Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1968, 38, 401-414

Waterborne Viral Infections and their Prevention

SHIH L. CHANG, M. D.[1]

Unless special measures are taken, community water supplies are likely to contain enteric viruses which may lead to sporadic cases, or even epidemics, of such diseases as infectious hepatitis or poliomyelitis. After a general discussion of waterborne viral infections, in which it is pointed out that subclinical infections may considerably outnumber clinical cases, the author proposes a method for the concentration and detection of enteric viruses in water by means of membrane filtration and growth on monkey-kidney-cell or other tissue cultures.

The various methods of disinfection of water which can reduce the virus concentration to an acceptable level are discussed, and it is concluded that flocculation and filtration followed by chlorination, or ozonation followed by chlorination, are adequate methods where large volumes of water are to be treated. In developing countries where relatively small volumes of water have to be treated, iodination appears to offer certain advantages, allowing the construction of a simple water-treatment plant requiring little supervision. However, until the long-term effects of iodine, in particular on pregnant women and young children, are known iodination plants should be used only on an experimental basis.

The subject of the transmission of viral agents of human infections by water is increasingly attracting the attention of both the layman and public health workers. While lay concern may emanate from fear of " viruses ", the concern of health workers is based on the repeated occurrence of waterborne outbreaks of infectious hepatitis and on the recovery, with ease, of enteroviruses in sewage and sewage effluent and even in large municipal water supplies. This paper is intended to give a brief account of the human viruses that may be transmitted by water, to define the role of water, especially municipal supplies, in transmitting viral infections, and to discuss the prevention of such infections by water treatment.

HUMAN ENTERIC VIRUSES

Although any human virus excreted in faeces is theoretically transmissible by water, the viruses that deserve consideration are limited to those that grow in the intestinal wall and are discharged in large numbers in the faeces. This criterion restricts us to the enteric group which comprise the enteroviruses (poliovirus, coxsackievirus, and echovirus), infectious hepatitis (IH) virus, the adenoviruses, and the reoviruses. The last-mentioned two groups cause respiratory and eye infections and will not be considered here.

The enteroviruses and IH virus are small (under 30 mμ) in size. The enteroviruses have a protein shell and a ribonucleic acid (RNA) core. Little is known of the structure of the IH virus, but its size and resistance to chlorine suggest that it probably has a protein coat. It is generally believed that the destruction of these viruses in disinfection results from denaturation of the protein shell, and that removal by flocculation results from the formation of a metallic-cation–viral-protein complex and its aggregation. The fate of the RNA core of the virus in the destructive process remains to be determined.

WATERBORNE VIRAL INFECTIONS

A viral infection means establishment of parasitism of a virus in a host, whereas a viral disease is an infection with overt symptoms and signs. Hence, a viral disease may be recognized while a viral infection without symptoms will not, unless the host is examined for the presence of virus. By the same token, an outbreak of a viral disease can be recognized without difficulty, while an outbreak of a viral infection may escape attention if it involves a small

---

[1] Chief, Etiology Section, Disease Studies and Quality Control Research, National Center for Urban and Industrial Health, US Department of Health, Education, and Welfare, Cincinnati, Ohio, USA.

number of clinical cases. This differentiation is important because both the IH virus and poliovirus are much more likely to cause unrecognizable infections than clinical disease in a general population. For instance, the number of subclinical cases in the Delhi epidemic of infectious hepatitis was modestly estimated at about 10 times the number of clinical cases (Viswanathan, 1957). Only 1%-2% of poliovirus infections in an epidemic could be diagnosed on clinical grounds, while 4%-8% were abortive cases and 90%-95% were cases of infection without symptoms; the percentage of clinical cases may be considerably less under endemic conditions (Paul, 1955).

On the basis of the above-mentioned criteria, it becomes apparent that so-called sporadic cases of either of these two viral infections are probably not isolated cases but the few clinical cases among a large number of subclinical cases in an outbreak of the infection. In such an event, sporadic cases deserve much more attention than they have received in epidemiological investigations.

In discussing the significance of the finding of poliovirus in sewage and the possibility of poliomyelitis being waterborne, Maxcy & Howe (1943) ruled out the water route of transmission on the following grounds: (*a*) poliomyelitis has never been correlated with poor drinking-water supplies, (*b*) there have never been explosive outbreaks of widely scattered cases in cities with municipal water supplies, and (*c*) cities with water sources located in remote spots far from human habitation suffer from poliomyelitis as frequently as those which obtain their water from polluted streams. While considerable space could be devoted to a discussion of the mechanism of transmission of poliomyelitis, there are, as Bodian & Horstmann (1965) have stated, numerous facts which support the view that poliomyelitis is an enteric infection spread primarily by contaminated excreta.

Another point that should be stressed is that water as a route of transmission of viral infections is not important when it causes outbreaks of disease; such outbreaks, resulting from the presence of viral agents in significant concentrations, are unlikely to occur in areas with good sanitation. What is of most concern in waterborne viral infections in these areas is the low-level transmission that causes sporadic clinical cases.

When studying water as a possible route of transmission in outbreaks of viral diseases, Mosley (1967) could gather from the literature only 8 episodes of poliomyelitis in which the water route could be presumed, and 50 episodes of infectious hepatitis in which the disease was stated to be waterborne—including the 1955-56 waterborne epidemic of infectious hepatitis in Delhi, India (Viswanathan, 1957), in which more than 28 000 cases were reported. Mosley concluded that " at the present time, we have no evidence that unrecognized transmission of any viral diseases is occurring under such circumstances or to such extent as to require revision of accepted minimum standards." At the same time, he stated that stringent epidemiological proof of water transmission involves difficulties which are inversely proportional to the size of outbreak and directly proportional to its duration, and that we must consider the possibility of low levels of viral transmission with the present standard treatment of water, producing sporadic cases of infectious hepatitis and other viral diseases. The question is, then, if we believe that a municipal water supply may be transmitting viral diseases at a low level, should we consider the treatment processes employed adequate and the minimum standards safe, or should we examine the situation seriously and eliminate or reduce to a practical minimum the water route of transmission?

When Coin et al. (1965) reported on the finding of poliovirus in Paris city water at the second International Conference on Water Pollution Research in Tokyo, several European participants expressed acceptance of rather than alarm at the report (personal communications). The reaction is typified by the comments of Bouquaix (1965) that the virological findings of Coin et al. are to be expected, that the presence of some viruses and bacteria in purified water actually represents certain advantages for the establishment of general immunity, and that if Coin and his associates consider the virus level in their municipal supplies unsafe, they should find more efficient methods of water treatment.

This philosophy is rather attractive to those who are interested in the prevention of infectious hepatitis, since a vaccine against this disease will have to wait until the IH virus (or possibly viruses) can be propagated in the laboratory. One may not object to the hypothesis that very low-level transmission of the IH virus by water supplies is responsible, at least partly, for the high percentage of immunity among the adult population. The point deserving consideration here is that if water is regarded as an immunization medium it must have the same degree of safety as, e.g., poliomyelitis vaccine. If clinical cases of infectious hepatitis occur sporadically and the occurrence can be blamed on the water supply, the safety of this medium is doubtful and more efficient methods of rendering it safe should be sought.

In regions with poorer sanitation, viral infections transmitted by water are more prevalent, owing apparently to more severe pollution of the raw water and lower efficiency of the water treatment. The writer visited India at the invitation of WHO in March–April 1967. His observations on the Jamuna River, the Delhi water treatment plants, the rapid disappearance of the brownish colour when 2 ppm–3 ppm of iodine (as tincture of iodine) was added to tap-water, and the water-drinking habits of the people, together with the information gathered from local physicians on the occurrence of infectious hepatitis, convinced him that the 1955–56 waterborne epidemic of infectious hepatitis was only a much more extreme phase of this viral infection, which occurs in Delhi owing to the existence of special social and climatic factors (Viswanathan, 1957). There was a significant increase in the number of cases every year following the monsoon season. The only difference between these annual episodes and the 1955–56 epidemic is that the latter involved so many more cases of the disease that it drew the attention of the health authorities. It would be absurd to consider the 1955–56 epidemic as unquestionably waterborne and these smaller episodes as unrelated to the municipal supplies.

Of particular interest is the fact that poliomyelitis occurs in India concurrently with infectious hepatitis in the fall. Although case records are lacking in most cities, the epidemiological investigation made by the Pasteur Institute in Coonoor on the occurrence of poliomyelitis in Dhalavoipuram (Balasubramanian, 1967) brought out features which typify the infection pattern. Over 50 suspected cases were treated by physicians in this southern Indian town, which has a population of 8000, in the fall of 1965 and winter of 1966, with maximum incidence in November—a seasonal pattern similar to that of the Delhi IH epidemic. Although the diagnosis was verified in only 10 cases, many if not all of the remainder could have been abortive (Paul, 1955). Taking into account the large number of cases of symptomless infection which are associated with paralytic cases (Paul, 1955), this frequency of poliomyelitis had a relative significance approaching, if not equal to, that of the Delhi IH epidemic.

The water supplies were not considered as a route of transmission in the investigation, and other sources of infection were not located. Personal communications revealed that the water supplies in Bhalavoipuram are exposed to heavy faecal pollution during the monsoon season. When all the facts are taken into consideration, it may be seen that this outbreak of poliovirus infection was most probably waterborne.

Large municipal water supplies in developed countries usually draw their raw water from sources without gross pollution, and the raw water is generally given more efficient treatment. With poliomyelitis cases reduced to a minimum through mass immunization, only the incidence of infectious hepatitis can be used to associate the water supply with viral infection. Clinical cases are usually so few in number and so sporadic, and the data on water treatment, such as the routinely recorded turbidity and residual chlorine, are so unreliable, that no convincing correlation can be established even by very careful analysis. It is not surprising that Hudson (1962) found no significant correlation between chlorination and finished water turbidity and the IH incidence in 12 cities in the USA in 1953; it may be mentioned that Taylor et al. (1966) obtained a suggestive correlation, unsupported by a correlation coefficient, between the residual chlorine and IH incidence, in a similar but more elaborate analysis made in 1961.

A meaningful correlation will have to await data on (*a*) virus concentrations in raw and in finished water, (*b*) enteroviral infection rates among children ascertained in rectal-swab surweys, (*c*) enteroviral antibody development patterns in children, from serological surveys, (*d*) incidence of clinical cases of infectious hepatitis and possibly other enteroviral diseases with carefully worked-out case records, and (*e*) carefully checked water-treatment data. Meanwhile, we must adopt an open-minded attitude toward the question of waterborne enteroviral infections and consider, as Mosley did, the possibility of low-level viral transmission with the present standard treatment, if polluted water is used as a source of municipal supply and sporadic cases of infectious hepatitis (or other enteroviral disease) occur in the population using such supply. The fact remains that sewage carries enteric viruses practically all the time; and sewage-treatment processes, with the exception of the activated sludge process which may effect up to 90% reduction of viruses (Clarke et al., 1962; England et al., 1967; Kelly et al., 1961; Mack et al., 1962), are relatively ineffective in reducing the virus concentration in the effluent

(Kelly & Sanderson, 1959; England et al., 1967; Mack et al., 1962; Malherbe & Strickland-Cholmley, 1967).

ENTERIC VIRUSES IN WATER AND METHODS FOR THEIR DETECTION

When a body of water receives sewage or sewage effluent after conventional treatment (virus concentration can be reduced by a factor of 10^6–10^9 by advanced waste treatment including chlorination of the clarified effluent), the presence of enteric virus in the polluted water cannot be excluded. The viral density in such water may be very low or it may be too high to be adequately treated by conventional methods for drinking purposes, depending chiefly on (*a*) the original viral density of the polluting material, (*b*) the dilution factor, (*c*) the time elapsing after discharge, and (*d*) the physical and chemical state of the water. Very few, if any, microbial processes are expected to change the enteroviral density in water or in sewage (Chang, 1967).

The IH virus does not lend itself to laboratory examination or cultivation; enteroviruses may be used as indicators in the same manner as coliform bacteria.

A method suitable for the detection of enteroviruses in water will have to fulfil the following conditions: (*a*) it should concentrate small numbers of viral units from large volumes of water, (*b*) the virus material thus concentrated should be suitable for inoculation into tissue cultures for determination of the viral density, and (*c*) ideally it should separate the virus from bacteria, protozoa, and other microbes, as well as from toxic material to avoid the detrimental effect of the latter on tissue cells. Several methods have been reported for such use (Anderson et al., 1967; Bier et al., 1967; Cliver, 1967; Godbole et al., 1966; Shuval et al., 1967; Wallis & Melnick, 1967a, 1967b). The methods that employ the Millipore (not Gelman GA) 0.45μ membrane appear most promising and most suitable for field use. Preliminary work, done by Cliver (1967) on enteroviruses suspended in deionized water, tap-water, and buffered phosphate saline, showed a 99% or better adsorption of viruses on to the membrane, and 80%–90% of the adsorbed viruses were eluted when the membrane was soaked for 30 minutes in 1 ml of phosphate-buffered saline containing 30%–50% chicken serum without gamma-globulin. Cliver's findings have been confirmed by tests done at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio (Berg et al., 1968) and by Wallis & Melnick (1967b).

The study carried out at the Taft Center employed a sterile 3% aqueous solution of beef extract as a virus-eluting agent, and a number of antibiotics to control microbial contamination when the beef extract solution was plaqued in monkey kidney-cell cultures. Complete recovery of poliovirus 1 (Mahoney) and echovirus 7 (D'Amori) was obtained when the membrane was soaked for 30 minutes in 5 ml of 3% beef-extract solution. In applying the technique to recover poliovirus 1 (LSc vaccine strain) in sewage effluent from secondary treatment, Berg and co-workers used 5.1 ml of beef-extract solution followed by 5.1 ml of Earle's balanced salt solution as a rinse, and recovered only 41%–59% of the virus from a litre of effluent. The presence of organic matter in the effluent might have reduced the adsorption sites on the membrane.

While some of the steps in the method are still being defined, the procedure described below appears practical and may be used on an experimental basis, with or without modifications.

Filter from 1 to 8 litres (depending on the filtrability) of a water sample through a Millipore HA-type (0.45μ) membrane at a rate not exceeding 150 ml/min. Disconnect the unit and place the holder with membrane on a sterile 200-ml suction flask. Pour 5 ml of sterile 3% aqueous solution of beef extract (Difco Bacto) over the membrane and allow it to filter through without pressure. If the filtration is not completed in 30 minutes, apply gentle suction at a pressure of about 2 lbf/in^2 (0.14 kgf/cm^2) and press the holder firmly against the rim of the flask to complete the process. The solution thus filtered is dispensed in 1-ml amounts over a monkey-kidney-cell sheet in each of 5 bottle cultures for enumeration of the plaque-forming units (PFU). A 10-day to 12-day incubation at 35° C is needed for full development of the plaques; but examination should start on the third day of incubation and be carried out daily until no new plaques are observed. Virus or viruses can be isolated in tube cultures from individual plaques and identified with the aid of specific antibodies; but more time and facilities are required for such identification.

Since the virus recovery efficiency of the membrane filter is influenced by the physical and chemical state of the water, inconsistency in virus recovery is to be expected in the use of this method on different types of water, and modifications may be necessary. For instance, if the water samples are too turbid, they can be filtered through coarse Gelman membranes prior to the Millipore-membrane filtration. If the water is too soft, about 100 ppm of calcium (as $CaCl_2$) can be added to facilitate virus adsorption. The pH, in the range normally encountered in natural surface waters, will not influence the capacity of the membrane for virus adsorption. Proteinaceous substances, if present in significant amounts, as in sewage effluent, may reduce the virus recovery efficiency of the membrane. The use of one or more cell systems in addition to the monkey-kidney cells should improve the virus recovery efficiency.

PREVENTION OF WATERBORNE VIRAL INFECTIONS BY WATER TREATMENT

The testing of both the raw and finished water of municipal supplies for the presence of viruses is highly desirable to provide factual information on the efficiency of the treatment as well as on the role of water in the transmission of enteric viral infections. It should be carried out whenever and wherever possible, once a standard method for virus detection has been worked out. Even when a method has been developed, there is still a long way to go before an enterovirus index can be developed for use in water treatment practice to protect the public against enteroviral diseases, particularly infectious hepatitis.

Until a virus detection method and an enterovirus index are developed, circumstantial evidence has to be used to ascertain the role of the water supply system in transmitting viral infections in a community. If polluted raw water is used and if cases of enteroviral diseases, i.e., infectious hepatitis, occur without a known mode of transmission, the water-treatment processes should be examined. If the adequacy of the treatment processes is questionable, steps should be taken to ensure the safety of the finished water.

A fair amount of information is available on the efficiency of various treatment processes in the removal and destruction of enteroviruses, but little is known in relation to the IH virus. However, general guidelines can be laid down for the latter in the light of the more complete information on the former.

The information available on virus survival and the relative efficiencies of various treatment processes in destroying or removing enteric viruses is presented here in a very condensed manner.

Storage

Owing to the existence of many unknown factors affecting virus survival in water, the differences in the survival times of different enteroviruses observed in storage (Clarke et al., 1964) are not sufficient to indicate that some enteroviruses survive significantly longer than others. In general, a 99.9% reduction requires from a few weeks in the warm season to a few months in the cold season in temperate regions and shorter time in the tropics and subtropics. The IH virus has been shown to survive more than 10 weeks in well water (8½ weeks at the temperature prevailing in the wells and 4 weeks at room temperature) (Neefe & Stokes, 1945), although there was a considerable prolongation in incubation time with storage time, a phenomenon regarded by these authors as an indication of reduction in virus concentration. It appears that only long storage is effective in reducing virus concentrations significantly, and even in large bodies of water there is no safeguard against currents, caused by convection or wind, carrying particles through the reservoir in a matter of hours rather than the weeks which might be the theoretical holding time.

The pH of the water used as a supply source usually lies within the range well tolerated by enteric viruses. Salts, especially with polyvalent cations, may even increase the survival time of enteroviruses in the high-temperature range (Wallis & Melnick, 1962). The aquatic flora and fauna appear to have very little, if any, influence on the survival of the enteric viruses (Chang, 1967). It may, then, be concluded that the storage time normally available in water treatment plants is not to be depended upon for any significant reduction of the enteric virus concentration in the water.

Flocculation

Since the chemical basis for the removal of enteric viruses by flocculation is the formation of a metallic-cation–protein complex and its aggregation, which is a relatively nonspecific metal–protein reaction, the efficiency of the process in removing one enteric virus should not differ significantly from that in removing another. The laboratory data obtained by Chang et al. (1958) and the data of Robeck et al. (1962) obtained in a pilot-plant study indicate that a 99% removal of enteroviruses can be achieved in the field if the process is carried out in a satisfactory manner. The data of Chang et al. (1958) also indicate that the removal of viruses parallels that of total bacteria, coliform bacteria, and turbidity. If allowance is made for a lower efficiency of the flocculation process under field conditions, a 90%–95% removal of waterborne virus should be expected. The observations of Neefe et al. (1947) on IH virus in volunteers indicate that alum flocculation significantly reduced the concentration of this virus: volunteers who drank water containing infective faecal material clarified by alum flocculation came down with the disease after a much longer incubation period than those who drank the infective water without alum flocculation; as stated above, the length of the incubation period appears to be inversely proportional to the concentration of the virus ingested.

Seasonal changes in temperature and moderate changes in the turbidity of the water exert an insignificant influence on the virus-removal efficiency of the flocculation process, but a large increase in turbidity requires an increase in the dosage of the flocculant to obtain a satisfactory removal of waterborne viruses (Chang et al., 1958). Clear water cannot be satisfactorily flocculated in the usual manner (Gilcreas & Kelly, 1955),unless special flocculation processes, e.g., "in-filter" flocculation, are employed, in which the water is dosed with a flocculant and flock formation is induced, e.g., by allowing the water to pass through a coarse medium on top of the sand filter (Robeck et al., 1964). The virus is not destroyed by flocculation and can be reactivated by dissociating it from the complex in the flock by special laboratory techniques (Stevenson et al., 1956).

It may be concluded that flocculation is effective in reducing the virus concentration as well as the total amount of organic matter in water, thus making the chlorination or other chemical disinfection more effective in destroying the viruses (and other pathogens). It should not be considered as a disinfection process by itself.

Filtration

Filtration is not to be depended on for any significant removal of viruses in water. Most of the earlier workers, as revealed in the literature survey by Clarke & Chang (1959), obtained poor virus removal in laboratory studies in which the virus suspensions were usually prepared from infected animals. The poor removal probably resulted from the high concentrations of extraneous organic matter in the suspensions, which competed with the virus particles for the limited adsorption sites on the sand grains.

The recent study of Robeck et al. (1962) showed that 1%–50% of added poliovirus was removed by rapid sand filtration alone at rates of 2880–8640 US gal/d/ft^2 (about 120 000–350 000 l/d/m^2) and 22%–90% removal by slow sand filtration at a rate of 50 gal/d/ft^2 (about 2000 l/d/m^2). The observations of Neefe et al. (1947) on the IH virus, on the basis of the incubation-period criterion stated above, indicate insignificant removal of this virus by diatomaceous earth filtration: incubation periods in persons infected with the flocculated and filtered water were no longer—in fact, sometimes shorter—than those in persons infected with the flocculated water.

It should be noted that the slow sand filter used by Robeck et al. (1962) and that used by Gilcreas & Kelly (1955) did not have the *Schmutzdecke* (biological film) which is generally believed to be essential for satisfactory performance of this type of filter—however, recent observations by Huisman and his associates in the Netherlands do not bear out this view that the *Schmutzdecke* is necessary (personal communication). In any case, little is known of the virus-removal efficiency of slow-sand filters with *Schmutzdecke.*

The virus-removal efficiency of flocculation and rapid sand filtration combined appears to be slightly better than the combined efficiency of the two processes conducted independently. For instance, Robeck et al. (1962) obtained better than 99.7% removal of poliovirus 1 by flocculation with settling followed by rapid sand filtration, while the combined efficiency of the two carried out independently was less. This improved performance may be attributed to the higher efficiency of the sand filter in removing virus particles in the unsettled flock rather than those freely suspended in water.

Rapid sand filters impregnated with preformed alum flock appear to remove viruses better than those without the alum (Carlson et al., 1942). Since there was a reduction of the filtration rate from about 30 ml/min/m^2 to 22 ml/min/m^2 in these authors' study, it appears that the improved virus removal could have resulted, at least partly, from the reduced filtration rate. Chang et al. (1958) obtained very little, if any, virus removal when the virus suspension was added to the water 1 minute after the dosing of alum. The effectiveness of rapid sand filters impregnated with preformed flock and operated at a high filtration rate remains to be determined.

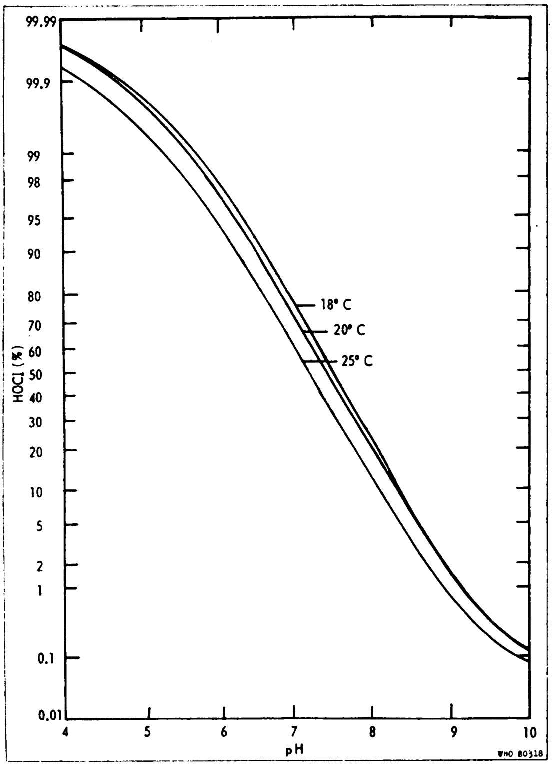
Virus destruction

Chlorination. When gaseous chlorine is dissolved in water, the Cl_2 hydrolyses into HOCl (hypochlorous acid) and HCl. The hydrolysis constant is so relatively large that practically all the Cl_2 is converted to HOCl in any water chlorination process.

The HOCl ionizes into H^+ and OCl^- ions; the degree of ionization depends chiefly on the pH of the solution and much less on temperature. These same reactions take place in the reverse direction when a hypochlorite solution is acidified. Below pH 6.0, practically all of the titratable chlorine exists as HOCl; above pH 9.0, nearly all the chlorine is to be found as OCl^- ions. Varying amounts of HOCl exist at pH values between 6.0 and 9.0. Although HOCl is a far more powerful disinfectant than OCl^- ion, they are lumped together as " free chlorine "; no practical method differentiates one from the other and the relative amounts of HOCl and OCl^- ion have to be computed from HOCl ionization curves; see Fig. 1 (Chang, 1947).

In water containing ammonia, amino compounds or both, HOCl reacts with these to form ammonia-chloramines, organic chloramine (N-chlor) compounds, or both. The titratable chlorine existing in these compounds is known as " combined chlorine ". When the molar ratio HOCl : (NH_3+RNH_2) is greater than 1, the excess HOCl goes to form dichloramines. In the absence of excess ammonia or amino compounds, the formation of dichloramine from monochloramine takes place as the pH is lowered from 7.0. The dichloramines are more active disinfectants than the monochloramines because they hydrolyse to form HOCl. The degree of hydrolysis depends on the hydrolysis constant of the particular species of dichloramines. The combined chlorine can be titrated by iodometric and colorimetric methods, but is much less active than HOCl. Combined chlorine can, however, be determined separately from free chlorine by *o*-tolidine-arsenite or amperometric titration.

FIG. 1
DISSOCIATION OF HOCl IN WATER



The data of Weidenkopf (1958) on the destruction of poliovirus 1 by free chlorine at 0° C reveal that HOCl is considerably more effective than the OCl^- ion. This is in line with the data of Kelly & Sanderson (1958) on the destruction of enteroviruses. Kelly & Sanderson (1958, 1960) found that the virucidal efficiency of HOCl is more than 50 times greater than that of the ammonia chloramines. Organic chloramines, such as chloramine-T, are even less virucidal than ammonia chloramines (Clarke & Chang, 1959; Kjellander & Lund, 1965). The limited data on IH virus destruction obtained by Neefe et al. (1945, 1947) also indicate that free chlorine is more active than combined chlorine.

The common use of the expression " free chlorine " in the literature, the calculation needed to compute concentrations of HOCl from pH and free chlorine values, and the theoretical nature of expressing titratable chlorine as HOCl prompt the writer to use the expression " free chlorine " in preference to " HOCl ".

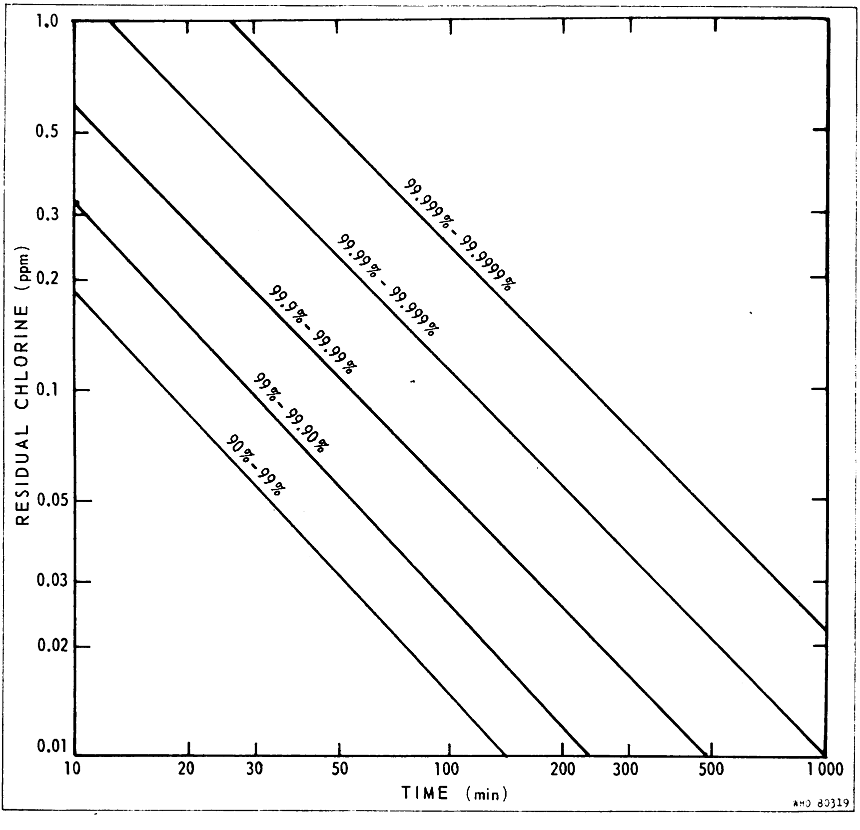
Enteric viruses differ in their resistance to free chlorine. Adenovirus 3 is less resistant than *Escherichia coli*, while poliovirus 1 and coxsackievirus A2 and A9 appear to be more resistant than any of the other enteroviruses studied (Clarke et al., 1964). Some of the differences in resistance among the " hardier " viruses could be attributed to the methods used in determining virus survival. In judging the virucidal efficiency of chlorination processes, these resistant viruses should be used as test organisms. The resistance of IH virus to free chlorine appears to be quite high if a residual chlorine concentration of 0.4 ppm after 30 minutes at room temperature (Neefe et al., 1947) is used as a criterion for satisfactory virus destruction. Assuming that the infective faeces used by these authors contained 10^6 infective organisms per gram, which is a reasonable assumption based on data obtained from poliomyelitis cases, and assuming that flocculation and diatomaceous-earth filtration removed 99% of the virus, it may be estimated that this residual chlorine concentration after 30 minutes means a better than 99.8% destruction of the virus. This is not too different from the figures found for the destruction of poliovirus 1 and coxsackievirus A2 and A9 under similar conditions (Clarke et al., 1959; Kelly & Sanderson, 1958).

It is difficult, if not impossible, to set a fixed free chlorine concentration and contact time for the chlorination of water in all cases met with in practice. The dosage-time combination depends on the percentage of virus destruction desired and on the pH and temperature of the water. To ensure satisfactory chlorination, the water must have very little ammonium ion and organic matter and a low virus concentration. A high ammonia or organic content will not only require a high chlorine dosage to leave enough free residual chlorine to obtain the desired level of virus destruction, but will also result in too much combined chlorine to provide a palatable water. Flocculation and filtration are needed to purify the raw water to a degree suitable for chlorination. If the water has too low a turbidity to facilitate conventional flocculation, " in-filter " flocculation or comparable processes may be used to render the water suitable for chlorination.

Taking all enteric-virus destruction data into consideration, it is however possible to derive a set of residual chlorine concentrations and contact times which will give the desired level of enterovirus destruction with water of a given quality. A slight margin of safety should be allowed for IH virus, which might possibly be a little more resistant to chlorine than the hardy enteroviruses. These combinations are shown in Fig. 2; 1 ppm of residual

FIG. 2

RESIDUAL CHLORINE CONCENTRATIONS AND EXPOSURE TIMES FOR THE DESTRUCTION OF POLIOVIRUS 1 AND COXSACKIEVIRUS A2 AND A9 AT TEMPERATURES ABOVE 4°C AND pH VALUES BELOW 8.0



chlorine should give better than 99.999% virus destruction in 30 minutes at pH values of 8.5 or less and temperatures above freezing. Under most practical conditions, where the pH is below 8.0 and the temperature above 4°C, residual concentrations of 0.1 ppm–0.2 ppm, 0.2 ppm–0.3 ppm and 0.3 ppm–0.4 ppm should attain virus destruction rates of 99%–99.9%, 99.9%–99.99% and 99.99%–99.999%, respectively, in 30 minutes. At pH values of 8.0 and over but below 9.0, there should be a 50% increase in either residual chlorine or contact time to achieve the same results.

Ozonation. The data of Kessel et al. (1943) and of Hettche & Schulz-Ehlbeck (1953) indicate that ozone is more virucidal than free chlorine when the comparison is made on a weight basis. Coin (1967) reported that 0.4 ppm of ozone can destroy poliovirus 1 in tap-water in 30 minutes, and that the virucidal concentration of ozone is slightly less for poliovirus 2 or 3. Trahtman (unpublished data, 1966) obtained a 99.9% destruction of enteroviruses with 0.2 ppm of ozone; no contact time was given.

Ozonation of water has the advantage of absence of odour and taste but the disadvantages that it is hard to disperse the ozone uniformly and to maintain a residual concentration in the treated water. It is ideal for water treatment prior to chlorination to reduce the chlorine demand and the pathogen load, thus making the chlorination process more satisfactory and less objectionable on the grounds of odour and taste.

Iodination. In a recent report, Chang (1966) showed that iodination can be satisfactorily applied to water under field conditions where proper chlorination may run into difficulty, such as in rural areas or underdeveloped regions. This is because elementary iodine can be added to water at a constant dosage without the need of a complicated set-up and knowledge of the chemical state of the water, and the residual iodine concentration can be roughly estimated from the colour imparted to the water. The virucidal efficiency of I_2 is about 200 times less, but that of HOI (hypoiodous acid) is only about 4–5 times less, than that of HOCl (Clarke et al., 1964). At pH values between 7.0 and 7.5 and residual iodine concentrations between 1 ppm and 2 ppm, 20%–60% of the titratable iodine will exist as HOI; the lower the pH and the higher the residual iodine concentration, the lower is the percentage of HOI formed.

The ease with which elementary iodine can be used to treat small water supplies is due to the fact that iodine is not very soluble in water. When water passes through a column of iodine crystals, it becomes a saturated solution of I_2 containing from about 200 ppm at 10°C to about 400 ppm at 30°C. This solution can be used for dosing water in calculated volume ratios to give the desired concentrations of I_2. A column of depth 30 cm–40 cm will ensure a saturated I_2 solution at almost any rate of passage of water through it. The loss of I_2 by sublimation can be prevented by maintaining a suitable depth of water above the column with a water-level control device. The constant depth of the I_2-crystal column can be maintained by periodic replenishment with a fresh supply. With a set-up consisting of a hand pump, an I_2-crystal column, a Venturi control valve and a reservoir tank to provide the detention time, a small water supply can be reasonably and satisfactorily disinfected without the need of preparing stock solutions of disinfectant, determining the strength of the stock solution and the residual concentration of disinfectant by titration, and other procedures that are essential for satisfactory operation of a process such as chlorination.

The combinations of residual iodine concentration and contact time required for the destruction of bacteria, enteroviruses, and protozoan cysts, which were presented graphically by Chang (1966), are reproduced in Fig. 3. As shown in the figure, 99.9% destruction of both amoebic cysts and poliovirus 1 requires residual iodine concentrations and contact times which lie within the range permissible in practice. To make the process reasonably safe for treating water over a long period of time, the dosage of iodine should not exceed 4 ppm, or preferably 3 ppm. If a residual iodine concentration of 0.6 ppm–1.0 ppm after 30 minutes is desired, and if the process is aimed at 99.9% destruction of viruses and amoebic cysts, the water suitable for use as a supply must have a relatively low content of organic matter, including pathogens. This limits the raw source to water no more than slightly polluted. Fig. 4 provides information on the hydrolysis of I_2 to form HOI at different pH values and iodine concentrations.

While dosages of iodine up to 4 ppm are unlikely to impart an objectionable odour and taste to the treated water or to produce untoward physiological effects among adults (Freund et al., 1965), the effect in pregnant women, infants, and small children if the iodination process is used on a permanent basis remains to be ascertained. Before such data are gathered, the process should, therefore, be used only on an experimental basis.

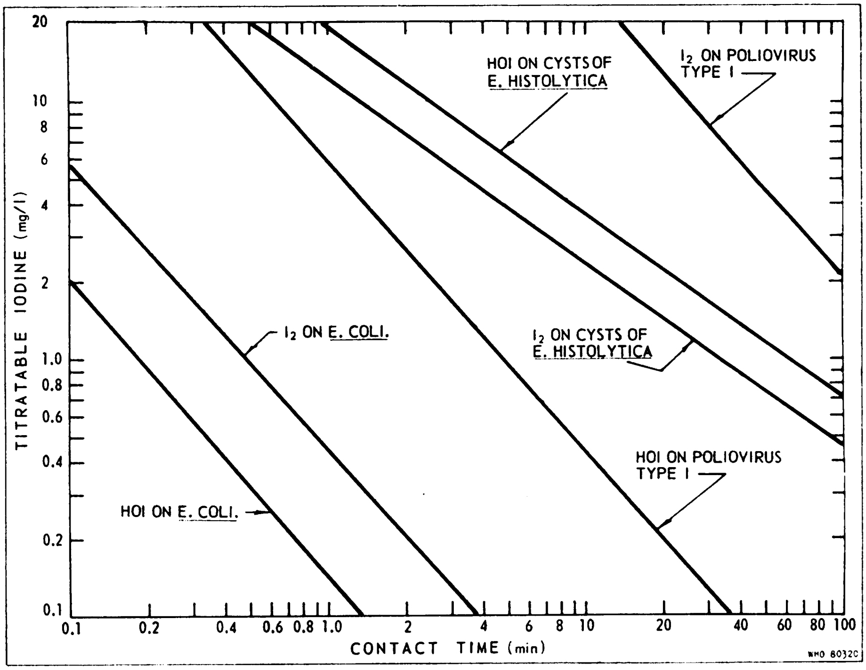


FIG. 3

CONCENTRATIONS AND EXPOSURE TIMES FOR 99.9 % DESTRUCTION OF CYSTS, VIRUSES AND BACTERIA BY I_2 AND HOI AT 18°C

Chlorine dioxide (ClO_2). Chlorine dioxide can also be used in water disinfection. The compound is frequently prepared by passing gaseous chlorine through a solution of chlorite. Unless purified, the resultant solution contains a mixture of ClO_2 and HOCl. Determination of the virucidal activity of ClO_2 relative to that of HOCl will have to await the preparation of pure solutions of ClO_2 and the development of a method for measuring ClO_2, not just titratable chlorine. Chlorine dioxide does have the advantage over HOCl that it is not as greatly affected by high pH and does not react with ammonia or amino compounds to form chloramines, nor with phenolic or quinolic compounds to give a chlorophenol taste.

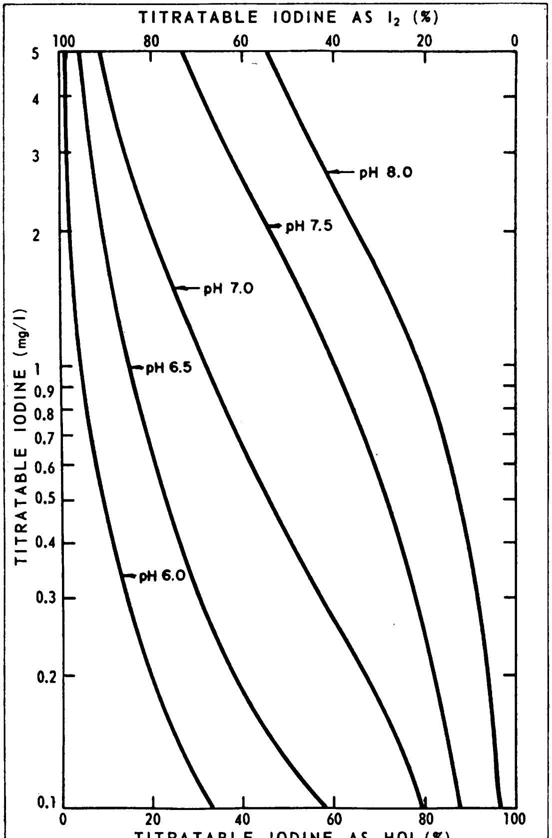
Other disinfecting processes. Bromine deserves mention. Its atomic weight lies between those of chlorine and iodine. It also hydrolyses, into HOBr and HBr, but the hydrolysis constant is not very large, so that Br_2 can exist in small amounts at water disinfection dosages if the pH is kept below 5.0. The HOBr dissociates into H^+ and OBr^- ions; the ionization constant is so relatively small that 85% of the HOBr formed by hydrolysis remains undissociated even at pH 8.0, and 30% at pH 9.0. Little is known of the virucidal efficiency of HOBr. The meagre information obtained by McLean (1967) on the elimination of low concentrations of enterovirus in swimming-pool water indicates that an initial concentration of 6.0 ppm–7.0 ppm of bromine produces a virucidal effect comparable to that produced with 1.0 ppm of free chlorine or 4.0 ppm–5.0 ppm of I_2. The relatively high dosage of bromine required may be attributable to the fact that water invariably makes higher demands on bromine than on either chlorine or iodine. At dosages of a few ppm, it is doubtful whether bromine will have any physiological effect worth mentioning.

DISCUSSION

It is not the writer's intention to create fear in the public of waterborne viral infections in towns; nor is it his intention to blame water-treatment plants for failing to provide purification which can prevent viral transmission. His intention is to point out the facts that water polluted by sewage, sewage effluent, or the like is likely to carry enteric viruses (and other

FIG. 4

PERCENTAGES OF TITRATABLE IODINE AS I_2 AND HOI IN WATER AT 18°C



enteric pathogens), that flocculation, filtration, marginal chlorination (leaving very little or no residual chlorine) or ozonation will be unlikely to reduce the virus concentration to a safe level for mass consumption, and that the coliform index used as the minimum standard will not ensure the safety of the water against viral infections (Coin et al., 1967; Foliguet et al., 1966).

A standard method for enterovirus detection in water is needed and an enterovirus index for a minimum standard is highly desirable. Before these two virological criteria are worked out, we must base ourselves on circumstantial evidence and not on epidemiological dogma in ascertaining the role of water supply in viral transmission in nonepidemic times. If a community uses a polluted source as a water supply and if sporadic cases of infectious hepatitis or other enteric viral diseases occur in the community, the water supply must be considered as a possible route of low-level transmission of viral diseases.

The water treatment and distribution services should then be improved—even if they are acceptable by current water-treatment standards.

In communities where virological services are available, co-operation with the virus laboratory should be sought for a virological survey of the raw as well as of the finished water, by the method outlined in this report with or without modifications or by any other suitable method. While such a survey will be experimental in nature, the data thus obtained are invaluable for the development of a standard method and an enterovirus index.

As stated above, the treatment efficiency desired depends highly on the percentage virus reduction aimed at, which in turn depends on the virus concentration expected in the raw water. In the absence of virological data and an enterovirus index as a minimum standard, a certain degree of intuitive judgement cannot be avoided in considering the levels of virus reduction that should be attained by the treatment process for the prevention of low-level transmission of virus diseases in municipal supplies.

The estimated virus concentration of from 1.5 PFU/litre (plaque-forming units per litre) of polluted water in cold months to 15 PFU/l in warm months (Clarke et al., 1964) was based on an enterovirus carrier rate of 10% among children under 14 years of age, and moderate pollution. The virus concentration in polluted water will be correspondingly higher if the carrier rate is higher or pollution heavier. Taking all matters into account and leaving a margin of safety in the estimation, an enterovirus concentration of 30 PFU/l may be regarded as a reasonable virus load in moderately polluted water.

To ascertain the percentage virus reduction desired in water treatment, the data of Coin (1967) on enteroviruses in Paris city water are used. Coin stated that poliovirus is present in most water supplies, but the virus concentration is unlikely to be more than 1 $TCID_{50}$ (tissue-culture-infective dose) in 250 litres of water. Since the $TCID_{50}$ is a smaller viral unit than the PFU (Chang, 1967), one may express Coin's data as 3 PFU/kl of Paris city water. Using the figure of 30 PFU/l as the initial virus concentration in the raw water, a final concentration of 3 PFU/kl implies a 99.99% reduction. If this is believed inadequate to prevent low-level transmission of virus diseases, a residual virus concentration lower than 3 PFU/kl may have to be considered as the goal of water treatment.

How low should the minimum residual virus concentration be? Disinfection is basically a rate process in the chemical sense, that is, theoretically speaking 100% elimination of the pathogen is impossible. While this principle does not hold in practice for the disinfection of small volumes of water, it does when the volume is huge, as in municipal supplies. For instance, a volume of 10 litres of water having a virus load of 30 PFU/l can be freed of virus by a process that possesses a 99.9% reduction efficiency, since the chances of having 1 PFU in the 10 litres are practically nil when the expected residual virus concentration is 3 PFU/100 litres. When tens of thousands of kilolitres of water are involved, as in municipal supplies, it is impracticable to treat such large volumes so thoroughly that there will be less than 1 PFU in the entire supply. Compromising between safety and practicability, it is felt that 1 PFU/10 kl may be considered as the minimum residual virus concentration in an adequately treated water supply. This virus level is 1/30 that in Paris city water and requires a treatment that can remove 99.9993% of the virus load from moderately polluted water.

One should expect a 99% virus removal from a satisfactorily performed flocculation process with settling followed by rapid sand filtration. The remaining virus reduction desired must, therefore, be obtained by disinfection. Chlorination is preferred to ozonation because of the difficulty of dispersing ozone uniformly and maintaining a residual concentration in the treated water. To obtain a 99.93% reduction of the remaining virus concentration, 0.25 ppm–0.30 ppm of free chlorine is needed for 30 minutes. If the pH is 8.5 or higher, the residual chlorine concentration should be increased to about 0.4 ppm or the contact time lengthened to 45 minutes.

Ozonation is, however, an ideal pretreatment process: it reduces the chlorine demand as well as the content of virus (and other pathogens) in water, thus allowing the chlorination to achieve the desired level of virus destruction more easily without creating odour and taste problems. For instance, if clear but polluted lake water is used as raw water, and if flocculation and filtration facilities are lacking or cannot function properly, a preozonation to leave 0.1 ppm of residual O_3 for 10 minutes should result in 99%–99.9% virus destruction. Chlorination with about 0.2 ppm of residual chlorine for 30 minutes should then reduce the virus concentration to the assumed safe level of 1 PFU/10 kl, if not lower.

If a virus reduction greater than that obtainable by flocculation and filtration followed by chlorination is desired, and if preozonation is considered impracticable, prechlorination of the raw water beyond the " breakpoint " (to destroy ammonia-chloramines and leave the residual chlorine as free chlorine) may be applied. This practice is quite common in the USA.

In developing countries where water supplies in villages and fringe areas around cities are just as important as municipal supplies, disinfection of such supplies to prevent waterborne viral and other infectious diseases is imperative but difficult. Raw water is usually obtained from shallow wells, streams or ponds (tanks in India and neighbouring countries) subject to faecal contamination, and treatment requiring electricity, machinery, and laboratory control of some sort is often impossible. Under these circumstances, iodination may be preferred to chlorination or permanganate treatment, owing to the ease with which iodination can be carried out and the greater disinfecting efficiency of iodine compared with permanganate. In the absence of information on the physiological effect of iodine on pregnant women, infants, and small children when very small amounts are ingested over a long period of time, iodination should be used in treating such supplies on an experimental basis to study its effectiveness in preventing waterborne infections as well as its physiological effect.

Viral infections associated with pollution of water other than municipal and small supplies, such as swimming pools, recreation beaches and shellfish beds located in harbours and estuaries, are not discussed in this report. Adenovirus infections have been associated with swimming pools (Bell et al., 1955; Ormsby & Aitchison, 1955; Cockburn et al., 1956), and the mode of infection has been believed to be respiratory or ophthalmic. Enteric viral infections, contracted either directly at polluted beaches or indirectly through shellfish harvested from polluted beds, have been discussed in detail in a report by Brisou (1968).

Any infection that is of faecal origin can, of course, be spread through a number of routes, such as water, person-to-person contact, contaminated foods, and contaminated domestic insects. The enteric viral infections are no exceptions. The water route is, however, stressed in this report because it has been neglected in epidemiological considerations of sporadic cases. The author has never stated in any part of the report that water is the only route of transmission; but he wants to make it clear here that he feels that the water supply should not be omitted from consideration when possible modes of transmission of sporadic cases of infectious hepatitis (and other enteric viral diseases) in a community are being considered.

Judging by the efficacies of the various treatment processes described in the report in removing or destroying enteric viruses in water, it appears that high levels of viral reduction are well within reach in water-treatment practice. This does not mean that water exposed to any degree of pollution can be satisfactorily treated. Under no circumstances can we neglect the fact that a good water supply depends not only on adequate treatment processes but also on rigid control of pollution of the raw source.

RÉSUMÉ

Parmi tous les virus qui peuvent être éliminés dans les matières fécales de l'homme et être en principe propagés par l'eau, certains doivent retenir spécialement l'attention, comme les entérovirus (poliovirus, coxsackievirus, échovirus) et le virus de l'hépatite infectieuse. En l'absence de mesures appropriées, on doit s'attendre à ce qu'ils soient véhiculés par l'eau destinée à la consommation humaine, avec comme conséquence l'apparition de cas sporadiques ou d'épidémies d'affections virales. Lors d'une épidémie d'hépatite infectieuse survenue à New Delhi, Inde, en 1955-1956, où l'on dénombra plus de 28 000 cas, l'origine hydrique de l'infection a été reconnue et l'on a estimé à cette occasion que le nombre des atteintes subcliniques était environ dix fois supérieur à celui des cas avérés.

Plusieurs méthodes permettent de déceler dans l'eau les entérovirus, qui servent alors d'indicateur de la pollution générale par les virus entériques. Celles qui utilisent la filtration sur membrane sont les plus prometteuses et les plus avantageuses en pratique. Après concentration du matériel viral, on procède à l'inoculation de cultures de cellules de rein de singe et à la numération des unités formatrices de plage. L'emploi de divers systèmes cellulaires permet d'accroître la récolte de virus.

On dispose d'un grand nombre de données sur l'efficacité des diverses techniques de traitement des eaux en ce qui regarde la destruction des entérovirus. Le stockage de l'eau, s'il est suffisamment prolongé, réduit dans une certaine mesure la concentration virale, mais ce facteur ne joue aucun rôle important dans les conditions réalisées dans les installations de traitement. La floculation, basée sur une réaction relativement non spécifique entre un cation métallique et une protéine, peut être utilisée contre toute espèce de virus et a l'avantage supplémentaire d'abaisser la teneur globale en matières organiques. Bien que ce procédé ne suffise pas à lui seul à assurer l'épuration de l'eau, on admet néanmoins que s'il est convenablement appliqué et complété par une sédimentation et une filtration rapide sur sable il élimine 99% du matériel viral. La destruction des virus restants doit être obtenue par stérilisation. La chloration est le procédé le plus utilisé pour le traitement de grandes masses d'eau. On estime que pour réduire la concentration virale dans la proportion de 99,93%, il convient d'appliquer le chlore résiduel libre à la concentration de 0,25 à 0,30 partie par million pendant 30 minutes. L'ozone est un excellent agent virulicide, qui ne communique à l'eau ni goût ni odeur, mais le manque de répartition uniforme et l'absence de concentration résiduelle sont des inconvénients. C'est un prétraitement idéal, s'il est suivi de chloration.

Dans les pays en développement, on peut recourir au traitement par l'iode qui convient très bien à la stérilisation de petites quantités d'eau. Il ne nécessite qu'un matériel simple et est applicable sans les contrôles et analyses chimiques périodiques que requiert la chloration. A la concentration optimale de 3-4 parties par million, l'iode ne donne à l'eau aucun goût ou odeur désagréables et n'a aucun effet physiologique chez l'homme adulte. Il reste cependant à déterminer si l'utilisation permanente de cette méthode est sans inconvénient chez les femmes enceintes et les jeunes enfants. Tant que l'on ne dispose pas d'informations à ce sujet, le procédé devrait rester expérimental.

REFERENCES

Anderson, N. G., Clive, J. B., Harris, W. W. & Green, J. G. (1967) *Isolation of viral particles from large fluid volumes.* In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 75

Balasubramanian, A. (1967) *Annual Report for 1965 and Scientific Report for 1966 of the Pasteur Institute of Southern India*, Madras, Diocesan Press, p. 131

Bell, J. A., Rowe, W. P., Engler, J. I., Parrot, R. H. & Huebner, R. L. (1955) *J. Amer. med. Ass.*, **157**, 148

Berg, G., Dean, R. B. & Dahling, D. R. (1968) *J. Amer. Wat. Wks Ass.*, **60**, 193

Bier, M., Bruckner, G. C., Cooper, F. C. & Roy, H. C. (1967) *Concentration of bacteriophage by electrophoresis*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 57

Bodian, D. & Horstmann, D. M. (1965) *Polioviruses*. In: Horsfall, F. L. & Tamm, I., ed., *Viral and rickettsial infections of man*, 4th ed., Philadelphia, Lippincott, p. 430

Bouquaix, J. J. (1965) *Advanc. Wat. Pollut. Res.*, **1**, 16

Brisou, J. (1968) *Bull. Wld Hlth Org.*, **38**, 79

Carlson, H. J., Ridenour, G. M. & McKhann, C. F. (1942) *Amer. J. publ. Health*, **32**, 1256

Chang, S. L. (1947) *J. New Engl. Wat. Wks Ass.*, **59**, 79

Chang, S. L. (1966) *Bol. Ofic. sanit. panamer.*, **61**, 317

Chang, S. L. (1967) *Proc. Amer. Soc. civ. Engrs.* (in press)

Chang, S. L. (1967) *Statistics of the infective units of animal viruses*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 219

Chang, S. L., Stevenson, R. E., Bryant, A. R., Woodward, R. L. & Kabler, P. W. (1958) *Amer. J. publ. Hlth*, **48**, 159

Clarke, N. A., Berg, G., Kabler, P. W. & Chang, S. L. (1964) *Advanc. Wat. Pollut. Res.*, **2**, 523

Clarke, N. A. & Chang, S. L. (1959) *J. Amer. Wat. Wks Ass.*, **51**, 1299

Clarke, N. A., Stevenson, R. E., Chang, S. L. & Kabler, P. W. (1962) *Amer. J. publ. Hlth*, **51**, 1118

Cliver, D. O. (1967) *Enterovirus detection by membrane chromatography*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 139

Cockburn, T. A., Rowe, W. P. & Huebner, R. J. (1956) *Amer. J. Hyg.*, **63**, 250

Coin, L. (1967) *Discussion*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 367

Coin, L., Menetrier, M. L., Labonde, J. & Hannoun, M. C. (1965) *Advanc. Wat. Pollut. Res.*, **1**, 1

England, B., Leach, R. E., Adame, B. & Shiosake, R. (1967) *Virologic assessment of sewage treatment at Santee, California*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 401

Foliguet, J.-M., Schwartzbrod, L. & Gaudin, O. G. (1966) *Bull. Wld Hlth Org.*, **35**, 737

Freund, G., Bird, E. D., Thomas, W. C., Jr, Kinman, R. N. & Black, A. P. (1965) *Clin. Res. Proc.*, **13**, 242

Gilcreas, F. W. & Kelly, S. M. (1955) *J. Amer. Wat. Wks Ass.*, **47**, 683

Godbole, S. H., Illaria, S. J. & Rawal, B. D. (1966) *Envir. Hlth*, **8**, 70

Hettche, O. & Schulz-Ehlbeck, H. W. (1953) *Arch. Hyg. (Berl.)*, **137**, 440

Hudson, H. R., Jr (1962) *J. Amer. Wat. Wks Ass.*, **54**, 1265

Kelly, S. M. & Sanderson, W. W. (1958) *Amer. J. publ. Hlth*, **48**, 1323

Kelly, S. M. & Sanderson, W. W. (1959) *Sewage ind. Wastes*, **31**, 683

Kelly, S. M. & Sanderson, W. W. (1960) *Amer. J. publ. Hlth*, **51**, 14

Kelly, S. M., Sanderson, W. W. & Neidl, C. (1961) *J. Wat. Pollut. Control Fed.*, **33**, 1056

Kessel, J. F., Allison, D. K., Moore, F. J. & Kaim, M. (1943) *Proc. Soc. exp. Biol. (N.Y.)*, **53**, 71

Kjellander, J. & Lund, E. (1965) *J. Amer. Wat. Wks Ass.*, **57**, 893

Mack, W. N., Frey, J. R., Rugle, B. J. & Mallman, W. W. (1962) *J. Wat. Pollut. Control Fed.*, **34**, 1133

Malherbe, H. H. & Strickland-Cholmley, M. (1967) *Quantitative studies on viral survival in sewage purification processes*. In Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 379

McLean, D. M. (1967) *Transmission of viral infections by recreational water*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 25

Maxcy, K. F. & Howe, H. A. (1943) *Sewage Wks J.*, **15**, 1101

Mosley, J. W. (1967) *Transmission of viral diseases by drinking water*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 5

Neefe, J. R. & Stokes, J., Jr (1945) *J. Amer. med. Ass.*, **28**, 1063

Neefe, J. R., Stokes, J., Jr & Baty, J. B. (1947) *Amer. J. publ. Hlth*, **37**, 365

Neefe, J. R., Stokes, J., Jr, Baty, J. B. & Reinbold, J. G. (1945) *J. Amer. med. Ass.*, **128**, 1076

Ormsby, H. L. & Aitchison, W. S. (1955) *Canad. med. Ass. J.*, **73**, 864

Paul, J. R. (1955) *Epidemiology of poliomyelitis*. In: Debré, R., et al., *Poliomyelitis*, Geneva (*World Health Organization*: *Monograph Series*, No. 26)

Robeck, G. G., Clarke, N. A. & Dostal, K. A. (1962) *J. Amer. Wat. Wks Ass.*, **54**, 1275

Robeck, G. G., Dostal, K. A. & Woodward, R. L. (1964) *J. Amer. Wat. Wks Ass.*, **56**, 198

Shuval, H. I., Cymbalista, S., Fatal, B. & Goldblum, N. (1967) *Concentration of enteric viruses in water by hydro-extraction and two-phase separation*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 45

Stevenson, R. E., Chang, S. L., Clarke, N. A. & Kabler, P. W. (1956) *Proc. Soc. exp. Biol. (N.Y.)*, **92**, 764

Taylor, F. B., Eagen, J. H., Smith, H. D. F., Jr & Coene, R. F. (1966) *Amer. J. publ. Hlth*, **56**, 2093

Viswanathan, R. (1957) *Indian J. med. Res.*, **45**, Suppl., p. 1

Wallis, C. & Melnick, J. L. (1962) *Virology*, **16**, 122

Wallis, C. & Melnick, J. L. (1967a) *Concentration of viruses on aluminum phosphate and aluminum hydroxide precipitates*. In: Berg, G., ed., *Transmission of viruses by the water route*, New York, Interscience, p. 129.

Wallis, C. & Melnick, J. L. (1967b) *J. Virol.*, **1**, 472

Weidenkopf, S. J. (1958) *Virology*, **5**, 56