Part Two

Environmental Biology and Epidemiology of Specific Excreted Pathogens

SECTION I

Excreted Viruses

9

Enteroviruses, Poliomyelitis, and Similar Viral Infections

OVER 100 DIFFERENT VIRUSES are known to be fecally excreted by man. New viruses are still being discovered, and several have yet to be fully characterized. Typically they infect the alimentary canal and are shed in very large numbers by infected persons (Madeley 1979). Diseases caused by these organisms range from the trivial to the serious or even fatal. The occurrence and medical significance of excreted viruses in the environment is the most rapidly changing field of knowledge reported here. A combination of heightened environmental concern (especially in the USA), improvements in laboratory techniques, and the recent discovery of important new human viral pathogens (especially rotavirus) have caused a marked increase in scientific activity, with several hundred papers yearly now being published on aspects of excreted viruses (EPA 1978; WHO 1979).

Table 9-1 presents a classification of some excreted viruses similar to that recently proposed by the World Health Organization (WHO 1979). This classification will undoubtedly undergo changes over the next few years as new viral agents are characterized and the taxonomy is revised. For the purposes of this book, excreted viruses are divided into three groups:

- The enteroviruses (chiefly polioviruses, coxsackieviruses and echoviruses), described in this chapter, which also contains some information on adenovirus and reovirus, as these are often considered jointly with enteroviruses; (see figure 9-1)
- The hepatitis A virus described in chapter 10
- The viruses possibly associated with gastroenteritis (rotavirus, Norwalk agent, and others) described in chapter 11.

Description of Pathogens and Diseases

The enteroviruses are an acid-stable subgroup of the small picornaviruses. They are a large group causing a wide variety of diseases (table 9-1). The polioviruses are probably the most important. They were the first enteroviruses to be fully investigated, and because they are relatively easy to culture they have been used in most experimental work.

Identification

Poliomyelitis is unique in being the major permanently crippling disease of infectious origin. It is caused by the infection of the central nervous system by poliovirus or occasionally another enterovirus. It is usually recognized by a sudden and unexpected onset of tiredness and weakness in the limbs. Fortunately, the clinical symptoms of poliomyelitis occur in only a very small proportion of the persons infected, usually a maximum of 2 and often less than 1 percent of the total.

The clinical effects of infection range from the asymptomatic through nonspecific minor illness to meningitis, paralysis and possibly death. There are two basic patterns of symptoms. The first is a minor illness arising a few days after infection, lasting 1–2 days and characterized by mild fever, listlessness, sore throat and vomiting. The second, developing 3–4 days later but often occurring without the first phase, is much more serious. Symptoms of aseptic meningitis, fever, severe headache, and vomiting are followed by stiffness of the neck and back. In paralytic cases the disease usually leads to progressive weakness resulting in severe paralysis. Death, usually caused by respiratory failure, may occur.

The disease is short-lived and most people recover fully, but many of the most severely affected are permanently disabled. Mortality among the paralytic cases varies between 4 and 10 percent depending on the virulence of the virus, the degree of medical care and the age of the patient. Diagnosis in asymptomatic cases is dependent on laboratory facilities in which the virus can be cultured from throat swabs or feces. Serological

Table 9-1. *Human Excreted Viruses*

Chapter in which described	*Virus group*	*Family*	*Size and composition*	*Number of types*	*Diseases or symptoms caused*
9	Enterovirus	Picornaviridae	About 20–30 nanometers diameter. Single-stranded RNA in a protein shell		
	Poliovirus			3	Poliomyelitis, meningitis, fever
	Coxsackievirus A			24	Herpangina, respiratory disease, meningitis, fever
	Coxsackievirus B			6	Myocarditis, congenital heart anomalies, meningitis, respiratory disease, pleurodynia, rash, fever
	Echovirus			34	Meningitis, respiratory disease, rash, diarrhea, fever
	New enteroviruses			4	Meningitis, encephalitis, respiratory disease, acute hemorrhagic conjunctivitis, fever
	Adenovirus	Adenoviridae	About 70–80 nanometers diameter. Double-stranded DNA in a protein shell	>30	Respiratory disease, eye infections
	Reovirus	Reoviridae	About 75 nanometers diameter. Double-stranded RNA in a double protein shell	3	Not clearly established
10	Hepatitis A virus	? Picornaviridae	About 24–29 nanometers diameter. Single-stranded RNA	1	Infectious hepatitis
11	Rotavirus	Reoviridae	About 70 nanometers diameter. Double-stranded RNA in a double protein shell	?	Vomiting and diarrhea
	Astrovirus	?	About 28 nanometers diameter	?	?
	Calicivirus	?	About 35–40 nanometers diameter. Single-stranded RNA in a protein shell	?	Vomiting and diarrhea
	Coronavirus	Coronaviridae	Between 20 and 220 nanometers diameter. Pleomorphic with petal-shaped projections 20 nanometers long. Single-stranded RNA in protein shell and lipid envelope	?	Common cold
	Norwalk agent and other small round viruses	?	About 20–35 nanometers diameter	?	Vomiting and diarrhea
Not described	Adeno-associated virus	Parvoviridae	About 19 nanometers diameter. Single-stranded DNA in protein shell	4	Not clearly established but associated with respiratory disease in children

tests can also be used. Treatment is supportive in nature.

The other enteroviruses can cause a wide variety of symptoms (table 9-1). These viruses are generally less dangerous than poliovirus and like poliovirus they only cause significant disease in a small proportion of cases. They normally infect the alimentary canal or the respiratory tract, giving rise to gastroenteritis or influenza-like symptoms. More severe disease is often associated with the spread of the virus to other organs such as the liver or central nervous system. As with polio, the effects are generally short-lived, and treatment is supportive.

Diagnosis is either based on symptoms or on laboratory culture and identification. Diagnosis is complicated by the fact that the same virus may cause different symptoms in different patients and that different viruses may give rise to similar symptoms.

Occurrence

These infections occur worldwide and are very common. Some isolated communities have been known that were not infected with poliovirus but few or none now remain. The other enteroviruses have a similar distribution, but there are local variations in both virus types and in the virulence of various strains.

Infectious agents

Poliovirus is a small spherical particle 28 nanometers in diameter and is therefore not visible by normal light microscopy (figure 9-1). It occurs in three serotypes, numbered 1 to 3. A most important characteristic is the degree of neurovirulence, which is known to vary from strain to strain in all three types. The infective dose is small: probably as little as one virus particle. Poliomyelitis can occasionally be caused by coxsackie- and echoviruses.

Like poliovirus, the other enteroviruses are submicroscopic spherical particles with sizes ranging between 20 and 30 nanometers in diameter. The number of types in each group is given in table 9-1.

Reservoir

The reservoir of poliovirus is man. Chimpanzees have been known to catch the disease in captivity, and monkeys may also act as natural hosts, but nonhuman reservoirs have not been shown to be significant.

As with poliovirus, man is the main reservoir of infection for the other enteroviruses. A number have been isolated from pets and other animals associated with man, but it is unclear whether or not they were naturally infected. Reovirus appears to be an exception, having been isolated from a surprisingly large range of animal species. Even so, animals have not been shown to be a significant source of infection for man.

Transmission

Infected persons can shed very large numbers of virus particles—more than 10^6 per gram of feces. Viruses are also present in throat secretions, especially during the early stages of infection. These particles are highly infectious and can remain viable for a considerable period under suitable conditions. Infection takes place when the virus is ingested, possibly in food or water. The primary sites of infection are the throat and the lower alimentary canal. Within a few days the virus spreads to the lymphatic system and the blood stream. This phase corresponds with the minor symptoms of infection. In the small proportion of severe cases the virus infects the central nervous system, possibly by spread along nerve fibers.

On infecting a suitable cell, the virus diverts the cell's metabolic activity to the production of large numbers of virus particles identical to the original virus. These are liberated when the cell breaks down, and they either infect other cells or, in the case of the cells lining the alimentary canal, are passed out in the feces. Transmission is mainly directly from person to person either by the oral-oral route or the fecal-oral route. There is some indication that in unhygienic conditions the latter route is the most common, with the former route being more important under more sanitary conditions. Children under the age of 2 years are the most potent disseminators by both routes.

There are indications that poliovirus is carried between family groups by young children who are both susceptible and mobile (2–6 year age group). Infection then spreads within the family downwards to nonmobile children and upwards to older children and adults. Up to 50 percent of persons having resistance from earlier infections may become reinfected, but in these cases the excretion of viruses is much reduced and symptoms absent.

The other enteroviruses are probably transmitted by the same route as poliovirus, whereas the adenoviruses, which are normally associated with upper respiratory tract infections, are mainly spread by an airborne route from contaminated throat secretions.

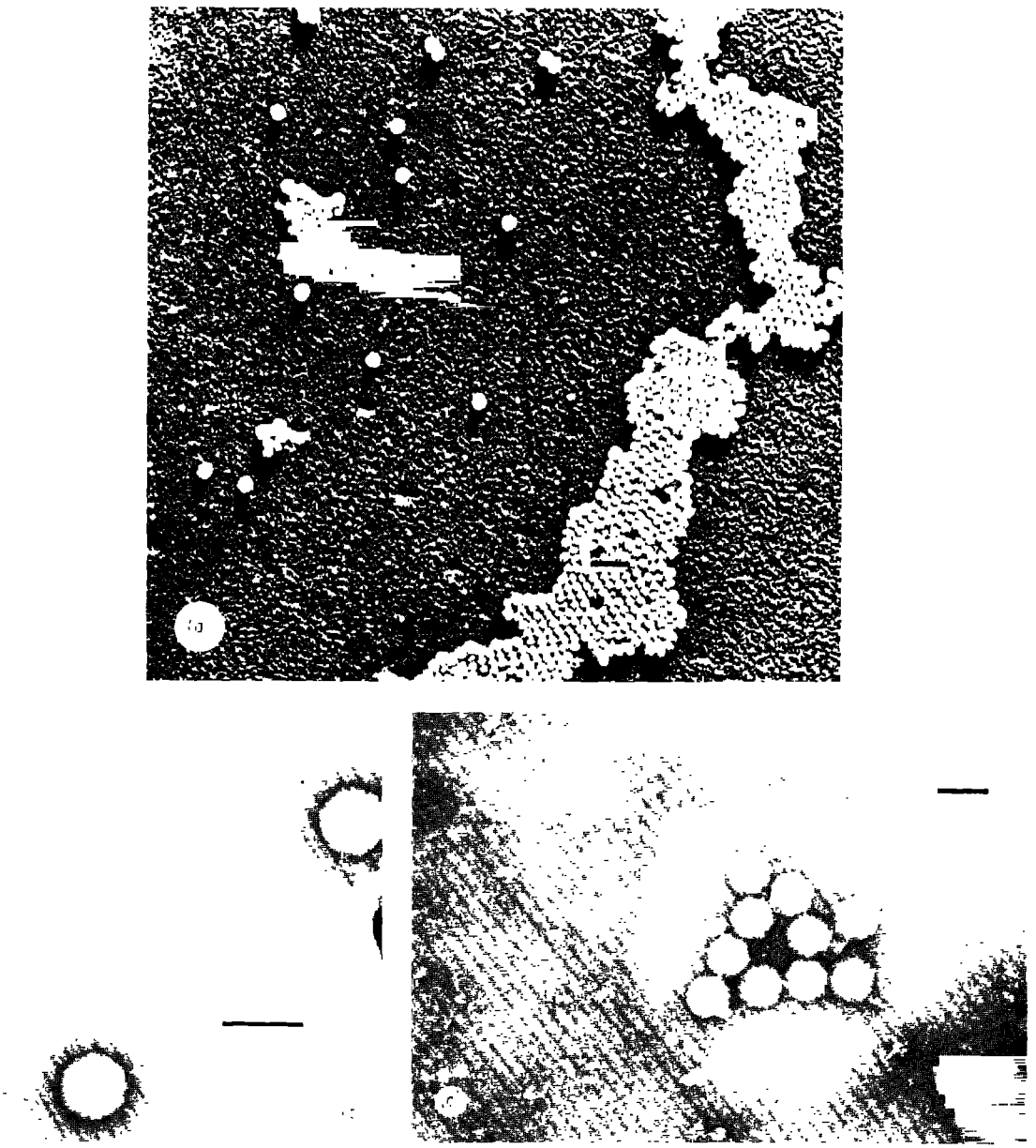

Figure 9-1. *Polio-, adeno-, and reoviruses under electronmicroscopy.* (*a*) Polioviruses under scanning electronmicroscopy. Other enteroviruses have a similar appearance. Scale bar = 0.1 micrometers. (Photo: World Health Organization, Geneva, Switzerland.) (*b*) Adenoviruses under transmission electronmicroscopy. Scale bar = 0.1 micrometers. (Photo: A. J. Zuckerman, London School of Hygiene and Tropical Medicine, London, U.K.) (*c*) Reoviruses under transmission electronmicroscopy. Scale bar = 0.1 micrometers. (Photo: A. J. Zuckerman, London School of Hygiene and Tropical Medicine, London, U.K.)

Incubation period

Minor illness when present occurs within 2–3 days of infection. Nervous system involvement possibly leading to paralytic poliomyelitis may occur between 5 and 35 days after infection—on average 17 days. The other enteroviruses are generally similar to poliovirus.

Period of communicability

Viruses have been found in the throat secretions and feces within 24 hours of infection, and contact cases have been observed within 3 days. Virus excretion, mainly in the feces, has been observed for as long as 17 weeks, and on average 7 weeks.

Most of the other enteroviruses are excreted for a shorter period than poliovirus. Coxsackie B viruses, for example, are excreted for 1 week or less. The adenoviruses, however, may persist in a latent form in the tonsils and adenoids.

Resistance

Young children are the most susceptible age group, most adults having acquired resistance to poliovirus during earlier infections or by vaccination. Infection leads to the development of life-long immunity to the infecting type of virus, but the individual may still be vulnerable to other types.

The severity of the disease is markedly dependent on the age of the patient; in a nonresistant population, teenagers and young adults show the most severe symptoms. Certain other factors have been found to increase the severity of the disease—including tonsillectomy, pregnancy, recent inoculations, physical exertion, and trauma.

Infection by the other enteroviruses usually confers resistance in a similar manner, but vaccines are not generally available.

Epidemiology

During this century the incidence of poliomyelitis has been observed to change from a constant background infection to epidemics of increasing severity. In temperate climates these occur in late summer and early autumn. In the tropics and subtropics the fluctuations are less marked, but the trend is the same. The reasons for this change are not fully understood but may be due to variations in hygiene or other factors affecting virus transmission and so leading to variations in the resistance within the community. In areas with poor hygiene children acquire immunity while very young, and the proportion of paralytic cases is therefore low and confined to this age group. Disruption of this pattern of infection may lead to a higher incidence of severe symptoms at a later period. Another possibility is that more virulent strains of virus have been introduced.

Many of the other enteroviruses act in a similar manner to poliovirus. Often serial waves of infection move through the community, fading away to a very low or undetectable level and being replaced by infection with another type of virus. Seasonal variations also occur.

Control Measures

In considering the control of enterovirus infections, one must distinguish clearly between reducing infection and reducing disease. In the case of poliovirus, for instance, environmental measures may reduce transmission and thus the incidence of infection. However, these measures may increase the incidence of serious clinical disease by deferring the age of first infection to that at which disease is more likely to be severe. A survey of 10,000 households in 25 villages in Gujarat (India) found that the number of paralytic poliomyelitis cases per 1,000 households was related to household income. Among low-income households there were 12 cases per 1,000 houses; among middle-income households there were 18 cases per 1,000 houses; and among high-income households there were 24 cases per 1,000 houses (Jhala, Goel and Dave 1979). Contrary evidence is presented in a recent review showing that the real (as opposed to reported) annual incidence of paralytic poliomyelitis in Burma, Egypt, Ghana and the Philippines is between 233 and 3,800 per 1 million children age 0–4 years, and between 37 and 589 per 1 million of the total population (Sabin 1980). About 90 percent of paralytic episodes in these countries occurred during the first 3 years of life. Despite the fact that for various technical reasons these annual incidences are underestimates, they are similar to or considerably higher than the rates occurring in the USA in the immediate prevaccine era. These data cast doubt on the belief that improved living conditions, in the absence of vaccination, increase the incidence of poliomyelitis disease.

Individual

Highly efficient vaccines are available for the three polioviruses. Both killed and live attenuated vaccines can be used. The live vaccine is probably preferable in developing countries because it is easily administered on a lump of sugar, whereas several injections are required for the killed vaccine. The live vaccines contain a mixture of attenuated (weakened) strains of virus that establish an infection which leads to resistance but, unlike many of the wild strains, does not infect the nervous system. These attenuated strains can also spread from person to person and so immunize a greater number of people but do not spread as efficiently as the wild virus. The wild viruses are suppressed but not eliminated from the community. It is therefore necessary to maintain the vaccination of young children to prevent the build up of a susceptible

group. Resistance appears 7–10 days after vaccination. The response is sufficiently rapid to be of great benefit during an epidemic. No vaccines are available for the other enteroviruses.

No specific drugs are available either for chemotherapy or prophylaxis. Good personal hygiene and the avoidance of contaminated food and water may reduce the risk of mild infection in early childhood but thereby increase the risk of severe infection later.

Environmental

Improvements in excreta disposal alone are unlikely to have a great impact. The highly infectious nature of viruses, the preponderance of young children among the cases, and the large proportion of symptomless infections indicate that the main route of infection will remain from person to person. The elimination of excreta as a source of infection may change the primary means of transmission from the fecal-oral to the oral-oral route. Improvements in both general hygiene and excreta disposal are likely to have an effect and may be responsible for the trend toward the epidemic type of poliovirus transmission. In these circumstances infection will probably be delayed rather than prevented, and the proportion of patients with severe symptoms may increase. This can be prevented in the case of poliomyelitis by the use of vaccines.

Polioviruses and other enteroviruses have been isolated from flies and cockroaches. For instance, in Texas (USA) polioviruses were isolated from 15 percent of flies, while coxsackieviruses were isolated from 45 percent, and flies experimentally fed polioviruses continued to excrete them for up to 2 weeks (Melnick and Dow 1953). It is clear from this and similar studies that insects can pick up viruses and may subsequently contaminate food, but it is unknown whether this mode of transmission is of any epidemiological significance (see chapter 37).

Occurrence and Survival in the Environment

Viruses are not capable of multiplying outside of living cells; therefore, in the environment their numbers can only decrease. In favorable conditions, however, they can survive for months. Their survival is aided by neutral pH and the presence of particulate or organic matter, moisture and, in particular, low temperatures. Resistance to inactivation varies considerably among different types of virus and even among different strains of the same virus. Inactivation is a rate process, and the removal of infectivity therefore depends on both the efficiency of removal and the numbers initially present. In feces and sewage these may be higher than 10^6 per gram and 10^6 per liter, respectively.

The enteroviruses (chiefly polio, coxsackie, and echo), and to a lesser extent adenoviruses and reoviruses, have been the only excreted viruses to be extensively studied in the environment. This is partly because certain other important excreted viruses (particularly hepatitis A virus and rotavirus) cannot be routinely grown in cell culture at the present time. However, as laboratory skills improve, and as models for human excreted viruses are developed (for instance, reoviruses and simian rotaviruses may provide models for human rotaviruses), more data will be obtained on the environmental behavior of excreted viruses other than the enteroviruses. Preliminary evidence from a few studies indicates that there may be significant differences between rotavirus, hepatitis A virus, and the enteroviruses in their environmental characteristics (Farrah and others 1978: Wallis and Melnick 1967), although a recent study has shown that simian rotavirus has survival properties in fresh and saline waters similar to enteroviruses (Hurst and Gerba 1980).

Throughout the rest of this chapter, data are presented on the numbers of viruses that researchers have isolated from various environmental samples. It must be stressed that these numbers depend very considerably on the techniques used; in general, as techniques improve reported concentrations of enteroviruses from a particular source (for example, river water) increase. The very earliest studies reported only the proportion of samples from which viruses could be isolated—for instance, 62 percent of sewage effluent samples contained enteroviruses. Subsequently, quantitive techniques were developed that were based on observed cell death following inoculation with varying dilutions of sample, and these yielded a count of median tissue culture infective doses ($TCID_{50}$) per volume of sample. More recently, most laboratories have adopted a technique whereby a direct count is made of plaques formed by viruses on cell monolayers, or in cell suspensions, which yields a count of plaque-forming units (PFUs) per volume of sample. In both the TCID and PFU techniques, counts depend upon the choice of cell line because different viruses will replicate with varying readiness in different primate cells (Schmidt and others 1978).

When viruses are present in small numbers in large volumes of sample, a variety of different methods can be used to concentrate them. When viruses are bound

to each other in clumps, or are adsorbed to solid particles, efforts must be made to disaggregate them. Even adopting the most careful and sophisticated techniques, recovery of seeded enteroviruses from environmental samples is typically below 60 percent. To add to the complexity and uncertainty, results obtained are still too dependent upon the personal technique of the laboratory staff and the whole tradition and routine of the particular laboratory. Quantitative data on viruses in the environment should therefore be taken as indicative only. Throughout this chapter concentrations are given as viruses per volume or weight of sample although, strictly speaking, they should be as infective units or $TCID_{50}$ or PFUs per sample.

In surface waters

Enteroviruses can be isolated in low concentrations from almost all surface waters receiving human wastes. For instance, the Thames at London (UK) contains up to about 100 enteroviruses per liter, with a peak usually occurring in winter (WHO 1979; the River Sowe (UK) in December contained up to 620 enteroviruses per liter (Morris and Waite 1980); the Missouri and Mississippi rivers (USA) have yielded up to 0.1 and 0.4 enteroviruses per liter respectively; the Seine at Paris and the Moselle at Nancy (France) have contained up to 170 and 280 enteroviruses per liter, respectively (Berg and Metcalf 1978).

Survival in water is dependent primarily upon temperature and the degree of contamination. Studies listed in the appendixes of Feachem and others (1980) show that at temperatures less than 10°C survival times of between 24 and more than 272 days are reported, while at temperatures above 20°C the range is 4 to 135 days. In a study of enterovirus survival in the Rio Grande (New Mexico, USA), at 23–27°C, 90 percent inactivation occurred in 25 hours for poliovirus 1, 19 hours for poliovirus 3, and 7 hours for coxsackievirus A13. At river temperatures of 4–8°C, the time for 90 percent inactivation of poliovirus 1 was 46 hours (O'Brien and Newman 1977). Niemi (1976) studied the survival of coliphage T7 in samples of Finnish river water and found that, after 64 days at 3°C, a 99.5 percent reduction occurred, whereas after 64 days at 20°C a 99.98 percent reduction was recorded. Joyce and Weiser (1967) found that poliovirus did not survive for more than 63–84 days at 20–25°C, but survived for more than 91 days at 4°C when stored in samples of various farm pond waters. Hurst and Gerba (1980) studied the survival of poliovirus 1, echovirus 7, coxsackievirus B3 and simian rotavirus in polluted and nonpolluted fresh water at 20°C and found that a 3 log unit reduction occurred in 3 to >14 days. For each virus, survival times were similar in polluted and nonpolluted waters. The strong influence of temperature on enterovirus survival is illustrated by the survival of 34 percent of enterovirus during 7.1 days of travel under the ice of a frozen 317 kilometer section of the Tanana River (Alaska, USA) (Dahling and Safferman 1979).

A number of workers (for instance, Cubbage and others 1979, Katzenelson 1978, Young and Sharp 1977) have noted that the observed loss of infectivity of viruses in water may be due in part to genuine damage to the virus and in part to an artifact caused by many viruses aggregating and simulating a single infectious particle. This aggregation may involve the adsorption of viruses onto suspended particulate matter or it may involve the formation of virus clumps.

An important aspect of the behaviour and survival of viruses in natural waters is their tendency to become adsorbed to organic or inorganic suspended particles. Adsorption is enhanced at slightly acidic pH and in the presence of divalent cations and is deterred by the presence of soluble proteins (Schaub and Sagik 1975; Schaub, Sorber and Taylor 1974). Furthermore, viruses adsorbed to solids retain their infectivity both to tissue culture cells and mice (Moore, Sagik and Malina 1975; Schaub and Sagik 1975; Schaub, Sorber and Taylor 1974). Adsorption to solids may cause an accumulation of viruses in bottom sediments, from which they may subsequently be resuspended in the overlying waters. Wellings, Lewis and Mountain (1976) isolated more than 15 excreted viruses per 100 grams of mud nearly 1 kilometer dowstream from a primary effluent discharge site. No virus was isolated from the overlying river water at this site.

There is very little evidence that the transmission of enteroviruses during recreation in polluted surface waters is of any public health importance. The spread of adenovirus in swimming pools has been demonstrated or suspected in several investigations (for instance, Heinz and others 1976).

In summary, a few enteroviruses may survive for many months, although 90 percent reduction usually occurs within a few days, and 99 percent reduction within 1 month. Temperature is the single most determining factor and 99 percent reduction at 20°C may be expected within about 10 days. Survival is longer in heavily polluted or in very clean waters. Very little data are available on virus survival in surface waters in the tropics (Lund 1979), and more research is required. Addy and Otatume (1976) isolated enteroviruses from 28 percent of surface water samples in

the Accra area (Ghana), with highest isolation rates (80 percent) obtained from polluted street drains. However, because surface waters in the tropics are typically in the temperature range of 20–30°C, it is reasonable to expect that survival times will be considerably shorter than those generally reported from temperate areas.

In groundwater

Enteroviruses have been isolated from groundwater, especially in situations where the groundwater is shallow and sewage effluent or sludge has been applied to the overlying soil. Survival of enteroviruses in groundwater may be somewhat longer than in similar surface waters at the same location because in hot climates the groundwater is cooler than the surface water (in cold climates the opposite may be true) and groundwater is not exposed to sunlight.

Wellings and others (1975) demonstrated enteroviruses in shallow groundwater beneath a wastewater irrigation site in Florida (USA) and showed that the viruses could survive in groundwater for at least 28 days. Wellings also reported a tentative association between the pollution of groundwater by septic tank effluent and an outbreak of disease associated with echovirus 22/23 complex at a migrant labor camp in Florida (Wellings, Mountain and Lewis 1976). Vaughn and others (1978) reported low levels of virus contamination (up to 3 viruses per liter) in wastewater recharged groundwater on Long Island (New York, USA) at sites where recharge basins were located less than 10 meters from the aquifer. This contamination occurred despite the fact that all wastewaters had undergone either chlorination or tertiary treatment by sand filtration. Slade and Edworthy (1981) isolated up to 1.3×10^4 viruses per liter from groundwater in chalk beneath groundwater recharge lagoons receiving raw comminuted sewage, but failed to detect viruses from boreholes 120 meters and 400 meters downstream (in relation to groundwater flow) from the recharge area. A study of groundwater pollution in Israel isolated enteroviruses in 20 percent of 99 samples and in 12 samples enteroviruses were isolated in the absence of fecal coliforms and fecal streptococci (Marzouk, Goyal and Gerba 1979).

Little is known about virus survival in groundwater, but estimates may be made from the data on survival in surface waters reported above. Yeager and O'Brien (1977) reported that a 90 percent reduction of enteroviruses in groundwater occurred in 11 to 14 days. Information on the travel of enteroviruses through soils to pollute groundwater is contained in the sections below on survival in soil and on sewage treatment by septic tanks and land application. The literature on viruses in groundwater has been reviewed elsewhere (Keswick and Gerba 1980).

In drinking water

Attention has been paid recently to the occurrence and epidemiological significance of enteroviruses in water supply systems (Anon. 1978: Committee on Viruses in Drinking Water 1979; EPA 1978; Mahdy 1979; Melnick, Gerba and Wallis 1978; WHO 1979). Enteroviruses have been isolated in very low concentrations from some treated, chlorinated water supplies in France, India, Israel, Italy, Rumania, South Africa, USA and USSR (Committee on Viruses in Drinking Water 1979; Gamble 1979; Kott and others 1974; Melnick, Gerba and Wallis 1978; Rao, Lakhe and Waghmare 1978; WHO 1979). A considerable debate has developed over whether these very low virus concentrations in treated drinking water constitute a cause for concern on public health grounds. There is strong support, especially among environmental virologists in the USA, for the concept that, because viral infectious doses may be very low, small numbers of viruses in large volumes of drinking or recreational water are important. It is postulated that viruses in water may cause low-level transmission which remains undetected due to the large proportion of asymptomatic infections and the varied symptomatology (table 9-1) in those individuals experiencing frank disease (Berg 1967; Committee on Viruses in Drinking Water 1979; WHO 1979). Some of those who take this view urge the adoption of stringent virus quality standards, such as less than one infective unit per 40–100 liters in recreational water and less than one infective unit per 100–1,000 liters in drinking water (Mahdy 1979; Melnick, Gerba and Wallis 1978; Shuval 1975; WHO 1979). This school of thought has been influenced by the unfortunate view that, because it is technically possible to achieve a certain level of water purity, it is therefore desirable.[1]

1. For example, Melnick (1976) writes: "I suggest ... a maximum of one detectable virus unit per 10 gallons of recreational water and a maximum of one infectious virus unit per 100 gallons of drinking water. As our methods for detecting and monitoring water supplies have continued to improve, I would suggest that we can do better, and raise the standards to a maximum of one infectious virus per 1,000 gallons of drinking water." Similarly, the WHO Scientific Working Group on Human Viruses in Water, Wastewater and Soil concluded that "the presence of even a few enteric viruses in a large volume of drinking water should be prevented, since treatment measures exist to achieve this goal and detection techniques are becoming available which can provide the required level of monitoring" (WHO 1979).

This point of view is refuted by others (especially European workers and those with an epidemiological perspective) who point out that there is no evidence for the existence of low-level waterborne transmission and that, even if it did exist, it might make no significant contribution to the maintenance of the endemicity of enterovirus infections (Gamble 1979). The authors of this book support this second viewpoint. There is strong epidemiological and theoretical evidence that enterovirus transmission is primarily by the person-to-person route[2] and may indeed be oral-oral as well as fecal-oral. It is unlikely that very low concentrations of enteroviruses in treated drinking water make any epidemiologically significant contribution to transmission[3], and any decision on increased water quality standards, implying increased treatment costs, must await evidence of the benefits to be expected from such a policy. This view is especially pertinent in developing countries where there are severe shortages of both financial and technical resources.

Some studies on the survival of enteroviruses in treated drinking water are listed in the appendixes of Feachem and others (1980). One study (Lefler and Kott 1975) found that at 18–25°C 99.9 percent of polioviruses were inactivated in 91 days in tap water and in 112 days in distilled water. At 4–8°C, poliovirus was completely stable in tap water and distilled water for 231 days. Kott, Ben-Ari and Vinokur (1978) reported a 99.9 percent reduction of poliovirus after 80 days in tap water at 18–23°C.

A quite distinct problem is that of enteroviruses in untreated and polluted drinking water. Very few data exist on virological aspects of water supplies in developing countries (Lund 1979), but the bacteriological data indicate the probability of substantial viral pollution of many sources. A study in Ghana (Addy and Otatume 1976) isolated enteroviruses from 3 out of 8 water samples taken from 3 drinking water wells near Accra. Poliovirus 1 was isolated from one well and, since vaccination rates are extremely low, it is likely that this was a wild strain of poliovirus. More data of this type are urgently required.

In seawater

Many coastal communities discharge untreated or partially treated wastes into the sea. This is not only the case in developing countries but is common practice throughout the world; for instance untreated sewage is discharged in large quantities at Honolulu and Miami Beach (USA) (Ruiter and Fujioka 1978; Edmond, Schaiberger and Gerba 1978). The potential health hazards are those of infection of bathers and marine sportsmen and the contamination of shellfish. Recent advances in laboratory techniques have permitted the concentration of small numbers of viruses from large volumes of turbid seawater (Payment and others 1976) and have encouraged a number of investigations into viral pollution of the marine environment.

Edmond, Schaiberger and Gerba (1978) studied enterovirus contamination of seawater along the Florida coast (USA), an area of exceptional importance for marine recreation. Between Palm Beach and Virginia Key there are 10 ocean outfalls discharging approximately 6×10^5 cubic meters per day of raw and treated sewage. The Miami Beach outfall discharges 1.8×10^5 cubic meters per day of untreated sewage at a depth of 44 meters. The authors found enteroviruses at the surface above the outfall at concentrations between 21 and 42 infectious units per 400 liters. Fecal coliforms at this site were in the range $0.9–1.4 \times 10^4$ per 100 milliliters, and fecal streptococci were $0.5–4.9 \times 10^3$ per 100 milliliters. At Miami the outfall discharges 2×10^5 cubic meters per day of sewage treated by activated sludge and chlorination at a depth of only 5 meters. At this site between 0 and 3 enteroviruses per 400 liters were detected, with fecal coliforms always less than 3 per 100 milliliters and fecal streptococci less than 33 per 100 milliliters. The marked difference in fecal pollution caused by discharging untreated and treated effluent was clearly demonstrated.

Loh, Fujioka and Lau (1979) reported that the city of Honolulu (Hawaii, USA) discharges 2.5×10^5 cubic meters of raw sewage per day into the Mamala Bay at a point 3.2 kilometers from Ala Moana beach and 6.5 kilometers from Waikiki beach. Up to 420 enteroviruses per liter were isolated from the sewage, and enteroviruses were isolated from the bay water at distances of up to 3.2 kilometers from the discharge site. At some sampling locations distant from the sewage outfall, enteroviruses were isolated from waters which contained negligible numbers of fecal indicator bacteria.

2. In this connection, it is unlikely that the assertion of Berg (1978*a*) that "the source of most of the viruses that infect man through the oral route is wastewater" is true. There is certainly no evidence to support it, whereas there is ample evidence of the vigorous transmission of enteroviruses, particularly vaccine-derived polioviruses, among members of the same household and especially among very young children (see the subsection below on enteroviruses in feces and night soil).

3. Further evidence of this is provided by unpublished data from Britain which shows that enterovirus levels in rivers and reservoirs (and therefore presumably in drinking water) tend to peak in winter, whereas enterovirus infections, and levels in sewage , tend to peak in late summer.

Survival of enteroviruses in seawater is generally reported to be shorter than in freshwater. However, enteroviruses survive for longer in seawater than coliform bacteria and there have been several reports of enterovirus isolations from seawater containing very few or no coliforms (Berg and Metcalf 1978). Won and Ross (1973) reported a 99.9 percent reduction of echovirus 6 in aerated seawater after about 2 days at 22°C and after about 6–7 weeks at 3–5°C. The survival in seawater was unaffected by the addition of organic substances. Akin and others (1976) reported a 99.9 percent reduction in poliovirus 1 in 5–6 days at 24°C in the Gulf of Mexico. Fujioka, Lau and Loh (1978) found a 90 percent reduction in poliovirus at 25°C in 1 day in seawater collected close to the shore, and in 2–3 days in seawater collected farther out. Further experiments revealed the strong possibility of a specific virucidal activity displayed by unidentified marine microorganisms. In another study the same authors (Loh, Fujioka and Lau 1979) reported 90 percent reduction of poliovirus 1 in 2 days, and 99.9 percent reduction in 4 days, in both sewage-contaminated and sewage-free seawater at 24°C.

Considerable attention has focussed upon the study of enteroviruses in estuaries and the associated risk of viral disease transmission via contaminated shellfish. Metcalf, Wallis and Melnick (1974) reported studies of pollution of the Houston ship canal (temperature 10–33°C; salinity 0.1–1.1 percent; fecal coliforms 6.6–500 $\times$ 10^3 per 100 milliliters) and Galveston Bay (temperature 9–30°C; salinity 1–2.4 percent; fecal coliforms <2–330 per 100 milliliters) in Texas (USA). Two activated sludge plants were discharging up to 1.7 $\times$ 10^9 enteroviruses per day into the ship canal, and the dominant virus types identified were polioviruses 1 and 2 and echovirus 7. In the ship canal, 0.8 kilometers downstream from the nearest effluent discharge site, enterovirus concentrations were 0.05–0.9 per liter, whereas 6.4 kilometers further downstream concentrations were 0.08–0.7 per liter. Enteroviruses were also isolated from the bottom sediments of the ship canal at concentrations of up to 4 per 100 grams. In Galveston Bay, 33 kilometers from the discharge site, no enteroviruses could be detected in the water, but polioviruses 1 and 2 were isolated from oysters (concentrations up to 26 per 100 grams). Further examination of 89 poliovirus isolates from the ship canal and oysters indicated that 8 percent might be wild, virulent strains. Survival tests in the laboratory indicated that, in ship canal water at 22°C, poliovirus 1, coxsackievirus B5 and echovirus 7 were eliminated within 21 days. Further studies in Galveston Bay (Gerba and others 1979) showed that the concentration of enteroviruses was weakly but significantly correlated with rainfall, total coliform counts and salinity (accounting together for approximately 16 percent of the variance in the virus data). Enteroviruses were isolated from 43 percent of recreational water samples judged acceptable by total coliform standards (<1,000 per 100 milliliters), and from 35 percent of shellfish-harvesting waters judged acceptable by total coliform standards (<230 per 100 milliliters). Similar studies in the same area are reported by Goyal, Gerba and Melnick (1978).

Studies of pollution in the Firth of Forth estuary (Scotland) revealed adenoviruses, coxsackieviruses, polioviruses and echoviruses at two sites where median fecal coliform levels were 2.1 $\times$ 10^4 per 100 milliliters and 4.2 $\times$ 10^3 per 100 milliliters (Watson 1977). Studies in an estuarine environment in the USA (salinity 0.9–2.8 percent, temperature 6–24°C) isolated enteroviruses from 6 percent of water samples and 17 percent of bottom sediment samples (Vaughn and Metcalf 1975). Survival experiments in the same study showed a 99 percent reduction in coxsackievirus B3 in about 12 days at summer temperatures (18–21°C), whereas this degree of reduction took approximately 28 days during winter and spring (4–15°C).

Colwell and Kaper (1978) reported viral stability for 322 days at 4°C, whereas at 25°C viruses were rarely detected after 56 days. These results were unaffected by salinity changes in the range 1 to 3.4 percent. Roberts, Haggerty and Johnson (1976) found that poliovirus 2 suffered an 84–88 percent reduction after 20 days at 17°C when held in seawater, estuary water, river water or lake water. Hurst and Gerba (1980) recorded a 99.9 percent reduction of poliovirus 1, echovirus 7, coxsackievirus B3 and simian rotavirus in estuarine water (temperature 20°C, salinity 1.2–2.8 percent) in 2–3 days. The rate of inactivation was unrelated to salt concentration. The same virus types in clean and polluted freshwater at the same temperature were reduced by 99.9 percent in 3 to >14 days. The unimportance of salinity in determining virus survival has been shown also by Berry and Noton (1976), Matossian and Garabedian (1967) and Metcalf, Wallis and Melnick (1974).

Of particular relevance to recreational hazards are the findings of Baylor and others (1977), which show that viruses can be transferred from surf to the air and blown onto the beach. This is caused by viruses adsorbing to air bubbles as they rise through the water. When they burst at the surface, tiny droplets rich in viruses are ejected into the air and are carried on the wind. The concentration of viruses in these droplets may be at least 100 times greater than in the seawater

from which they came. This presents a potential health risk similar to that postulated for aerosol droplets produced by activated sludge plants, spray irrigation systems and flush toilets (see the section below on the occurrence and survival of enteroviruses in air).

A number of other studies (for instance, Gerba and others 1977; Hetrick 1978; Metcalf and Stiles 1968; Pietri and Breittmayer 1976; Shuval 1978; Vaughn and others 1979*a*) have investigated enteroviruses in marine and estuarine environments and have reached broadly similar conclusions. Survival in seawater is generally shorter than in freshwater, and a specific virucidal property of seawater (possibly of microbiological origin) has been postulated. The evidence for this has been reviewed by Kapuscinski and Mitchell (1980) and Katzenelson (1978). Sunlight may have some slight virucidal action on viruses suspended near the surface. Viruses are found associated with bottom sediments in a greater proportion of samples, and at higher concentrations, than in the overlying waters, and their survival is prolonged in this state (Gerba and others 1977; LaBelle and Gerba 1979; Smith, Gerba and Melnick 1978; Vaughn and Metcalf 1975). Sediment may serve as a reservoir of enteroviruses that may be resuspended into the overlying water by wind, currents, dredging or boats.

Temperature is the most determining factor in viral survival, with greatly increased inactivation rates in warmer waters (Berry and Noton 1976; Colwell and Kaper 1978). Few or no data are available on marine pollution near major tropical coastal towns (Lund 1979). However, these towns typically discharge considerable volumes of untreated or partially treated wastes into the sea, and so substantial viral pollution is to be expected. Survival times are probably shorter than those reported from temperate areas.

In feces and night soil

The source of enteroviruses in the environment is the feces of infected individuals, which may contain 10^6 or more viruses per gram. There is extensive information on the proportion of people, especially children, excreting enteroviruses at a given time. Little is known directly about the occurrence of viruses in night soil, although typical concentrations may be inferred from data on enteroviruses in feces (given here) and in sewage (given below). Enterovirus survival in feces and night soil may be estimated from data on survival in sludge (see below and the appendixes to Feachem and others 1980).

Under conditions of poverty and poor hygiene the incidence of enteroviral infections, and the prevalence of enterovirus excretion, are high. Otatume and Addy (1975) isolated enteroviruses from 44 percent of 386 fecal specimens collected from 45 healthy infants in Accra (Ghana). Virus isolation rates were not affected by seasons, were higher in urban (44 percent) than in rural (21 percent) areas, and were higher among infants from houses without flush toilets (50 percent) than among infants from houses with flush toilets (30 percent). Out of 138 typed virus isolates, 12 percent were poliovirus (presumed to be wild), 4 percent were coxsackieviruses, and the remainder were echoviruses. In Ibadan (Nigeria) three separate studies showed 39 percent of infants excreting enteroviruses or adenoviruses (Montefiore and others 1963), 49 percent of children under 3 years excreting enteroviruses other than polioviruses (Poliomyelitis Commission 1966), and 45 percent of children between 3 and 24 months excreting enteroviruses (Peradze, Montefiore and Coker 1968). In Yaounde (Cameroon), 39 percent of 524 children age 0–6 years were excreting enteroviruses, and 11 percent were excreting polioviruses (Boche, Millan and Le Noc 1973). Sabin and others (1960) found that 11 percent of children 1–5 years old in Toluca (Mexico) were excreting wild poliovirus, while 51 percent were excreting other viruses. Rao, Lakhe and Waghmare (1978) reported that 45 percent of children between 1 and 15 years old in Nagpur (India) were excreting enteroviruses. These data on the prevalence of enterovirus excretion among children in Africa, Asia, and Central America show a quite remarkable consistency.

Another gauge of the very high incidence of enterovirus infections among young children in developing countries is data on the proportion of unvaccinated children having antibodies to poliovirus. For instance, John and Jayabal (1972) found that 79 percent of 191 unvaccinated infants and children under 5 years old in Vellore (India) had antibodies to one or more types of poliovirus, thus indicating a history of infection. Sabin and others (1960) found that 100 percent of children in Toluca (Mexico) developed antibodies to one or more polioviruses before the age of 4 years, and that over 90 percent of 4 year olds had antibodies to all three polioviruses. Montefiore and others (1963) reported that 100 percent of children over 3 years in Ibadan (Nigeria) had immunity to poliovirus 1. In a subsequent study in Ibadan (Poliomyelitis Commission 1966), the prevalence of immunity to polioviruses 1, 2, and 3 in children between 24 and 36 months old was 68, 48, and 68 percent respectively. Studies in Kabul (Afghanistan) showed that over 90 percent of unvaccinated children had acquired immunity to polioviruses by the age of 5 and that peak

transmission occurred in the early summer (Šerý and others 1970; Thraenhart and others 1970).

By contrast, enterovirus isolation rates from healthly individuals in industrialized countries are much lower. Froeschle, Feorino and Gelfand (1966) isolated enteroviruses (excluding polioviruses, which they assumed to be vaccine derived) from 4.9 percent of healthy 1–5 year old children in six cities in the USA. Isolation rates peaked in late summer and early fall with a maximum of 12.4 percent positive in September. Infection rates were higher in males than in females and highest in 1 year olds, with rates decreasing with increasing age. Cooney, Hall and Fox (1972) studied over 14,000 fecal specimens in Seattle (USA) and found polioviruses (presumed to be vaccine derived) in 8.8 percent, adenoviruses in 2.2 percent, coxsackieviruses in 0.7 percent, and echoviruses in 0.6 percent. From 18,000 respiratory specimens, polioviruses were isolated in 0.8 percent, adenoviruses in 0.9 percent, coxsackieviruses in 0.1 percent, and echoviruses in 0.5 percent. Isolation rates were inversely related to age.

In summary, it is clear that enteroviruses spread vigorously by the fecal-oral route (and possibly also by the oral-oral route) in conditions of poverty and low personal and domestic hygiene. Thus the prevalence of enterovirus excretion is high (40–50 percent among infants and young children), as is the prevalence of antibodies to enteroviruses, which indicate past or current infection. Under conditions of relative affluence and optimal hygiene (as in the USA) wild enteroviruses, and vaccine-derived polioviruses, continue to circulate in the community and among members of the same family. However, the prevalence of enterovirus excretion is very much lower (around 5 percent in young children) than in the developing countries. In all countries studied, the prevalence of virus excretion is inversely related to age, so that the highest virus excretion rates are found among infants. As hygiene improves in the absence of vaccination programs, the age distribution of infections tends to shift upwards, and the amount of clinically serious disease may increase (see, for instance, Anon. 1971, Hillis 1979, Metselaar 1968).

In sewage

Since enteroviruses are not normally excreted for prolonged periods by healthy individuals, their occurrence in sewage is subject to wide fluctuations. However, nearly all sewages contain enteroviruses, and the larger the contributing population the less variable is the concentration of viruses. Communities with poor hygiene and a high proportion of children will produce a sewage especially rich in enteroviruses, and reported concentrations of enteroviruses will continue to rise as laboratory techniques improve and as more studies are carried out in developing countries. Sewage in developing countries must be assumed to contain at least 10^5 enteroviruses per liter, and sewage effluents produced by conventional treatment plants will also contain high concentrations of enteroviruses (see the section below on enterovirus inactivation by sewage treatment processes).

In an early study of this subject, Bloom and others (1959) investigated the enteroviruses in the sewage of Lansing and East Lansing (Michigan, USA) between 1955 and 1957. East Lansing sewage yielded enteroviruses in 14 percent of samples, compared with 7 percent in Lansing, which had large volumes of industrial wastes in its sewage. Peak isolations occurred during July through November. Thirty-three percent of samples of influent at the East Lansing activated sludge plant were positive for enteroviruses, while only 10 percent of samples of the final effluent were positive.

Haifa (Israel) sewage between 1972 and 1974 contained a monthly average of between 6×10^3 and 4.9×10^5 viruses per liter. The highest value recorded was 1×10^6 viruses per liter (Buras 1976). Fattal and Nishmi (1977) reported a predominance of polioviruses amongst enteroviruses isolated from the sewage of six Israeli towns and found that 13 percent of isolated polioviruses were wild strains rather than attenuated vaccine strains. By contrast, Katzenelson and Kedmi (1979) reported detecting poliovirus in only about 50 percent of 25 samples of raw sewage and sewage effluents in Jerusalem, Tel Aviv and elsewhere in Israel. The proportion of polioviruses to all enteroviruses was low.

In Seattle (USA) Heyward and others (1979) isolated up to 1.3×10^3 viruses per liter from combined sewer-stormwater overflows. In Ottawa (Canada) Sattar and Westwood (1977) detected pathogenic viruses in 79 percent of sewage samples. Of 72 isolates identified, 56 (78 percent) were reoviruses; the remaining 16 were enteroviruses. Of the 16 enteroviruses, 1 was coxsackie, 10 were vaccine strains of poliovirus, and 5 were wild polioviruses. The authors point out that the presence of these wild strains in sewage, at a time when immunity against poliovirus is declining due to a fall-off in vaccination, is a cause for concern. Fujioka and Loh (1978) isolated enteroviruses from 100 percent of raw sewage samples investigated in Hawaii (USA) at concentrations between 27 and 1.9×10^4 per liter. In the same study, 26 poliovirus isolates were assayed for virulence and 3

(all from chlorinated effluent) were found to be wild.

Rao, Lakhe and Waghmare (1978) reported virus concentrations of up to 11,500 per liter in Indian raw sewage. There was a pronounced diurnal and seasonal variation in virus load; maximum concentrations occurred between 8 and 10 a.m. and during the rainy season. Nearly 80 percent of viruses isolated were polioviruses and 60–80 percent of these were wild strains. The authors noted that about 60 percent of recorded cases of paralytic poliomyelitis cases in India are reported during the rainy season.

Virus survival in sewage has been investigated in several studies (see the appendixes of Feachem and others 1980). The results generally indicate longer survivals than in river water, with survival times of over 231 days at cool temperatures (<10°C) and up to 110 days at warmer temperatures (20°C). Lefler and Kott (1975) showed 99.9 percent reduction of poliovirus in sewage, after 42 days at 18–25°C, and after 231 days at 4–8°C. Kott, Ben-Ari and Vinokur (1978) reported the complete disappearance of enteroviruses in lagooned trickling filter effluent within 73 days, and the disappearance of poliovirus in oxidation pond effluent at 18–23°C within 110 days (99.9 percent reduction in about 70 days). Rao and others (1977) found up to 1,250 enteroviruses per liter in Bombay sewage and report that, when stored at 8°C for 2 days, a 22 to 40 percent loss occurred.

Prolonged survival of enteroviruses in sewage may be due in part to the protective effects of adsorption to solids. Wellings, Lewis and Mountain (1976) found that between 16 and 100 percent of viruses in raw sewage and sewage effluent, at two treatment plants in Florida (USA), were solids associated. Gerba, Stagg and Abadie (1978) reported that 3 to 49 percent of viruses were solids associated in treatment plant effluents near Houston (Texas, USA).

In a unique study Ruiter and Fujioka (1978) investigated the sewage produced by two communities in Honolulu (Hawaii, USA). Kuhio Park Terrace had a total population of 2,745, of whom 46 percent were children under 14 years. The density of settlement was 376 persons per hectare, 73 percent of households had an income of under US$5,000 per year, and 23 percent of heads of household were unskilled laborers. Nuuanu had a total population of 2,302, of whom 10 percent were children under 10 years. The density of settlement was only 19 persons per hectare, 4 percent of households had an income of under US$5,000 per year, and 4 percent of household heads were unskilled laborers. Kuhio Park Terrace produced a sewage with an average of 345 enteroviruses per liter (maximum 820 per liter), while Nuuanu produced an average 93 enteroviruses per liter (maximum 218 per liter). This difference was attributed to the differences in socioeconomic status and age structure between the two communities. Enteroviruses in sewage reflect the levels of infection and vaccination within the contributing population and may be used as an aid to epidemiological surveillance (see, for instance, Zdražílek, Šrámová and Hoffmanová 1977).

Little information is available on the concentration of enteroviruses in tropical sewage. It is to be expected that poor communities, living in conditions of inadequate hygiene, will produce a sewage with 10^7 or more infectious virus units per liter (Lund 1979), although, of course, most such communities produce no sewage at all because they are not connected to a sewerage system. However, even fairly affluent communities in developing countries will almost certainly produce a sewage with a greater concentration of pathogenic viruses than in Europe and North America, because the incidence of viral infections is higher, water use is lower, and a greater proportion of the population is under 15 years old (see the previous section on viruses in feces).

In sludge

The sludges of sewage works are rich in enteroviruses because a high proportion of viruses in sewage are, or become, solids associated and are therefore concentrated into both primary and secondary sludges (Lund 1973, 1976; Lund and Ronne 1973; Wellings, Lewis and Mountain 1976). It is probable that most of the difference between the virus concentration of the influent and the effluent of a sewage treatment plant is accounted for by the viruses in the sludge. The few studies on virus survival in sludge are listed in the appendixes to Feachem and others (1980).

Subrahmanyan (1977) studied the survival of several types of enteroviruses added to sewage sludge at a concentration of 10^7 per liter and kept at 22°C. Survival times ranged from a minimum of 2 weeks (coxsackievirus A9) to a maximum of over 12 weeks (coxsackievirus B5 and echovirus 9). Polioviruses survived from 8 to 12 weeks. Damgaard-Larsen and others (1977) inoculated digested and dewatered sludge with coxsackievirus B3 at a concentration of 10^6 per gram. The sludge was applied outdoors in Denmark to sandy and clay soils during December to May, when rainfall totalled 300 millimeters and temperatures ranged from -12 to 26°C. Virus inactivation took place at a rate of about 1 log unit per month, and it took 23 weeks before viruses could not be

detected. Viruses remained bound to the sludge and did not travel downward through the soil. Nielsen and Lydholm (1980) reported the survival of naturally occurring coxsackievirus B5 for 4 months (March–July) in digested sludge applied to land in Denmark.

Sattar and Westwood (1979) studied the viruses present in raw sludge (5 percent solids), anaerobically digested sludge (20 days at 35°C), and lagoon-dried sludge (minimum of 6 months drying time) from the largest sewage treatment plant in Ottawa (Canada). Excreted viruses were isolated from 84 percent of raw sludge samples, 53 percent of digested sludge samples, and 39 percent of dried sludge samples. Viruses were isolated from sludge that had been drying for over 8 months. Most virus isolates were reoviruses, the remainder being enteroviruses.

Hurst and others (1978) isolated viruses from various activated sludge samples at a sewage treatment plant at Houston (Texas, USA) in concentrations of around 30 viruses per liter of sludge. After sludge thickening, aerobic digestion and centrifugation the concentration of viruses rose to 231 per liter. This sludge was then applied to land, where the virus concentration was monitored over two separate periods. During the first 7 day period (during September with no rain), the solids content of the sludge rose from 6.9 to 18.4 percent, and 97 percent of the viruses were inactivated. During the second 7 day period (during September with rain on day 6), the solids content of the sludge stayed almost constant at 13–14 percent, and 99.5 percent of the viruses were inactivated. A sample of sludge which had been on the field for 3 months had a solids content of 59 percent and no demonstrable virus. Thus in a Texas summer a 2 log reduction per week was recorded, compared with a unit log reduction per month during a Danish winter (Damgaard-Larsen and others 1977).

In soil

Increased interest in the health aspects of the agricultural use of sewage and sludge has generated several studies on virus survival in soil. However, little information is yet available. More is known about virus travel and adsorption in soils, and this subject is reviewed below in the section dealing with land treatment.

A review by Gerba, Wallis and Melnick (1975*a*), and the studies listed in the appendixes to Feachem and others (1980), indicate that survival times of over 175 days are possible. Hurst, Gerba and Lance (1979) found that enterovirus survival in soil was prolonged by low temperatures but was unaffected by whether the soil was wetted with distilled water or with various concentrations of sewage effluent. Virus inactivation occurred much more rapidly under nonsterile aerobic conditions than under sterile aerobic conditions or under sterile and nonsterile anaerobic conditions. Duboise, Moore and Sagik (1976) found that polioviruses held for 84 days in loamy sand were reduced by less than 90 percent at 4°C but by 99.999 percent at 20°C. In studies into the disposal of septic tank effluent it was found that poliovirus 1 adsorbed to sandy soils was reduced by 97.5 percent after 28 days at 20°C and by less than 50 percent after 56 days at 7°C (Small Scale Waste Management Project 1978).

Lefler and Kott (1974*a*) studied the survival of poliovirus 1 in sand kept in the dark at room temperature (18–22°C). With the sand saturated in distilled water, no poliovirus was detected after 112 days, and 99 percent inactivation was achieved in about 63 days. With tap water or oxidation pond effluent, complete inactivation took 105 days, while 99 percent reduction took 42 days. Coliphage f2 survived for longer than poliovirus 1. When the saturated sand was kept at 4–8°C, 20 percent of poliovirus was still active after 175 days. On dried sand at 4–8°C, 96 percent inactivation occurred in 21 days and virus was still detectable (at 0.02 percent of the original concentration) after 77 days.

Tierney, Sullivan and Larkin (1977) inoculated poliovirus 1 into samples of activated sludge and secondary effluent to produce a viral concentration of 2.5×10^8 viruses per liter. The fluids were sprayed over soil plots so as to flood them to a depth of 25 millimeters. Runoff water from the plots contained 10^6 viruses per liter on the day of flooding but this fell to 0 by day 6. In winter (-14 to 27°C) viruses applied in effluent survived in soil for between 89 and 96 days, whereas viruses applied in sludge survived between 96 and 123 days. In early summer (19°C to 34°C), viruses were not detected for more than 11 days after flooding with either effluent or sludge.

Yeager and O'Brien (1979*a*) elucidated several aspects of virus survival in soil. They found that poliovirus survival was heavily temperature dependent, with virus survival in saturated soil being up to 12 days at 37°C, up to 92 days at 22°C, and up to 180 days at 4°C. Viruses survived longer in soils saturated with septic tank liquor (90 percent reduction in 8–21 days at 22°C) than in soils saturated with river or groundwater (90 percent reduction in 5–7 days at 22°C). Viruses survived for longer in sandy loam (90 percent reduction in 6–21 days at 22°C) than in sand (90 percent reduction in 4–8 days at 22°C). Soil drying

was found to be highly virucidal, irrespective of soil type, and speed of soil drying depended on temperature. Soil moistures of below 2.9 percent appeared to be especially virucidal. In an accompanying study (Yeager and O'Brien 1979*b*) the same authors investigated the nature of virus inactivation in soil. They concluded that loss of infectivity is due to irreversible damage to the virus particles. They speculate that two general mechanisms may underly the inactivation of picornaviruses in the environment: under temperate, saturated conditions RNA degradation may occur, whereas in dried soil (and also perhaps in aerosols and under a variety of hostile circumstances such as heat, irradiation and desiccation) the virions may dissociate into intact RNA and isoelectrically altered capsids (see also O'Brien and Dacus 1978).

Virus survival in soil depends upon many factors including the type of soil, its pH, the sterility of the soil, and the type of liquid in which the viruses are applied. However, temperature and moisture appear to be the dominant factors. While very long virus survival times (over 5 months) are possible at cool temperatures ($<10°C$), at warmer temperatures ($>25°C$) viruses are likely to be eliminated within 2 weeks. Soil drying is also highly virucidal, and the evidence suggests that, in warm climates, intermittent agricultural application of sewage, night soil or sludge, with drying periods of 3–5 days between applications, would result in little or no build-up of viable pathogenic viruses in the soil. This contention is further supported by the studies of Sadovski and others (1978)—reported in the next subsection, concerning viruses on crops.

On crops

The interest in virus survival in soil has been accompanied by interest in virus occurrence and survival on vegetables fertilized or irrigated with sludge or sewage.

Konowalchuk and Speirs (1975*a*) studied virus survival on vegetables stored at 4°C. In a humid atmosphere, coxsackievirus, poliovirus, echovirus, reovirus, and adenovirus inoculated in a water droplet onto lettuce, celery, green peppers, tomatoes, radish, and carrots were undetectable after 4–5 days. When inoculated in dilute feces, 4–5 percent were detectable after 5 days; when inoculated in feces, 7–12 percent were detectable after the same time. Additional studies on virus survival in vegetable infusions indicated that the vegetables contained no antiviral agents. In a later study Konowalchuk and Speirs (1975*b*) investigated the survival of various viruses on strawberries, cherries, and peaches at 4°C and investigated the effect of inoculating the viruses in water or in dilute feces and of storing the fruit in humid or dry atmospheres. Viruses were inactivated far more rapidly under dry than under humid conditions, and virus survival was prolonged by inoculation in feces rather than in water. Viruses survived longer on cherries and peaches than on strawberries, and coxsackieviruses and echoviruses survived for longer than polioviruses and reoviruses. Under dry conditions survival of viruses was between 40 and <1 percent after 1–2 days, and viruses were undetectable after 4–6 days. The short survival times are notable in view of the cool temperature (4°C), and the authors consider that an antiviral substance produced by the fruit is active. The authors also consider that the longer survival of viruses inoculated in dilute feces is due to delayed desiccation as compared with viruses inoculated in water. Subsequently, the same authors (Konowalchuk and Speirs 1977) reported that the concentration of poliovirus 1 and coxsackievirus B5 was reduced by about 99 percent after 5 days on grape bunches hung indoors at 22°C.

Kott and Fishelson (1974) investigated the effect of effluent chlorination and sunlight on the survival of seeded poliovirus 1 on tomatoes and parsley. The maximum recoveries of polioviruses from vegetables 6 hours after application in waste stabilization pond effluent were: 2.2 percent when applied in unchlorinated effluent with exposure to sunlight, 1.6 percent when applied in chlorinated effluent with exposure to sunlight, 12.7 percent when applied in unchlorinated effluent and kept in darkness, and 8.5 percent when applied in chlorinated effluent and kept in darkness. Poliovirus applied outdoors did not survive for more than 1 day on tomatoes or 2 days on parsley at 15–31°C. Poliovirus survival was considerably prolonged when viruses were applied in phosphate-buffered saline rather than effluent.

Larkin, Tierney and Sullivan (1976) planted lettuces and radishes and, 8–10 days later, sprayed them with secondary effluent or sludge seeded with 2.5×10^8 polioviruses per liter. These experiments were conducted in Ohio (USA) in the summer of 1973 and 1974 when there was extensive direct sunlight, periodic rain, air temperatures of 19–34°C, and soil surface temperatures rising to 45°C. On the days immediately after spraying, large amounts of virus (up to 2.9×10^4 per 100 grams) could be recovered from the vegetables. Two weeks after spraying no more than 100 viruses per 100 grams could be detected, and small numbers (10 per 100 grams) persisted for at least 36 days. In similar

studies, Tierney, Sullivan and Larkin (1977) were still able to isolate 60 polioviruses per 100 grams of lettuce 23 days after spraying. (Lettuces and radishes in this climate are normally harvested 3 to 4 weeks after planting.)

Sadovski and others (1978) studied the survival of polioviruses inoculated into waste stabilization pond effluents and applied by drip irrigation to cucumber plots on two farms in Israel. At one site (air temperature 13–30°C, soil temperature at noon 22–30°C, sunlight 9.5 hours per day, relative humidity 27–55 percent), a single irrigation was performed with inoculated effluent containing 9×10^7 polioviruses per liter. Viruses were still detectable in the irrigation system, at a concentration of $>10^4$ per liter, 8 days after the flow of inoculated effluent. The soil contamination immediately after irrigation with inoculated effluent was 10^4 viruses per 100 grams of soil (dry weight) and persisted at a level of $>10^3$ per 100 grams for at least 8 days. Cucumbers grown in exposed soil were contaminated by 2.2×10^3 viruses per 100 grams immediately following irrigation, and this contamination fell to 30 per 100 grams on day 8. However, when the soil and drip lines were covered with plastic sheets no viruses could be isolated from the cucumbers after a few hours had elapsed after inoculated irrigation. At the second site (air temperature 23–28°C, soil temperature at noon 40–43°C, sunlight 11.8 hours per day, relative humidity 62–70 percent), three irrigations were performed with inoculated effluent containing 2.2×10^{12} polioviruses per liter. After the third inoculated irrigation the soil contained 9.1×10^3 viruses per 100 grams (dry weight), and this contamination fell to 47 per 100 grams after 10 days and to 0 after 15 days. Unlike at the first site, where irrigation with uninoculated effluent had continued throughout the study, in this case irrigation terminated 5 days after the third inoculated irrigation, and consequently the soil moisture content fell from 15 to 3 percent. Virus contamination of the cucumbers grown in exposed soil rose to 0.13 per 100 grams, but was undetectable 6 days after the last inoculated irrigation. In covered soil no viral contamination of the vegetables could be detected. At both sites, virus survival in the soil was unaffected by whether plastic sheets were lain over the soil and the drip lines. Earlier studies at the first site by the same workers (Sadovski, Fattal and Goldberg 1978) showed that no viruses could be isolated from cucumber or eggplants drip-irrigated with uninoculated waste stabilization pond effluent containing 10^3 enteroviruses per liter.

These studies, and others listed in the appendixes of Feachem and others (1980)[4], indicate highly variable survival times for enteroviruses on vegetables and fruit. Type of virus and type of crop are clearly important, but the dominant factors are temperature, sunlight and humidity (which will control the degree of warming), and radiation and desiccation experienced by the viruses. Survival times of up to 2 months are possible, but at day temperatures above 25°C (and especially in dry climates) one may anticipate negligible survival of enteroviruses on crops for more than 2 weeks. Indeed, it may be that almost complete elimination will occur in under 5 days and that the longer survival times reported by some investigators are only achieved by the untypically high concentration of seeded enterovirus in the applied effluent [for instance Larkin, Tierney and Sullivan (1976) employed 2.5×10^8 polioviruses per liter, and Sadovski and others (1978) had 2.2×10^{12} per liter]. It has been clearly demonstrated by Israeli workers that drip irrigation, particularly when combined with soil covered with plastic sheets, is a method of effluent application that minimizes the risks of crop contamination by enteroviruses.

In fish and shellfish

The primary hazard associated with estuarine and marine discharge of fecal wastes may not be risks to bathers and water sportsmen but to those who eat the fish and shellfish that are harvested in polluted waters. The greatest risks of viral infection are associated with the ingestion of contaminated molluscs (such as oysters, mussels, cockles, and clams) and crustacea (such as crabs, lobsters, shrimps, and prawns) in a raw or partially cooked state. Most attention has focussed upon oysters because they are commonly eaten raw and their method of filter feeding (common to all bivalve molluscs) concentrates pathogenic organisms from the water into their tissues (Anon. 1976; Gerba and Goyal 1978; Hughes, Merson and Gangarosa 1977; Metcalf 1978; Wood 1979). Attention has also been paid to improved laboratory techniques for isolating enteroviruses from shellfish (Gerba and Goyal 1978; Metcalf 1978; Vaughn and others 1979*b*).

Mitchell and others (1966) placed 600 eastern oysters (*Crassostrea virginica*) in seawater at 20°C containing 10^6 polioviruses per liter and found that viruses rapidly accumulated in the oyster tissue such that after 1 hour the virus concentration in the oyster was 27 times higher than in the surrounding water.

4. See also Engley (1956) and Berg (1978*b*) for reviews of literature on virus survival in food (as opposed to on crops).

When the seawater contained 3×10^5 polioviruses per liter, viruses accumulated less rapidly but were always concentrated in the oyster by at least 10-fold after 3 hours. When the oysters were rinsed and placed in sterilized seawater, over 95 percent and 99.9 percent of viruses were eliminated after 8 and 24 hours respectively. Viruses were sometimes undetectable after 48 hours and sometimes detectable in very small numbers (2 per gram) for up to 96 hours.

Hoff and Becker (1969) studied the accumulation of poliovirus by the Olympia oyster, the Pacific oyster, and the Manila clam and found that these species concentrated the virus to a level between 10 and 180 times higher than in the surrounding waters. When the contaminated shellfish were held in disinfected seawater (6–16°C), poliovirus concentrations in the meat were reduced by at least 99.9 percent after 96 hours. Hedstrom and Lycke (1964) found that poliovirus survived for 3.5 days in seawater but for well over 6 days in oysters in contaminated seawater. Oysters did not cleanse themselves of poliovirus within 22 hours when transferred to uninfected water or to water containing up to 1.7 milligrams per liter of free chlorine.

DiGirolamo, Liston and Matches (1975) placed oysters in seawater (salinity 2.8 percent, temperature 13°C) containing 1.9×10^7 polioviruses per liter and found that after 2 days the oysters had accumulated about 10^4 polioviruses per gram of meat. Most viruses were concentrated in the digestive organs and feces. When placed in stationary sterilized seawater (salinity 2.8 percent, temperature 13°C), contaminated oysters lost between 79 and 84 percent of polioviruses in 5 days. When placed in flowing sterile seawater, oysters lost over 99 percent of accumulated poliovirus after 3 days. Gerba and Goyal (1978) reviewed 17 other studies on the accumulation and depuration of excreted viruses by shellfish.

The isolation of enteroviruses from oysters living in lightly contaminated waters has been frequently reported. Metcalf and Stiles (1965) isolated coxsackie B4 and echo 9 viruses from oysters in estuary waters at distances of up to 4 miles from the nearest sewage outlet. Vaughn and Metcalf (1975) found that 7.6 percent of oyster samples contained enteroviruses in waters from which only 5.6 percent of samples were positive for viruses. Vaughn and others (1979*a*) isolated up to 30 viruses per 100 grams of flesh from clams in Great South Bay (New York, USA), the waters from which contained no more than 2 viruses per liter.

Goyal, Gerba and Melnick (1979) studied the presence of enteroviruses in oysters and oyster-harvesting waters in Galveston Bay (Texas, USA). Of a total of 44 water samples, 26 yielded viruses in concentrations of up to 0.4 per liter, whereas of 40 pools of 10 to 12 oysters each, viruses were isolated from 14 pools at concentrations of up to 224 per 100 grams. On five occasions viruses were found in oysters but not in the overlying waters. Gerba and Goyal (1978) reviewed 17 reported isolations of viruses from shellfish.

It is generally agreed that no human enterovirus multiplication takes place in shellfish (Chang and others 1971) and that the dangers lie in the uptake, concentration, and survival of viruses in shellfish tissue. Uptake, depuration, and survival in oysters has been found to be temperature dependent. Below a given temperature, a particular species of shellfish will cease to filter. The European flat oyster (*Ostrea edulis*) appears to filter at temperatures down to 5°C, whereas the eastern oyster (*Crassostrea virginica*) will not filter below 7°C. The edible mussel (*Mytilus edulis*) filters at temperatures down to 2°C, but the hard clam (*Mercenaria mercenaria*) ceases active filtration at about 12°C (Metcalf and Stiles 1968; Wood 1979). As the temperature falls, the rate of filter feeding declines and so does the rate of accumulation of viruses. Below the critical temperatures mentioned above, virus accumulation should cease. The same is true of depuration, and so a contaminated shellfish will cleanse itself more slowly as temperature falls and will cease to cleanse itself at all below a critical temperature.

Survival of viruses in stored shellfish is very much prolonged by low temperatures. Vaughn and Metcalf (1975) reported that coxsackievirus B3 in oysters survived for up to 42 days at 1–11°C, but for only 22 days at 14–21°C. Metcalf and Stiles (1965) found that viruses in oysters stored at 5°C remained relatively stable for at least 28 days. DiGirolamo, Liston and Matches (1970) found that 10 percent of polioviruses survived for 84 days in oysters after freezing at −36°C and storage at −17.5°C. DiGirolamo and Daley (1973) froze crabs at −20°C and found that 17–35 percent of seeded coliphage T4 survived after 30 days. Other studies of viruses in refrigerated shellfish have shown that survival times of up to 120 days are possible (Gerba and Goyal 1978).

These and many other studies (reviewed in Gerba and Goyal 1978) show that viruses in water are readily accumulated by shellfish. In edible bivalve molluscs (clams, mussels, oysters) the viruses are concentrated mainly in the digestive system and may be present in concentrations over 100-fold higher than in the surrounding waters. This is because these shellfish are filter feeders, and one oyster may filter as much as 1,500

liters of seawater per day in its quest for food. Crustacea that inhabit polluted waters or that feed on contaminated molluscs may also accumulate enteroviruses, although less work has been done on this. DiGirolamo and others (1972*a*) showed that crabs kept in contaminated seawater for 2 days at 10°C, or allowed to feed on contaminated mussels for 12 hours, accumulated over 10^3 polioviruses per gram of meat.

Depuration or cleansing of viruses from shellfish is a mechanical process induced by the filter feeding of the mollusc in clean water. Maximum depuration occurs when feeding activity is greatest. Thus, cleansing is more rapid at warmer temperatures, at optimal salinities, and in flowing water. Depuration of commercially harvested oysters by placing them in clean water is practiced. Chlorination of the water to maintain its purity has been advocated, but this is antagonistic to the oyster, inhibits feeding, and thus delays depuration. Preliminary experiments have been conducted on virus removal from contaminated seawater prior to aquaculture by adsorbing viruses to magnetite and removing them in a magnetic field (Bitton and others 1977).

When contaminated shellfish reach the market, the risks are obviously greatest if they are eaten raw. However, a residual risk remains even with cooked shellfish. Studies have shown that a proportion of polioviruses in oysters survived stewing (after 8 minutes, 10 percent survived), frying (after 10 minutes, 13 percent survived), baking (20 minutes, 13 percent survived), steaming (30 minutes, 7 percent survived), and irradiation (4 kilogray,[5] 7–13 percent survived) and that up to 20 percent of coliphage T4 in crabs survived boiling (DiGirolamo, Liston and Matches 1970, 1972; DiGirolamo and others 1972*b*).

Nearly all the documented disease outbreaks associated with excreted virus contamination of shellfish are outbreaks of hepatitis A (see chapter 10) or viral gastroenteritis (see chapter 11). These outbreaks have been reviewed by Gerba and Goyal (1978) and Levin (1978). However, as reported above, most studies on viruses in shellfish have focussed on the enteroviruses because it is for these viruses that well-developed laboratory isolation methods exist. Before the development of isolation techniques for hepatitis A virus and rotavirus, it must be assumed that these viruses behave in shellfish similarly to the enteroviruses.

5. The SI unit of radiation dose is the Gray (Gy), which is equal to 100 rads and is the equivalent of 1 joule per kilogram.

In the air

Airborne droplets of water and wastewater may contain enteroviruses, and these viruses may cause infection when inhaled. Droplets containing viruses may be formed by the flushing of a toilet, by spray irrigation, or by any occasion in which bubbles are rising through contaminated waters and bursting at the surface (such as activated sludge plants, waves and surf, or the passage of boats). As a bubble rises through water, viruses become adsorbed to its surface. The bubble bursting at the surface ejects a tiny jet of water that breaks into many droplets, and these droplets contain most of the viruses that were adsorbed to the bubble. Thus the droplets contain a much higher concentration of viruses than the water from which they came.

Baylor, Peters and Baylor (1977) bubbled air through a column of liquid containing coliphages T2 and T4 and produced droplets that contained a concentration of phage 50 times that in the column. Baylor and others (1977) seeded the breaking surf with coliphages T2 and T4 at beaches near New York. Droplets were formed in the surf that contained a concentration of phages 100 to 250 times higher than the seawater, and these droplets were carried by the wind for at least 30 meters.

The aerosolized excreted viruses most encountered by people in developed countries are those produced by the flush toilets in their houses. Gerba, Wallis and Melnick (1975*b*) seeded 10^8 polioviruses into a toilet bowl and found that flushing ejected at least 2.8×10^3 infectious units to the level of the seat. Further experiments with seeded coliphage MS2 showed that these organisms remain airborne long enough to settle out in large numbers on surfaces throughout the bathroom, and presumably also to be inhaled by people in the bathroom. In an unventilated bathroom, 94 percent of recovered coliphage had settled out within 2 hours of the flush, and most of the remainder had settled within 4 hours. Small numbers of viruses could apparently remain airborne for much longer.

Fannin and others (1977) studied airborne viruses produced by a trickling filter plant and an activated sludge plant in Michigan (USA). The naturally occurring level of animal viruses in sewage at the trickling filter and activated sludge units was 100 per liter, while the coliphage concentration was 5×10^5 per liter. No animal viruses were recovered from air samples at the plants, but coliphage was recovered at concentrations up to 0.5 per cubic meter of air. Airborne coliphage recovery was correlated with relative humidity (higher humidity associated with

higher recovery) but was not correlated with wind speed or ambient temperature (see also Cochran and Fannin 1976 and Fannin and others 1976). Earlier laboratory studies by de Jong and Winkler (1968) had shown that the inactivation of poliovirus 1 during spraying was greatest at low humidities.

Sorber, Schaub and Bausum (1974) developed a theoretical model of the transmission of viruses in aerosols produced by spray irrigation with effluents containing various enterovirus concentrations. The model indicates that a healthy young male working at the wetted perimeter when strong effluent (6,000 viruses per liter) is being sprayed may inhale as many as 240 viruses in 10 minutes; whereas if he is working 200 meters from the wetted perimeter when weak effluent (10 viruses per liter) is being sprayed, he may inhale only 0.0006 viruses in 10 minutes. These findings depend upon assumptions made about climatic conditions. The authors conclude that spray irrigation with chlorinated effluents from conventional treatment plants poses considerable risk of virus inhalation and that better virus removal systems need to be applied prior to spray irrigation.

Moore, Sagik and Sorber (1979) were able to isolate small numbers of coliphages (up to 1.5 per cubic meter of air) and enteroviruses (up to 1.7×10^{-2} per cubic meter of air) from large volumes of air sampled 50 meters downwind of the wet-line edge of a wastewater spray irrigation site in California (USA). Teltsch and Katzenelson (1978) isolated echoviruses from 4 out of 12 air samples collected 40 meters downwind of wastewater sprinklers in Israel. Bausum, Schaub and Kenyon (1978) studied a spray-irrigated golf course in Arizona (USA) and isolated seeded coliphage f2 from aerosol droplets 563 meters downwind of sprinklers delivering secondary effluent and 137 meters downwind of sprinklers delivering chlorinated effluent.

The possibility of virus transmission via aerosol droplets will undoubtedly be the subject of a considerable amount of research over the next few years. Attention will focus upon risks to workers at sewage treatment facilities and at agricultural sites employing spray irrigation with wastewater. A study from Israel (Katzenelson, Buium and Shuval 1976) showed that the populations of kibbutzim that practiced spray irrigation with waste stabilization pond effluent had a higher incidence of hepatitis A infection than kibbutzim in which no form of wastewater irrigation was used. However, it is most unlikely that transmission by aerosol droplets plays any major part in the maintenance of endemic enteroviral infections in poor communities where basic hygienic facilities are lacking.

Inactivation by Sewage Treatment Processes

The realization that raw sewage is rich in pathogenic viruses, and recent advances in laboratory techniques (for instance, Lydholm and Nielsen 1980), have given rise to many investigations into the effectiveness of various treatment processes in reducing viral concentrations in sewage effluents. These studies have almost exclusively examined the removal of enteroviruses and coliphages, and these two groups of viruses do not always behave in a similar way (nor may they be good models for rotavirus or hepatitis A virus). Several reviews have been published (for instance Berg 1973; Sproul 1976; WHO 1979).

By primary and secondary sedimentation

Primary sedimentation tanks, with retention times of 2–6 hours, allow a proportion of the viruses in the sewage to adsorb onto solids and settle. Many viruses will already be adsorbed to settleable solids in the influent. Removals reported in the literature, listed in the appendixes of Feachem and others (1980), suggest between 0 and 83 percent removal from influent to effluent.

Rao and others (1977) recorded a 24 to 33 percent removal of enteroviruses by primary settling tanks in the wet season in Bombay. At other times of the year removal was between 41 and 83 percent with a 2-hour retention time. Rao, Lakhe and Waghmare (1978) reported a 50 percent reduction of viruses in a pilot plant settling tank at Nagpur (India). Sherman and others (1975) and Naparstek and others (1976) studied removal of seeded coliphage f2 in treatment plants in Maryland (USA) and found 35–47 percent average removals in primary sedimentation tanks and 30 percent removal during secondary sedimentation. One report suggests that factors other than settlement may be operative in removing viruses from sedimentation tank effluent (Clarke and others 1961).

Similar performance may be expected from secondary sedimentation tanks, except that these are often designed with higher overflow rates. The sludge removed from sedimentation tanks will normally contain a 10–100 times higher concentration of enteroviruses than the raw sewage.

By storage

Storage is an effective method of virus inactivation, especially at temperatures above 20°C. In any storage vessel, some sedimentation will also be taking place

that will remove a proportion of viruses to the sludge layer. Expected removal rates in stored sewage may be derived from the data given above on the survival of enteroviruses in sewage (see also the appendixes of Feachem and others 1980), although little is known about survival under tropical climatic conditions.

By septic tanks

Removal of enteroviruses by septic tanks has been very little studied, and not at all in developing countries. A septic tank is simply a settling chamber (or chambers) with a mean retention time of 3 days or less. In poorly designed tanks, or those requiring desludging, there is very considerable carryover of solids into the effluent. Viruses will be removed both by inactivation in the anaerobic liquor and by adsorption to solids that settle to the sludge layer. Some studies of enterovirus removal have been conducted, and estimates may also be derived from information on survival in sewage and on removal by primary sedimentation (see the appendixes to Feachem and others 1980). A series of laboratory experiments showed that a 99 percent reduction of poliovirus 1 in septic tank effluent took 14 days at 20°C and 43 days at 7°C (Small Scale Waste Management Project 1978). Therefore, if all influent was held for 3 days at 20°C (because of short circuiting, it never is), a 64 percent virus reduction might be expected. In practice, enterovirus reductions of 50 percent and under are to be expected. Septic tanks usually serve small populations (5–200 people), and so influent and effluent virus concentrations will fluctuate dramatically.

The ultimate fate of viruses entering a septic tank depends on the disposal of the effluent and the sludge. Effluents are normally discharged to drainfields, where viruses may be retained and inactivated in the soil. Cliver, Green and Bouma (1975) reported that septic tank effluent (containing 10^9 seeded polioviruses per liter) was rendered virus free after travelling 0.4 meters through sand, with an application rate of 0.05 cubic meters per square meter per day at 20°C. Higher application rates or lower temperatures greatly reduced virus removal. This and other studies relevant to enterovirus removal from septic tank effluent have been recently reviewed in detail (Small Scale Waste Management Project 1978). More information on virus removal by sand and soil is given in the subsections below on filtration and land treatment.

Septic tank sludge will be rich in accumulated enteroviruses and requires treatment by digestion, drying or composting (see the subsections below on sludge treatment). The inactivation of enteroviruses, both in sludge within the septic tank and in the drainfield, will be considerably enhanced by warm temperatures.

By trickling filters

The basic mechanism for virus removal by trickling filter plants is adsorption onto the biological slime; retention times are too brief for other processes to be significant. However, reported removal rates are low, and this suggests that there is poor contact between viruses and slime surface, that adsorbed viruses are subsequently eluted by the flow of sewage passing through the filter, or both.

Sherman and others (1975) found that 9 percent and 19 percent of seeded coliphage f2 were removed by the trickling filter beds in two treatment plants. When primary sedimentation trickling filters and secondary sedimentation were considered together, coliphage removals were 55 percent and 64 percent. Buras (1976) studied the performance of the Haifa (Israel) trickling filter plant over a two year period. Average influent biochemical oxygen demand by the standard test (BOD_5) was 500 milligrams per liter, while average effluent BOD_5 was 70 milligrams per liter. The monthly average enterovirus concentrations in the influent varied between 6×10^3 per liter and 4.9×10^5 per liter, with a 2-year mean of the monthly means of 1.3×10^5 per liter. The monthly average concentrations in the effluent varied between 3×10^3 per liter and 4.5×10^5 per liter, with a 2-year mean of the monthly means of 9.6×10^4 per liter. An overall removal efficiency of only 26 percent is derived. Kott, Ben-Ari and Vinokur (1978) isolated between 2.4×10^3 and 1.2×10^4 enteroviruses per liter of trickling filter plant effluent at Haifa.

Clarke and Chang (1975) studied the performance of bench-scale, rotary-tube trickling filters. At medium filtration rates poliovirus 1, echovirus 12, and coxsackievirus A9 were reduced by 85, 83, and 94 percent, respectively. At higher filtration rates removals were 59, 63, and 81 percent, respectively. The authors failed to disassociate viruses from the biological slime in the filters and concluded that either the slime-virus complex is very stable or that the virus is somehow inactivated by adsorption to the slime. Fecal coliform and fecal streptococci removal rates closely paralleled those for enteroviruses and lead the authors to suggest that these bacteria may be used as indexes of viral removal by trickling filters. Gerba, Stagg and Abadie (1978) investigated the association with solids of enteroviruses in the effluent of a trickling

filter plant in Houston (Texas, USA). Between 9 and 196 enteroviruses per liter were contained in the effluent, and between 3 and 20 percent of these were adsorbed onto solids. This is a much lower solids-associated proportion than that reported by the same authors for activated sludge effluent (49 to 100 percent) and supports the contention that the poor virus removal efficiency of trickling filters is due to the system's providing insufficient opportunity for virus adsorption to solids or slime.

Few data are reported on the removal of enteroviruses by trickling filters in developing countries. Nupen (1970) reported that the trickling filter plant (together with primary and secondary sedimentation) at Windhoek (Namibia) reduced an influent concentration of 2×10^4 viruses per liter by 82 percent. In a subsequent report (Nupen, Bateman and McKenny 1974) it was stated that the outflow from the primary sedimentation tanks at Windhoek contained 7×10^4 viruses per liter and that, following trickling filtration and secondary sedimentation, this was reduced by 70 percent in winter and by 95 percent in summer.

Removal rates reported in the literature listed in the appendixes of Feachem and others (1980) vary between 0 and 95 percent. It is not always clear from the literature whether removal in the trickling filter alone, or across the whole treatment plant, are being recorded. Predictably, removal achieved in laboratory models (for instance Clarke and Chang 1975) is far higher than that achieved in practice (for instance, Buras 1976), and removal is reduced at higher loading rates. A typical removal rate for a trickling filter unit alone might be 5 to 20 percent, whereas a complete trickling filter plant (with no tertiary processes) could be expected to remove 25–60 percent of enteroviruses. Many of the viruses removed from the sewage will be concentrated into the primary and secondary sludges.

By activated sludge

The most significant variables in the removal of enteroviruses from activated sludge effluent are temperature, retention time (Heyward and others 1977; Malina and others 1975), the degree of adsorption of viruses onto activated sludge flocs (which may vary considerably between different virus types—Farrah and others 1978; Gerba and others 1980), and the efficiency of removal of suspended solids from the final effluent. Studies on virus removal by activated sludge are listed in the appendixes of Feachem and others (1980).

Since retention times in activated sludge plants are short (typically 6–12 hours), it is to be expected that most removal of virus is by adsorption to flocs that are subsequently removed by sedimentation. Glass and O'Brien (1980) calculated that the inactivation rate of enteroviruses in activated sludge mixed liquor at 25°C was about 12 percent per hour. Therefore, in an activated sludge tank with a mean retention of 9 hours, only 68 percent virus removal would be obtained by inactivation even if all liquor were retained for the mean retention time.

Moore and others (1974) studied a contact stabilization plant (contact time of 20–30 minutes followed by a 4-hour stabilization period) near Austin (Texas, USA). Incoming enterovirus concentrations were 250–1,500 per liter. Between 80 and 90 percent of enteroviruses became solids associated in the mixed liquor, and overall removal varied from 80 to 90 percent. In subsequent laboratory studies it was found that 99 percent of poliovirus in mixed liquor became solids associated after 1 hour's aeration. The same authors (Malina and others 1974) also reported laboratory model studies on seeded poliovirus removal by activated sludge and contact stabilization processes. Poliovirus removals by the activated sludge model were 92–99.9 percent and were not especially sensitive to changes in aeration time (range of 5–15 hours) or to mixed liquor suspended solids concentration (range of 1940–2710 milligrams per liter). Poliovirus removal was also not affected by whether pure oxygen or compressed air was used. Contact stabilization (contact time of 16–32 minutes followed by 2.1 hours stabilization period) removed 84–99.8 percent of poliovirus. Sludges, from both the activated sludge and contact stabilization models, contained between 70 and 5,800 enteroviruses per gram.

Balluz, Jones and Butler (1977) studied a laboratory-scale activated sludge plant that received raw settled sewage from Guildford (UK) with a mean BOD_5 of 270 milligrams per liter and produced an effluent with a mean BOD_5 of 11 milligrams per liter. The temperature was 15°C. An average poliovirus removal of 99.8 percent was recorded, with 85 percent of virus associated with the solids fraction of the mixed liquor. The authors stress that the efficiency of the plant in removing viruses may be closely related to the ability to remove suspended solids and that the subsequent treatment of the virus-rich sludge is of the utmost importance. In similar experiments with coliphage f2, the same authors (Balluz, Butler and Jones 1978) found a removal of only 68 percent and that a mere 16 percent of phage was associated with the solids fraction. It was concluded that coliphage is an

unsuitable indicator of enterovirus behavior in sewage treatment processes (see also Butler and Balluz 1979).

These findings on coliphage in activated sludge are important in interpreting the results of studies in which coliphage has been seeded into treatment plants to study virus removal. Naparstek and others (1976) recorded the removal of seeded coliphage f2 at an activated sludge plant in Maryland (USA). On average, only 11 percent of coliphage was removed by the aeration tanks and secondary sedimentation units, and the removal across the whole plant (which included chlorination) was only 80 percent. Safferman and Morris (1976) studied the coliphage removal ability of a sophisticated pilot plant that incorporated high-rate activated sludge, clarification, nitrification, denitrification, aeration, and filtration. Average flow was 200 cubic meters per day, and the final effluent had a BOD_5 of 2 milligrams per liter. Removal of coliphage by the high-rate activated sludge unit was between 90 and 99 percent, whereas removal across the whole plant averaged 99.97 percent.

Gerba, Stagg and Abadie (1978) found between 0.1 and 7 enteroviruses per liter in the effluents from two activated sludge plants in Houston (Texas, USA). Between 49 and 100 percent of viruses in the effluent were adsorbed onto solids. Fujioka and Loh (1978) investigated a treatment plant in Hawaii (USA) that employed settling and activated sludge. Influent contained 27–19,000 viruses per liter, while effluent contained 7–5,222 per liter. Rao and others (1977) studied virus removal at the Dadar sewage treatment plant (Bombay, India) where about 19,000 cubic meters of sewage per day are treated by activated sludge prior to marine discharge. Effluent BOD_5 over a 2-year period averaged 6 milligrams per liter (98.5 percent reduction), and effluent suspended solids averaged 20 milligrams per liter (97.2 percent reduction). Raw sewage contained 250–1,250 enteroviruses per liter, and final effluent contained 5–60 per liter. Removal rates were between 90 and 99 percent.

Both laboratory and field experience indicate that activated sludge systems are not particularly effective in removing enteroviruses but are more effective than trickling filters (see above and Heyward and others 1977). Enterovirus removal in activated sludge treatment works is in the range 0 to 99 percent, although better results (up to 99.9 percent) have been achieved in laboratory and pilot-scale models. It is reasonable to assume that a well-run activated sludge plant may reduce the enterovirus concentration by 50–95 percent, but that a poorly operated plant will achieve negligible removal. Many of the viruses removed from the sewage will be concentrated into the primary and secondary sludges.

By oxidation ditch

Practically no information is available on enterovirus removal by oxidation ditches (see the appendixes of Feachem and others 1980). The process is essentially similar to activated sludge, but the longer hydraulic retention times (1–3 days), and the higher proportion of sludge recycling giving a solids retention time of 10–30 days, are features that should produce improved virus removal. This is supported by laboratory studies in the USSR indicating the elimination of seeded enteroviruses following 2 day's aeration (Goncharuk and others 1970) and by pilot-plant studies in India showing 97–99.7 percent reduction of naturally occurring enteroviruses (Rao and others 1973). However, full-scale ditches will achieve considerably lower removal rates, and poorly operating plants will most likely remove a negligible proportion of enteroviruses.

By waste stabilization ponds

Very few systematically compiled data exist on the virus removal properties of well-constructed waste stabilization pond systems in warm climates. Removal rates reported (see the appendixes of Feachem and others 1980), vary widely, which is partly due to poor pond design, poor experimental procedures and short-circuiting of sewage flow across the ponds (Malherbe and Strickland-Cholmley 1967).

Rao, Lakhe and Waghmare (1978) reported that even very poorly designed stabilization ponds in India achieved virus removal rates similar to those of activated sludge plants. A single pond with 3–10 days retention removed 89.9–96.2 percent of viruses; a single pond with a 2.7 days retention time removed 94.8–97.3 percent, and 4 ponds in series with a 17.2 days retention time removed 88–98.9 percent of viruses.

There is ample evidence of reduced survival of enteroviruses in stabilization ponds at warm summer temperatures when compared with the same ponds at cooler winter temperatures (Funderberg and others 1978; Kott, Ben-Ari and Betzer 1978; Lefler and Kott 1975; Slanetz and others 1970). Viruses adsorbed to settleable solids will fall to the sludge layer at the base of the facultative pond where they may survive for extended periods (Funderberg and others 1978). Other biological and physical factors—such as a virucidal increase in pH to 9 or above caused in part by blooms

of algae (Funderberg and others 1978)—also may determine virus survival.

Lund (1979) estimated that virus inactivation in heavily polluted water might proceed at approximately 1 log unit in 5 days at 32°C and 1 log unit in 1 day at 35°C. Funderberg and others (1978) reported poliovirus removal in model outdoor ponds near Austin (Texas, USA). During the summer over 99 percent of added virus was lost within 5 days, whereas this degree of removal took 15 and 25 days in spring and winter, respectively.

These data suggest that well-designed stabilization ponds in the tropics (with minimal short-circuiting, water temperature above 25°C, and overall retention time of 30 days or more) should achieve very high levels of virus removal (at least a 4 log reduction). Confirmation of this must await further experimentation on well-designed waste stabilization ponds in developing countries.

By aerated lagoons

An aerated lagoon on its own may be expected to have a virus removal rate similar to, or a little better than, an oxidation ditch. If the effluent is treated in maturation ponds, removal rates as in waste stabilization ponds are expected. No specific data are available, but warm temperatures will certainly increase enterovirus inactivation rates. The sludge drawn off from secondary sedimentation tanks or settling ponds will be rich in enteroviruses.

By tertiary treatment

Some tertiary, or advanced physicochemical, treatment processes are effective in removing viruses. However, they add cost to sewage treatment and in some cases are too technically and mechanically sophisticated to be appropriate in developing countries.

LAGOONING. Secondary effluents may be further treated in maturation lagoons. Enterovirus removal rates and processes are the same as in waste stabilization ponds, except that little or no sedimentation takes place. High rates of virus removal can be achieved if several lagoons are employed and short-circuiting is avoided.

Kott, Ben-Ari and Betzer (1978) investigated the use of lagoons for the tertiary treatment of trickling filter effluent at Haifa (Israel). In winter (temperatures down to 8°C), initial enterovirus concentrations of 1.1×10^4 per liter were reduced to zero in 47–73 days. In summer (18–20°C), with initial concentrations of 2,000 per liter, inactivation was complete within 11–35 days. Nupen (1970) and Nupen, Bateman and McKenny (1974) reported a 95 percent reduction in enteroviruses in a chain of 9 maturation lagoons (total retention time 14 days) receiving the trickling filter plant effluent at Windhoek (Namibia). Lagoon effluent contained up to 25 enteroviruses per liter in summer and up to 842 per liter in winter.

COAGULATION. Coagulation is one of the more effective chemical processes for removing viruses from wastewater. Alum [$Al_2(SO_4)_3$], lime [$Ca(OH)_2$], iron salts, and polyelectrolytes have all been used. Wolf and others (1974) reported a greater than 99.6 percent removal of seeded coliphage f2 and poliovirus 1 in a laboratory model coagulation-sedimentation system employing alum. Lime is probably the most effective coagulant, since the high pH values produced are strongly virucidal (particularly above pH 11—see, for example, Nupen, Bateman and McKenny 1974). For maximum efficiency, coagulation should be followed by slow sand filtration (Berg 1973; Berg, Dean and Dahling 1968; Derbyshire and Brown 1979; Grabow, Middendorf and Basson 1978; Nupen 1970; Shelton and Drewry 1973; Sproul 1976). A comprehensive review of virus removal by coagulation and pH adjustments has been recently published (Sproul 1980).

FILTRATION. Sand filters can remove a high proportion of viruses from secondary effluents, but reported performances are erratic. Higher removal rates are achieved at lower filtration rates. Removal of viruses is also enhanced by low or high pH and by the presence of cations (Jenkins and others 1980) and very much enhanced by coagulation prior to filtration (Berg 1973).

Sproul (1976) reported 99.7 percent removal from activated sludge effluent at a filtration rate of 0.04 cubic meters per square meter per day and 100 percent removal at 0.007 cubic meters per square meter per day. Safferman and Morris (1976) reported very poor removal of coliphage (0 to 48 percent) by dual and multimedia filters without precoagulation. Berg, Dean and Dahling (1968) recorded an 82–99.8 percent removal of viruses in lime coagulated effluent at a filtration rate of 131 cubic meters per square meter per day.

Very significantly from the viewpoint of developing country operating problems, Vaughn and others (1978) stated that the treatment plant at Holbrook (New York, USA), which features extended aeration,

denitrification, and gravity sand filtration, was experiencing "operating difficulties" and they isolated up to 283 enteroviruses per liter from the final effluent. Assuming a raw influent concentration of about 1,000 per liter, a removal rate of only 72 percent was achieved in filtered tertiary effluent in an industrialized country. Similarly, Rao, Lakhe and Waghmare (1978) reported that a sewage reclamation plant at a factory in Bombay incorporated extended aeration, alum coagulation, rapid sand filtration, and deionization but achieved a virus removal of only 81–99 percent. These are powerful illustrations both of the operating difficulties frequently experienced with advanced wastewater treatment plants even in developed countries and of the inapplicability of much virus removal data obtained in laboratory or pilot-scale plants to full-scale operating treatment plants.

DISINFECTION. Enterovirus removal from secondary or tertiary effluents by disinfection has been the subject of numerous investigations in recent years. The most widely used disinfection technique for sewage effluents is chlorination, and there is considerable evidence that viruses are less readily destroyed by effluent chlorination than enteric bacteria (Berg and Metcalf 1978; Snead and others 1980), although, unlike some bacteria, viruses cannot regrow in the effluent subsequently.

As with the bactericidal effects of chlorine in water treatment, free chlorine (especially in the form of hypochlorous acid at low pH and particularly at warm temperatures) is a far more potent virucide than combined chlorine (monochloramine, dichloramine, and other compounds), which is formed in the presence of ammonia and organic matter (Olivieri, Donovan and Kawata 1971). Chlorine added to most sewage plant effluents is rapidly converted to combined chlorine, and this, together with the protective effect of virus association with solid particles, may result in very poor virus removal.

In clean water at pH 7–8 1–2 milligrams per liter of free chlorine maintained for 1–2 hours will be more than sufficient for complete virus inactivation. Englebrecht and others (1978) showed that 6 different enteroviruses in water were all inactivated by 99 percent in under 5 minutes when 0.5 milligrams per liter of free chlorine were applied at 5°C and pH 7–8. In a secondary sewage effluent (BOD_5 of 45 milligrams per liter), however, poliovirus was reduced by 50 and 90 percent in 6 hours after applying 5 and 11 milligrams per liter of chlorine, respectively (Shuval and others 1966). Similarly, Berg and Metcalf (1978) reported that, at 22–24°C, 11–23 milligrams per liter of combined chlorine added to primary effluent inactivated only between 1 and 2 log units of enterovirus in 15 minutes, whereas in the same experiments fecal coliform reductions ranged from 3 to more than 5 log units, and total coliform reductions ranged from 5 to more than 7 log units.

Different species of human excreted virus have different sensitivities to inactivation by chlorine. Reoviruses are among the most sensitive, and polioviruses are among the most resistant (Drulak, Wallbank and Lebtag 1979; Englebrecht and others 1978; Shuval and others 1966; Sproul 1976).

Boardman and Sproul (1977) studied the inactivation of coliphage T7 by chlorine when the phage was adsorbed to particles of clay, aluminium oxide, or calcium carbonate in water. It was concluded that adsorption of virus to the surface of inorganic particles offered no protection against inactivation by chlorine, but that encapsulation by a particle may afford protection. This conclusion has been confirmed by studies by Hejkal and others (1979) into the inactivation by chlorine of poliovirus in fecal homogenates. They found that the virus that was closely associated with, or occluded within, small fecal particulates was protected from chlorine inactivation. A combined chlorine residual of 6.6 milligrams per liter (at pH 8 and 22°C) achieved a 50 percent inactivation of solids-associated virus in 15 minutes, whereas only 1.4 milligrams per liter of combined chlorine were sufficient to obtain the same reduction of free virus in the same time. However, these differences were small compared with differences in inactivation due to dissolved organics that determined whether any free chlorine, as opposed to combined chlorine, was present. Stagg and others (1978) studied three treatment plants in Houston (Texas, USA) and found that between 2 and 21 percent of coliphages in plant effluent prior to chlorination were solids associated. Passage through chlorine contact chambers inactivated freely suspended phages to a greater extent than solids-associated phages, and increased the proportion of solids-associated phages in the final effluent to between 6 and over 99 percent. Only about 15 percent of the solids-associated viruses were embedded; the remainder were adsorbed.

It is clear from the above that the efficacy of effluent chlorination in virus removal depends considerably upon the quality of the effluent prior to chlorination. The better the quality of the effluent, the higher the virus inactivation attained by a given chlorination system; tertiary treatment (for instance, by filtration) is therefore often recommended to reduce further suspended solids and dissolved organics prior to

chlorination (Dryden, Chen and Selna 1979; Kirkpatrick and Presecan 1978).

Several reports indicate that well-run chlorination units, receiving high quality effluents, can produce a virus-free final effluent. Kott, Ben-Ari and Betzer (1978) investigated the effect on enteroviruses of chlorinating a trickling filter effluent at Haifa (Israel). At a chlorine dose of 20 milligrams per liter and a contact time of 2 hours, enterovirus concentrations were reduced from up to 5,900 per liter to zero (see also Kott, Ben-Ari and Vinokur 1978; Lindeman and Kott 1971).

The possibility of a virus-free chlorinated effluent is also illustrated by data from some of the advanced wastewater reclamation plants. Culp (1974*a*, 1974*b*) reports complete virus inactivation at a Lake Tahoe (USA) sewage treatment plant by carefully controlled chlorination. Grabow and Isaäcson (1978) failed to isolate any enteroviruses from 144 10-liter samples of water produced by the advanced wastewater reclamation plants at Windhoek (Namibia) and Pretoria (South Africa). These plants incorporated a train of advanced processes and included break-point chlorination sufficient to produce 0.2–0.6 milligrams per liter of free residual chlorine after 2–3 hours of contact time (Nupen 1970; Nupen, Bateman and McKenny 1974).

However, chlorination in no way guarantees a virus-free effluent. Sherman and others (1975) found that the chlorination of trickling filter plant effluents from two treatment plants in Maryland (USA) reduced seeded coliphage by 60 percent. Overall reductions of phage across the two plants were 82 percent and 86 percent. Fujioka and Loh (1978) reported isolating 25–34 and 2–750 enteroviruses per liter from the chlorinated effluent of two treatment plants in Hawaii (USA). Influent concentrations were 5–268 and 27–19,000 enteroviruses per liter, respectively. Wellings and others (1975) found on average 0.13 enteroviruses per liter (53 percent of samples positive) in the chlorinated effluent from a package treatment plant receiving sewage containing 161 viruses per liter from a mobile home park. Wellings, Lewis and Mountain (1974, 1976) isolated up to 12 and 98 enteroviruses per liter, in two studies on the chlorinated effluent from an activated sludge plant in St. Petersburg (Florida, USA). Metcalf, Wallis and Melnick (1974) isolated up to 4 enteroviruses per liter from a chlorinated effluent (1.2–1.9 milligrams per liter of residual chlorine after 10 minutes of contact) at an activated sludge plant near Houston (Texas, USA). Vaughn and others (1978) isolated up to 26 and 98 viruses per liter from the chlorinated secondary effluents from two sewage treatment plants on Long Island (New York, USA). Bausum, Schaub and Kenyon (1978) found that chlorination of a trickling filter effluent in Arizona (USA) reduced the concentration of seeded coliphage by only 95 percent, as compared with a bacterial reduction of 99.97 percent. Kott and others (1974) studied chlorinated waste stabilization pond effluents in Israel (8 milligrams per liter of applied chlorine for 1 hour at 20°C) and found an average enterovirus reduction of only about 10 percent, whereas the total coliform reductions were 2 log to >6 log units. The chlorinated pond effluents contained between 300 and 1,000 enteroviruses per liter. Other experiments found that, at pH 6.0 with a 2-hour contact time, seeded poliovirus 1 in stabilization pond effluent was reduced by 86 percent with 20 milligrams per liter of applied chlorine, by 87 percent with 40 milligrams per liter of chlorine, and by 100 percent with 60 milligrams per liter of chlorine.

The chlorination of inadequately treated sewage, in the hope of thereby removing much of the microbial hazard, is disturbingly widespread despite clear evidence that it is generally ineffective and represents bad engineering practice. The authors of this book have observed this practice on several occasions, and have frequently heard it recommended in developing countries when concern is being expressed about the discharge of highly polluted effluents from improperly designed or malfunctioning sewage treatment plants. An interesting case study of this problem is the investigations by Sattar and Westwood (1978) in Ottawa (Canada), a city of 500,000 people that discharged 90 percent of its sewage into the Ottawa River after the wastes received only primary treatment (sedimentation) and chlorination. Two treatment plants were studied that received a raw sewage with a BOD_5 of 79–98 milligrams per liter and that produced a chlorinated primary effluent with a BOD_5 of 44–48 milligrams per liter. Raw sewage samples were 80 percent positive for enteroviruses, with an average concentration of 1,000 per liter. Samples of sedimentation tank effluent were 72 percent positive for enteroviruses and also contained 1,000 viruses per liter. Samples of final chlorinated effluent were 56 percent positive for enteroviruses and contained 27 viruses per liter. The Ottawa River is used for recreation and provides the raw water source for about 600,000 people.

Various other wastewater disinfection systems have been studied with respect to their ability to inactivate viruses. Ozone is a very potent virucide, and its activity is less disturbed by organic pollution than is the case for chlorine (De Michele 1974; Dryden, Chen and Selna 1979; Evison 1978; Katzenelson and

Biedermann 1976; Munger, Heyward and Swartz 1977; Pavoni and Tittlebaum 1974; Rakness and Hegg 1979; Sproul 1976). Bromine chloride and paracetic acid have been evaluated and are also little affected by organic matter in the effluent (Hajenian and Butler 1980). Chlorine dioxide also effectively inactivates enteroviruses in water at a rate similar to hypochlorous acid. Unlike hypochlorous acid, chlorine dioxide is more potent at higher pH values (Cronier, Scarpino and Zink 1978). Radiation at a level of 3–5 kilogray[6] inactivates approximately 90 percent of viruses in sewage effluent, whereas only 1–1.5 kilogray are required to achieve the same inactivation of viruses in distilled water (Metcalf 1977; Sullivan and others 1971). Photodynamic oxidation has also been evaluated for the disinfection of wastewaters (Gerba, Wallis and Melnick 1977*a*, 1977*b*). However, all these techniques are currently at the experimental stage; in any case, they may involve a level of technical sophistication and cost that would make them inappropriate in many situations in developing countries.

LAND TREATMENT. Land treatment by soil filtration or groundwater recharge can be highly effective in removing viruses from primary or secondary sewage effluents, but results reported in the literature vary widely.

Lance, Gerba and Melnick (1976) showed, in laboratory studies, that poliovirus in secondary effluent was almost completely removed by filtration through loamy sand after flowing to a depth of 1.6 meters and was reduced by 99 percent after 0.4 meters of flow. These results were obtained at filtration rates of both 0.55 cubic meters per square meter per day and 0.15 cubic meters per square meter per day. Flooding the soil with deionized water (to simulate a rainstorm) caused some downward movement of the viruses, but this was greatly reduced when $CaCl_2$ was added to the deionized water. Drying of the soil between effluent application and a simulated rainstorm considerably reduced desorption of viruses; 5 days drying prevented subsequent desorption completely. The authors concluded that viruses would move through 2.5 meters of calcareous sand only if heavy rains fell within a day following the cessation of sewage application. In follow-up studies (Gerba and Lance 1978; Lance and Gerba 1980; Lance, Rice and Gilbert 1980), similar adsorption and elution results were obtained in loamy sand whether polioviruses were suspended in primary or secondary effluent. In one series of experiments, 99 percent of poliovirus in primary effluent was removed in the top 0.2 meters of loamy sand, and no poliovirus was detected below 0.8 meters, when the flow rate was approximately 0.2 cubic meters per square meter per day. In other experiments it was shown that poliovirus adsorption by loamy sand is reduced when the flow rate is increased above some critical value, whereas flow rate changes above and below this value do not affect adsorption. The critical value for loamy sand was about 1 cubic meter per square meter per day; below this flow rate poliovirus penetration was less than 1.6 meters, whereas above it viruses penetrated the entire 2.5-meter column.

Duboise, Moore and Sagik (1976) reported that poliovirus and coliphage adsorbed to cores of loamy sand were eluted less by intermittent application of effluent or water than by continuous application. In addition, the chemical quality of the effluent improved more by soil filtration when the effluent was applied intermittently than when it was applied continuously (see also Duboise and others 1974).

Bitton, Masterton and Gifford (1976) found that under experimental conditions coliphage T2 and poliovirus were adsorbed more readily when suspended in tap water than in secondary effluent, and that effluent is a more potent eluent than tap water for washing adsorbed viruses out of soil columns. Lefler and Kott (1976) also found higher elution of poliovirus and coliphage from sand with effluent as compared with tap water. However, Duboise, Moore and Sagik (1976) reported that flooding cores of loamy sand with dechlorinated effluent eluted fewer polioviruses than when distilled water was used.

Landry and others (1979) found that 72–100 percent of polioviruses, coxsackieviruses, and echoviruses were retained when tertiary sewage effluent was passed through 1.25-meter natural cores of gravelly sand at a rate of 20 cubic meters per square meter per day. Flooding the cores with artificial rain water released 0–67 percent of the adsorbed viruses, whereas flooding with sewage effluent released 0–14 percent. A total of eight laboratory and field strains of enteroviruses were used in these experiments. Some differences in adsorption among the strains were recorded (with echovirus 1 showing the greatest soil affinity), and the proportion of adsorbed viruses being eluted by rainwater or sewage differed markedly (with echovirus 1 being the least mobilized and a wild strain of poliovirus 3 being the most readily eluted). The authors concluded that soil adsorption-elution behavior is strain dependent and warn against the application of laboratory data from experiments using only a single strain of poliovirus 1. Gerba and others (1980), Goyal and Gerba

6. See footnote 5, above.

(1979), and Goyal and Melnick (1978) also reported wide intertypic and intratypic variation in adsorptive behavior among enteroviruses in various soils.

Burge and Enkiri (1978*a*) reported substantial differences in the ability of five soils to adsorb coliphage viruses. More acidic soils had higher adsorption rates, and one loamy sand adsorbed no viruses (see also Burge and Enkiri 1978*b*; Vilker and Burge 1980). Similarly, Lefler and Kott (1974*b*) found that many coliphage f2 and poliovirus 1 were able to pass through a 0.2-meter sand column (at an application rate of 1.7 cubic meters per square meter per day) and that only a high concentration of bivalent cations (Ca^{++} and Mg^{++}) prevented this. Funderberg and others (1979), Goyal and Gerba (1979), and Moore and others (1979) have also reported wide variation in virus adsorption behavior dependent on soil properties.

Studies by Scheuerman and others (1979) showed that some organic soils have poor virus adsorption potential due to the presence of water-soluble humic substances (humic and fulvic acids), which may compete with viruses for adsorption sites on soil particles or may react with certain surface groups on virus particles that are functionally important in adsorption to soil.

Wellings, Lewis and Mountain (1974) studied an effluent spray irrigation site near St. Petersburg (Florida, USA). The effluent from the activated sludge plant contained up to 240 enteroviruses per liter, and the final chlorinated effluent contained up to 98 per liter. The effluent was applied to a sandy soil at between 0.007 and 0.04 cubic meters per square meter per day. Water collected in drains 1.5 meters under the soil contained polioviruses, echoviruses, and reoviruses (2 out of 9 samples positive). Enteroviruses were also isolated from wells 3 and 6 meters deep at the site following heavy rain.

Wellings and others (1975) studied the discharge of effluent from a 154-unit mobile home park into a cypress dome in the wetlands of Florida (USA). The population of the park varied between 310 and 337 during the study and produced a raw sewage with an average of 161 enteroviruses per liter (range between 0 and more than 700 per liter). Polioviruses 1, 2, and 3 accounted for 40 percent of isolates identified, with coxsackieviruses (B3 and B4) making up 43 percent, and echoviruses (7, 11, and 14) the remaining 17 percent. Chlorinated effluent from a package treatment plant yielded polioviruses and coxsackieviruses in 8 out of 15 samples taken. The effluent was discharged into the cypress dome, and groundwater quality was monitored in 18 3-meter-deep wells constructed in and around the dome. Enteroviruses were isolated from 3 out of 48 well-water samples, and horizontal movement of viruses through the saturated soils of at least 7 meters was demonstrated.

Gilbert and others (1976*a*, 1976*b*) studied virus removal from activated sludge effluent (up to 75 viruses per liter) applied to loamy sand (infiltration rate of 0.27 cubic meters per square meter per day) at a 7-year-old wastewater renovation plant near Phoenix (Arizona, USA). No viruses were detected in the observation wells, indicating at least a 99.99 percent removal after flow through 3–9 meters of soil. In contrast, Schaub and Sorber (1977) reported very low viral removal by soil filtration (infiltration rate 0.07 cubic meters per square meter per day) of primary effluent at a 30-year-old treatment plant in Massachusetts (USA). The soil was unconsolidated silty sand and gravel. Seeded coliphage f2 viruses were reduced by an average of 53 percent after 18 meters of percolation, and seeded f2 and indigenous enteroviruses were sporadically detected in the groundwater at horizontal distances of 180 meters from the application zone.

It is clear from these and other studies that adsorption to soil particles, rather than viral death, is the dominant removal mechanism. Adsorption is increased by low infiltration rates (say <0.1 cubic meters per square meter per day), by low pH, and by the presence of divalent cations (Ca^{++} and Mg^{++}). Adsorption is reduced in the presence of soluble organic matter which competes for adsorption sites on the soil particles. Adsorption and elution behavior depend very much upon the characteristics of the soil and the particular strain of virus. A great deal of further experimentation will be required before the optimal soil structures—and their relationships to infiltration rates, application schedules, and virus removal performance—are fully understood. It is already apparent, however, that by passing even raw sewage through less than 1 meter of a suitable soil it is possible to reduce the virus concentration by as much as, or more than, that normally achieved by wastewater chlorination (Gerba 1979). Because of higher temperatures there, the effectiveness of land application in virus removal in the developing countries is likely to be greater than that generally reported from temperate areas. It must be remembered, however, that without efficient management, operation, and maintenance land application systems will become insanitary bogs.

The above discussion has dealt exclusively with soil filtration and groundwater recharge as methods of land treatment for wastewaters. The other major type of land treatment technology is the grass plot or overland run-off method. In these systems a significant

proportion of the effluent may run over the surface of the soil and not flow through it, and it may be expected that virus removal will be poor compared with the soil filtration data reported above. Experiments by Schaub and others (1980) confirmed this, showing a removal of only 30–60 percent of seeded f2 bacteriophage and only 76–88 percent of indigenous excreted virus during treatment on 36 meter long grass plots with a 3 percent slope.

OTHER PROCESSES. A variety of other treatment processes are associated with advanced wastewater treatment plants and with water reclamation or renovation projects. Some of these processes have been assessed for virus removal capability. Carbon adsorption and nitrification do not seem particularly effective, whereas denitrification was reported to remove 97 percent of coliphage (Berg 1973; Gerba and others 1974; Safferman and Morris 1976; Sproul 1976). Complete water reclamation plants, incorporating a train of advanced processes, are generally reported to achieve total virus removal when operating perfectly. The Lake Tahoe (Nevada, USA) reclamation plant has occasionally let through viruses beyond the carbon adsorption stage, but they were eliminated by chlorination (Berg 1973). Similarly, the Windhoek (Namibia) reclamation plant is reported to have achieved a virus-free effluent, despite an influent virus concentration of up to 2×10^4 per liter (Grabow and Isaäcson 1978; Nupen 1970; Stander and Clayton 1977).

Inactivation by Night soil and Sludge Treatment Processes

Raw night soil contains all the viruses being excreted by the contributing population. Sewage works sludges are rich in viruses because a high proportion of viruses in sewage are, or become, solids-associated and are therefore concentrated into both primary and secondary sludges (Lund 1973; Lund 1976; Lund and Ronne 1973; Wellings, Lewis and Mountain 1976). Interest in viruses in sludges has been stimulated by the fact that a large proportion of sewage sludge is applied to the land as a method of disposal and soil enrichment.

Information on this subject is restricted to laboratory studies and a few field studies conducted in North America and Europe. Little is known about viruses in sludge in developing countries or about sludge treatment under tropical climatic conditions. Even less is known about the virological aspects of night soil and night soil treatment.

By pit latrines

Little information is available, but it is probable that enteroviruses survive for several weeks in pit latrines (see the sections above on occurrence and survival in feces and night soil and in sludge; see also the appendixes to Feachem and others 1980). In warm climates, the pit contents should be free of enteroviruses if they are left for at least 3 months before digging out.

A pit latrine may act as a source of viral groundwater pollution depending on the type of soil, groundwater levels, and the proximity of local wells (see the sections above on occurrence and survival in groundwater and on virus removal by land treatment).

Francis, Brown and Ainslie (1953) isolated polioviruses (16 out of 220 samples positive) and coxsackieviruses (10 out of 63 samples positive) from pit latrines in poor areas of four towns in southern Texas (USA). Pit latrines with polioviruses were not associated with known cases of poliomyelitis, an epidemic of which was taking place at the time (March–July of 1948), but were associated with the isolation of polioviruses from flies in the vicinity.

By anaerobic digestion

Although some form of anaerobic digestion is used to treat sludge from most larger sewage treatment plants, very little information is available on the virus removal performance of full-scale digesters. (Some laboratory studies are discussed below and are listed in the appendixes of Feachem and others 1980).

Ward and Ashley (1976) investigated the inactivation rate of poliovirus in digested sludge and found that it was greater than 1 log unit per day at 28°C and about 1 log unit per 5 days at 4°C. They concluded that anaerobically digested sludge contains a specific virucidal agent; in a subsequent study (Ward and Ashley 1977*a*) they identified this agent as ammonia (see also Fenters and others 1979). Ammonia is not virucidal in its charged state, but free ammonia, which is formed at pH values of 8 and above, is highly virucidal to enteroviruses but much less so to reoviruses. Ward and Ashley concluded that ammonia acts as a potent enteroviricide in raw and digested sludges with high pH values. At pH 9.5 and 21°C, greater than 3 log unit and 5 log reductions in poliovirus concentrations were obtained in 72 hours in raw and digested sludges, respectively. A later study

confirmed that reovirus 3 was unaffected by the presence of ammonia (Ward and Ashley 1977*c*).

Eisenhardt, Lund and Nissen (1977) studied the inactivation of coxsackievirus B3 in a laboratory-scale anaerobic sludge digester at pH 7. At 32°C a 5 log reduction in virus concentration occurred in about 14 days, whereas at 35°C the same reduction took only 4 days. Inactivation was slightly faster when the virus was held in pasteurized sludge. Bertucci and others (1977) ran a laboratory anaerobic digester (pH 7.2–7.4) at 35°C and compared inactivation rates of various enteroviruses. Inactivation rates varied from 75 percent per day for echovirus 11 to 97 percent per day for coxsackievirus A9.

Sanders and her coworkers (1979) pointed out that most previous laboratory studies investigated the inactivation of free viruses inoculated into sludge immediately prior to digestion. However, to simulate more exactly real operating conditions it is necessary to allow the viruses to become associated with solids prior to commencing digestion. Sanders therefore investigated the inactivation by anaerobic digestion of solids-associated poliovirus and found that survival was enhanced by solids incorporation. The inactivation rates at 34 and 37°C were 84 to 99 percent per day, respectively, for the first 24 hours. After that time inactivation slowed considerably to between 30 and 60 percent per day. At 50°C the inactivation rate was high at more than 7 log units per day.

Berg and Metcalf (1978) reported the destruction of between 76 and 96 percent of viruses by mesophilic digestion (35°C for 20 days) and between 98.9 and >99.9 percent by thermophilic digestion (50°C for 20 days). Enterovirus concentrations in raw sludge were 4×10^3 to $>1 \times 10^5$ per liter, 300 to 4,100 per liter in mesophilically digested sludge, and 0 to 170 per liter in thermophilically digested sludge. In these experiments, samples of digested sludge were taken shortly after the addition of fresh sludge to the digesters, and so theoretical retention times would not have applied to all aliquots of digested sludge.

Wellings, Lewis and Mountain (1976) isolated enteroviruses and reoviruses, at concentrations of up to 34 per liter, from sludge from an anaerobic digester in Florida (retention time >60 days at 34°C) to which no raw sludge had been added for the previous 7 days. Sattar and Westwood (1979) found excreted viruses in 53 percent of samples of digested sludge (20 days at 35°C) and in 39 percent of dried sludge samples (>6 months' drying time) at a large sewage treatment plant in Ottawa (Canada). Hurst and others (1978) isolated viruses, at concentrations of up to 231 per liter, from sludge that had been thickened, aerobically digested, and centrifuged at a Houston (Texas, USA) treatment plant.

Investigators who have looked for viruses in digested sludge have generally found them in considerable numbers (Berg and Metcalf 1978; Grigoryeva, Korchak and Bey 1969; Hurst and others 1978; Sattar and Westwood 1979; Wellings, Lewis and Mountain 1976). Some laboratory studies have reported an inactivation rate of around 1 log unit per day at 30–35°C (for instance, Eisenhardt, Lund and Nissen 1977; Fenters and others 1979; Ward and Ashley 1976). At this rate of inactivation, typical anaerobic digestion at 35°C for 35 days should produce a virus-free sludge with a wide margin of safety. However, Sanders and others (1979) have shown that inactivation rates of solids-associated viruses after the first day of digestion may be very much slower (around 1 log unit every 2–7 days). In addition, most digesters are operated by continuous, or regular, addition and removal of sludge. Therefore, some sludge has a retention time of very much less than the design value and will contain significant concentrations of viruses after digestion. It is probable that only batch digestion at 35°C, for 35 days, or digestion at temperatures of around 50°C, will produce a virus-free sludge. More field data are required, on the actual virus removal performance of operating plants of these types, to confirm this assumption.

By drying

Both raw and digested sludges are normally dewatered prior to disposal, and the most common technique is spreading on outdoor sludge drying beds. Very little information is available on virus removal by sludge drying (see below and the appendixes of Feachem and others 1980), and no studies have been reported from developing countries. However, the data on enterovirus survival in sludge are also relevant (see the section above on the occurrence and survival of enteroviruses in sludge and the appendixes of Feachem and others 1980).

Ward and Ashley (1977b) investigated the inactivation of viruses in sewage sludge that occurs during dewatering by evaporation. Sludge, with a solids content of 5 percent and a pH of 6, was inoculated with 2.7×10^7 viruses per milliliter and air dried at 21°C in 1 centimeter thick layers over 4 days. As evaporation proceeded, poliovirus 1 was inactivated at a low but constant rate until, at a solids concentration of 65 percent, approximately 75 percent inactivation had occurred. At this stage inactivation

increased rapidly so that, during concentration up to 83 percent solids, a further 99.9 percent inactivation occurred. Further concentration up to 91 percent solids produced little more inactivation. A similar result was obtained with coxsackievirus B1 and reovirus 3. In a subsequent study (Ward and Ashley 1978*b*), it was found that sludge drying increased the heat required to inactivate enteroviruses and reovirus. Sattar and Westwood (1979) isolated viruses from anaerobically digested sludge that had been drying for 8 months in Canada. Wellings, Lewis and Mountain (1976) isolated 10 enteroviruses per 100 grams of sludge that had been on sludge drying beds for two weeks during February in Florida (USA).

These studies, and the reports on virus survival in sludge indicate that during cool, wet weather enteroviruses may survive in drying sludge for several months. Data on rapid virus inactivation at solids concentrations between 65 and 83 percent may be irrelevant, since under temperate conditions sludges may achieve a solids content of only about 25 percent after about 2 months on a drying bed. Even in Texas (USA) in the summer, a sludge that had been applied to land for 3 months had dried to only 59 percent solids (Hurst and others 1978). However, a comparison between virus inactivation rates in sludge during Danish winters (1 log unit per month), and during Texan summers (2 log units per week), clearly indicates that enterovirus inactivation is far more rapid in hot climates under bright sunshine. A good virus removal performance may therefore be obtained by sludge drying beds in many developing countries, and field studies are required to confirm this possibility.

By heating

Under certain circumstances enteroviruses can be remarkably resistant to heating. For instance, Larkin and Fassolitis (1979) reported that infectious ribonucleic acid (RNA) liberated from poliovirus 1 and coxsackievirus B2 could withstand 65 minutes at 70°C. In general, however, heat is a potent virucide, and the heating of sludges, or their digestion at elevated temperatures, is an effective method of virus inactivation.

Ward, Ashley and Moseley (1976) studied the effect of raw and anaerobically digested sludge on the heat inactivation of poliovirus. Raw sludge was found to be very protective of poliovirus inactivation whereas digested sludge was not, and subsequent studies (Ward and Ashley 1977*a*) determined that this difference was due to the virucidal activity of uncharged ammonia present at the high pH levels found in anaerobically digested sludge. At 43°C after 200 minutes, poliovirus concentration was reduced by over 3 log units in digested sludge, but it was almost stable in raw sludge. At 51°C, the poliovirus concentration was reduced by over 5 log units in under 5 minutes in digested sludge and by over 4 log units after 50 minutes in raw sludge. In a second study (Ward and Ashley 1977*c*), the heat inactivation of reovirus 3 in sludge was investigated. Reovirus was found to be quite heat resistant compared with poliovirus but was not protected against heat inactivation by sludge. At 50°C for 20 minutes, reovirus concentrations were reduced by 4 log units in digested sludge and by 2 log units in raw sludge. At 60°C after 20 minutes, the reductions were 5 log units in digested sludge and 4 log units in raw sludge. A virucidal agent against reoviruses was discovered in the sludge that had greatly increased activity at pH values above 8. Unlike the case of the enteroviruses (Ward and Ashley 1977*a*), this agent was not ammonia. A follow-up study (Ward and Ashley 1978*a*) determined that ionic detergents found in sewage sludges reduce the heat required to inactivate reoviruses (cationic detergents being more active than anionic detergents, and nonionic detergents having no activity). In contrast, some detergents were found to protect poliovirus against heat inactivation. (More information on the virucidal activity of detergents, and the effects of differing pH values, is given in Ward and Ashley 1979). In a subsequent study (Ward and Ashley 1978*b*), a reduction in moisture content was found to reduce significantly the rates of heat inactivation of both enteroviruses and reovirus. Poliovirus in raw sludge at 51°C was reduced by over 5 log units in 5 minutes when the sludge had 5 percent solids but by less than 2 log units after 100 minutes when the sludge had an 80 percent solids content. Reovirus in raw sludge at 51°C was reduced by 4 log units in 50 minutes when the sludge had 5 percent solids and by less than 2 log units after 50 minutes when the sludge had a solids content of 80 percent.

When compared with the reality of sludge treatment processes, however, these are trivial differences and distinctions. Figure 9-2 presents data on the survival of different types of enteroviruses under different conditions for various time-temperature combinations. A conservative upper bound is drawn, above which the combinations of time and temperature should guarantee enterovirus elimination. From this figure it is postulated that holding sludge at 30°C for 3 months, at 40°C for 2 weeks, at 50°C for 1 day, or at 60°C for 2 hours, will inactivate all enteroviruses, reoviruses, and adenoviruses.

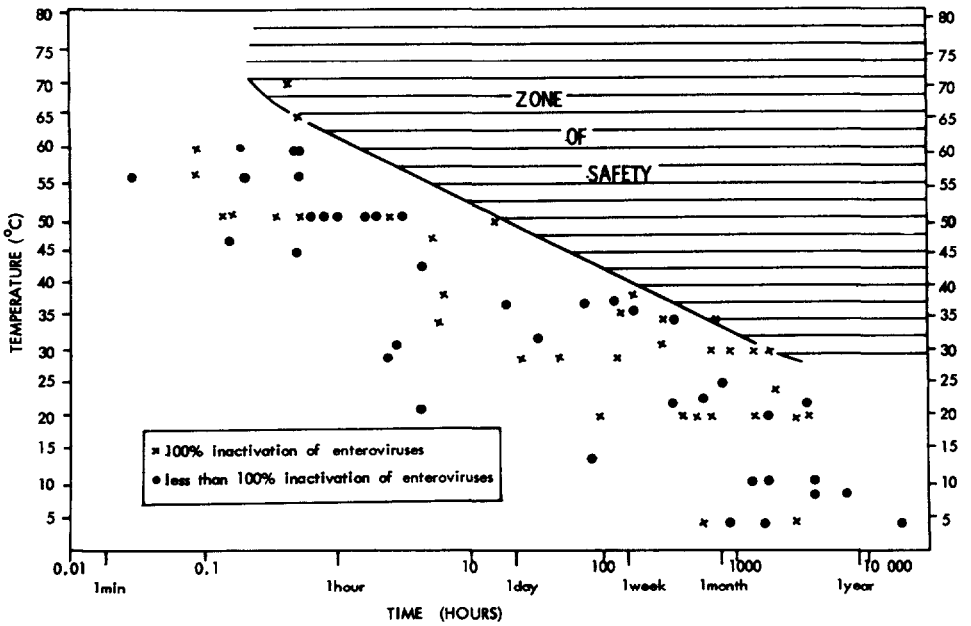

Figure 9-2. *The influence of time and temperature on enteroviruses.* The data probably also apply to adenoviruses and reoviruses. The points plotted are the results of experiments done under widely differing conditions. The line drawn represents a conservative upper boundary for death

By composting

Aerobic thermophilic composting is an effective method of inactivating viruses in sludge if all parts of the pile or mass are heated to 50°C or above for sufficient time (see figure 9-2 and the appendixes of Feachem and others 1980).

Krige (1964) reported that seeded poliovirus 1 was eliminated from a sludge, grass, and refuse mixture composted at 38–58°C for 7 days. Wiley and Westerberg (1969) determined that the thermal death points for poliovirus 1 were 60°C for 5 minutes or 55°C for 30 minutes. When poliovirus 1 was added to a forced-air sludge-composting unit operating at 60–76°C, it could not be detected after 1 hour.

Kawata, Cramer and Burge (1977) reported the inactivation of seeded bacteriophage f2 in a sludge and wood chips mixture composted at a plant in Maryland (USA). The mixture was formed into windrows, which were turned regularly (up to once per day depending upon the temperature within the mass) for 2 weeks and were then made into large piles for 4 weeks of curing. When raw sludge was composted, the temperatures rose to 50–70°C within 3 days and remained there except for short periods following rainstorms. When digested sludge was composted, the temperature rose gradually to 40–60°C after 10–14 days, and during cold wet winter weather the temperatures rose only to the 20–30°C range. Complete inactivation of the seeded bacteriophage (originally present at a concentration of 10^6 per gram) took about 50 days in composting raw sludge and up to 70 days in composting digested sludge. Naturally occurring enteric viruses were isolated throughout the windrow phase of the composting but were never isolated from the curing piles. All these experiments were conducted during the cold and wet months of October–March. Experiments were later conducted into the inactivation of seeded coliphage f2 by a forced air composting system (21 days of aerated composting followed by 30 days of curing) at the same site (Burge, Cramer and Epstein 1978). Temperatures rose to 50°C and above within the first five days, and coliphage destruction deep in the pile was complete within 13 days. However, at the edge of the pile, very small proportions (around 0.001 percent) of virus survived after 21 days. The inactivation rate in the pile was approximately 1 log unit per 2 days. Pile temperatures in the forced air system were unaffected by ambient temperature or rainfall.

Much more research is required on virus removal from various types of composting system using night soil, sludge, refuse, woodchips, and other materials. Pending this work, virus inactivation may be tentatively predicted from figure 9-2. Even where the time–temperature characteristics in the pile are well within the safety zone in figure 9-2, virus survival may still be occurring at the edges of the pile, which are

usually much cooler, especially during rain. Complete elimination of enteroviruses is therefore dependent upon pile management techniques such as turning, lagging, or forced aeration.

By other sludge treatment processes

Any sludge treatment process that involves temperatures of 50°C or above should yield a virus-free product if the process is well controlled and carried out for sufficiently long to ensure that all parts of the mass are heated. This latter point is particularly important when continuous, rather than batch, processes are being used. Examples include pasteurization (70–80°C), anaerobic or aerobic thermophilic digestion (46–55°C), wet oxidation (180–220°C), incineration (over 650°C), and pyrolysis, as well as heating and composting (discussed above).

Sludge disinfection by irradiation with high-energy electrons is attracting increasing interest. (Osborn and Hattingh 1978). The few data available on virus inactivation in sludge by irradiation indicate a rather poor removal of 75–90 percent after the application of 3–5 kilograys.[7] (Lessel and Suess 1978; Sullivan and others 1971; Ward 1977). Sludge protects poliovirus from irradiation, but little or no extra protection is afforded by increasing solids content above about 1 percent (Ward 1977). Superchlorination or chlorine oxidation (the application of 700–4,000 milligrams per liter of chlorine under pressure) may inactivate most viruses in sludge but has been objected to because it proliferates chlorinated organic compounds in the environment (Kamlet 1979).

Most of these technologies for sludge disinfection are still in the research and development stage, and many of them will prove to be too costly and too technically complex to be appropriate in most situations in developing countries.

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7. See footnote 5, above.

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