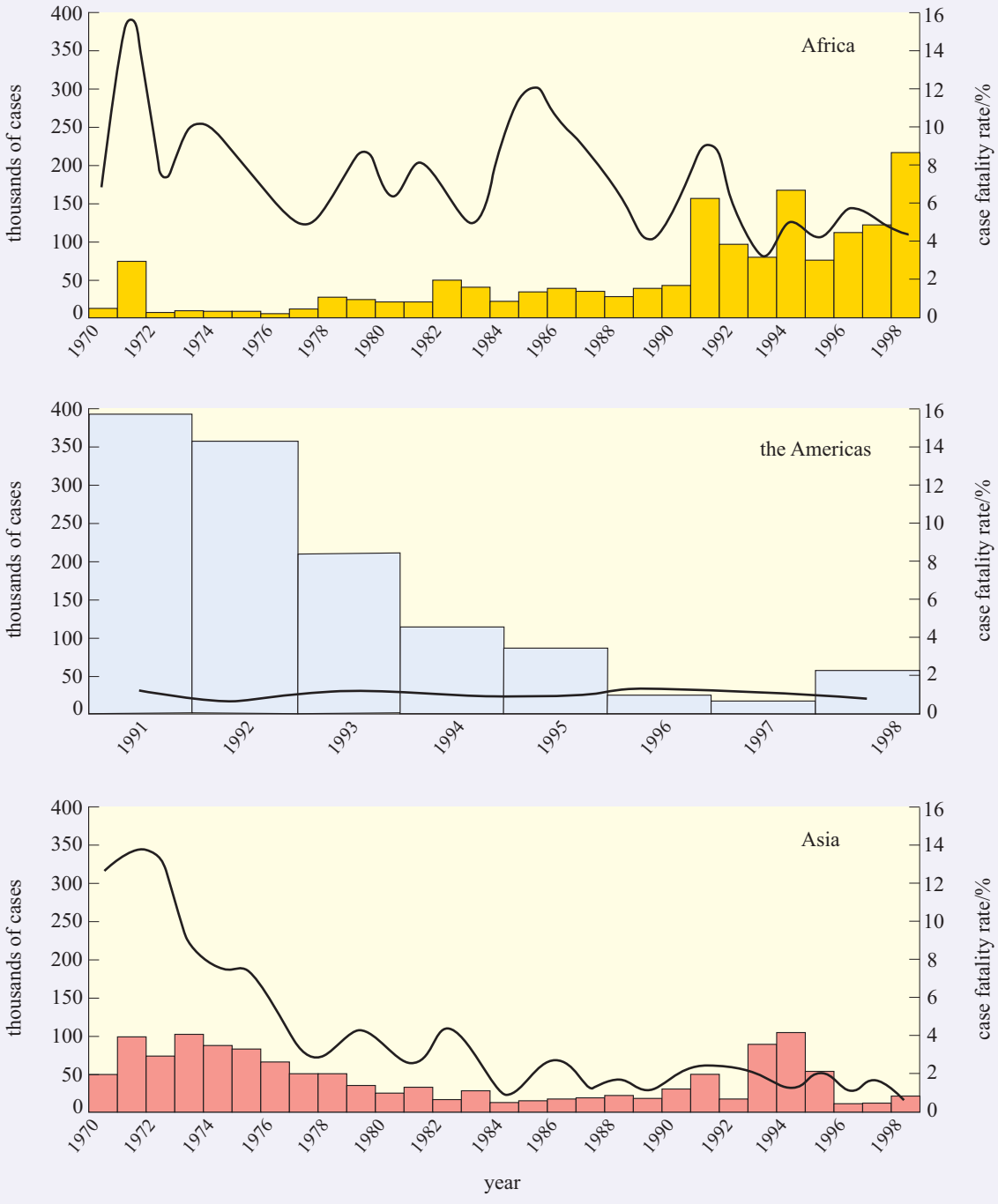


FIGURE 7.1 Numbers of reported cases (bars) and case fatality rates (solid lines) for cholera in Africa, the Americas and Asia between 1970 and 1998.

9 Learning outcomes

When you have completed this case study, you should be able to:

1 Define and use, or recognize definitions and applications of, each of the terms printed in **bold** in the text.

2 Outline the main steps in the identification of *V. cholerae* as the causative organism of cholera.

3 Describe the symptoms of cholera.

4 Explain the molecular mechanisms underlying the disease.

5 Describe the body's response to infection with *V. cholerae*.

6 Outline measures to prevent and treat cholera.

7 Describe epidemiological evidence that indicates the appearance of cholera pandemics.

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Figures

Figure 1.1: Brody, H. *et al.* (2000) 'Map-making and myth-making in Broad Street: the London cholera epidemic, 1854', *Lancet*, **356**, pp. 64–68, Elsevier Science; *Figure 2.1*: Wellcome Photo Library/TMR/R. H. Behrens; *Figure 2.2*: Wellcome Photo Library/TMR/International Centre for Diarrhoeal Disease Research, Bangladesh; *Figure 3.1*: Wellcome Photo Library/TMR; *Figures 5.1, 7.1*: WHO Report on Global Surveillance of Epidemic-prone Infectious Diseases, World Health Organization.

Table

Table 5.2: Greenwood, D. *et al.* (1997) 'Formulation of oral rehydration solution recommended by the WHO', *A Guide to Microbial Infections*, 15th edn, p. 31, Churchill Livingstone.

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