SOME VIROLOGICAL ASPECTS OF WATER PURIFICATION

by

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INTRODUCTION

The goal, of course, is virus-free water for drinking, for recreation, for shellfish beds. This goal is added to the other goals for the production of high quality water, but many of the procedures already used to produce clear, colorless, odorless, chemically and bacteriologically safe water are useful, even essential, to the elimination of viruses. Since more and more water must be reused it is essential that the elimination process be carried out before waste water is discharged into a water source.

It is said that proven outbreaks of water-borne virus disease are extremely rare, and that these rarities can usually be traced to inadequate water treatment. That, in itself, constitutes an argument for adequate wastewater treatment. But there is another, more subtle argument which hinges on a peculiar characteristic of virus infections. One detectable virus particle can infect a human being but many, in some cases most, of the infections with low doses are subclinical. In the infected, apparently healthy host the virus can multiply and be excreted in enormous numbers. The contacts of the original host may be exposed to disease-causing doses. Or they may in turn develop mild or inapparent infections and transmit the virus to an ever widening circle of contacts. By the time the number of clinical cases becomes excessive, the epidemiological association with water may be obscured.

I should like to begin by giving a brief description of viruses in general, and then proceed to the more specific aspects of viruses in water. The very basic structure of a virus consists of a strand, which may be doubled, of nucleic acid, enclosed in a protein shell called a capsid. The capsid usually is in the form of a helix, making a tube-like structure, or of an icosahedron- a solid with twenty equilateral triangles as faces. The capsid maybe the outermost surface of the particle, or the nucleocapsid - the basic structure - may be enclosed in an envelope, a lipoprotein membrane.

Some viruses have been found to carry with them, inside the capsid, a few molecules of one specific enzyme, but most depend on the host cell to make for them any enzyme that may be necessary for their replication. The nucleic acid of the virus contains the code for the necessary enzymes and structural components and the infected cell is forced to translate the code and produce more virus.

The infection of the host cell depends on a specific adsorption of the virus to the cell membrane and the formation of chemical bonds

between the outermost layer of virus particle and cell membrane. Animal viruses are then taken into the cell by a normal cell process called pinocytosis and stripped of their capsid by normal cellular proteolytic enzymes. The bare, unecapsidated nucleic acid - and, rarely, the virus-associated enzyme - are all that are necessary for the formation of hundreds of new virus particles within that cell. The capsid and envelope, if present, have served their purpose by getting the nucleic acid into the right cell.

Animal viruses have no recognizable organ of attachment to the cell, but it is known that one molecule of specific antibody can prevent attachment and thus infection. Since the outermost layer, whether capsid or envelope, is or contains protein, the virus particle will carry an electrical charge at most pH values. Sizes range from about 20 to about 200 nm but the effective size for filtration will depend on the charge.

Although practically any of the viruses which are capable of infecting man could find their way into wastewater, those which can multiply in the intestinal tract or could reach the intestinal tract directly from, for example, the liver, are the ones which must be of primary concern.

The viruses which meet these criteria and the further criterion of being infectious by the oral route fall into four groups: Adenovirus, picornavirus, reovirus, and the virus(es) of infectious hepatitis. Infectious hepatitis agent requires a special discussion, but the other three groups are fairly well characterized. Intestinal flu viruses, if there are such things, have not been isolated.

Adenoviruses consist of a double strand of DNA enclosed in a protein shell, or capsid. The capsid is in the form of an icosahedron with special appendages, sometimes called wands, at each of the twelve corners of the structure. They are not known to carry preformed enzymes with them so presumably their vulnerability to chlorine is due to an alteration in the attachment site or integrity of the capsid.

Reoviruses are also icosahedral viruses, but are unique in that they have a double capsid and have double-stranded RNA as gentic material. They do carry with them a preformed enzyme, an RNA polymerase, so their susceptibility to chlorine might be due to inactivation of the enzyme or to some vital alteration of external capsid protein.

Among the picorna viruses is the very important subgroup, the enteroviruses, including poliomyelitis viruses, Coxsackie viruses, and Echoviruses. All are very small - 20-30 nm- icosahedral viruses, containing single-stranded RNA, and apparently no virus-associated preformed enzymes. They are relatively resistant to chlorine and, because of their small size, capable of passing through many kinds of filters.

These viruses, or at least some members of these groups of viruses, can be cultivated in living cell cultures and many of them form areas of cell destruction, known as plaques, which permits their enumeration. Cell culture and virus assay and identification in cell cultures can not be considered routine laboratory operations but they can be done.

The virus of infectious hepatitis, hepatitis A virus, has never been isolated. It does not apparently grow in known cell culture systems. In the infected host it apparently does not accumulate in sufficient numbers in any tissue to be seen in biopsy or autopsy specimens. Because of its epidemiological characteristics it is traditionally placed among the enteroviruses. The virus of serum hepatitis, hepatitis B virus, which may or may not be related to the A virus, has possibly been cultivated (1). There is some evidence that the "candidate" B virus contains DNA, which would remove it from the enterovirus classification.

The problems involved in producing virus-free waters and wastewaters are all complicated by difficulties of actual detection of viruses which may have escaped the water treatment. Until a satisfactory, practical, and economically feasible method of detection is developed, the adequacy of specific treatment methods under field conditions can not be monitored. Laboratory studies have shown that waters meeting bacteriological standards can not be assumed to be free of potentially dangerous enteroviruses (2,3). Epidemiological studies suggest that this is also true with regard to infectious hepatitis virus (4,5,6).

The enteroviruses can usually be cultivated in tissue cultures and a direct quantative assay for their presence is possible. But not practical! Not economically feasible! Except in the hands of highly trained personnel, not even satisfactory. Assuming that the technical difficulties entailed in the concentration of large volumes of water for virus recovery are solved and mechanized, tissue culture technique verges on being a mystique, and its reduction to a few simple, routine operations lies far in the future. At best, the water could be seeded, before treatment, with a selected, highly resistant virus, and assayed, after treatment, for survival of that one virus. Then only one cell culture type, one medium, one method of cultivation would be needed. However, the most resistant enterovirus described in the literature is Coxsackie B, which is potentially virulent and not a very desirable thing to add deliberately to a water system.

The alternative is, of course, to develop an indicator, the disappearance of which parallels the disappearance of virulent virus during treatment.

Most of the research has been concentrated on the isolation, or development, of an easily grown, easily identified bacterium, either naturally occurring in polluted waters or to be seeded in the water before treatment. Spores of both Clostridium and Bacillus species are so chlorine resistant that their presence in treated water could not be considered an indication of inadequate treatment. Vegetative cells of most bacteria, on the other hand, are much less resistant to chlorine than are enteroviruses (reviewed in 7). Toenniessen and Johnson proposed that heat shocked spores of Bacillus subtilis could provide an index for virus removal (8). Greening (7) conducted a comprehensive survey of stock bacterial cultures, bacteria isolated from tap water, and different microorganisms isolated from secondary effuents of a waste water treatment plant. Only two bacteria, unidentified Mycobacteria, and a yeast showed chlorine resistance great enough to be considered as candidates for indicators of virus removal.

Bacteriophages constitute another class of easily cultivated organisms, and, since they are viruses, they might seem to be ideally suited as indicators of virus pollution and removal. Metcalf (9) showed that the natural occurrence of bacteriophage in water did not parallel the natural occurrence of enteroviruses, so presumably phage would have to be added before treatment. The T phages of Escherichia coli, phage 34 of Salmonella typhimurium, phage E 1 of Salmonella typhi, and phage IV of Shigella sonnei have been found to be as sensitive to chlorine as are the most sensitive bacteria (10,11). A bacteriophage of Serratia marcescens was investigated with the purpose of demonstrating its suitability as an indicator of viral pollution (12) but the conclusions derived from the work have been challenged (7). It should be noted that all of these bacteriophages are complex structures, containing DNA, and carrying with them at least one enzyme. They have no similarity to the enteroviruses physically, chemically or morphologically.

There is a group of bacteriophages which does resemble the picornaviruses, in fact, is included in the picornaviruses in some classification schemes. The ones which have received the most attention are parasites of Escherichia coli. They are very small (20 nm) icosahedral viruses containing RNA. Under laboratory conditions one of them, f2, has been shown to have a resistance to chlorine which would seem to make it a promising indicator organism for removal of polioviruses during the disinfection of waste water (13), but it is quite sensitive to the high lime process used in some advanced waste water treatments (14).

A suitable method of monitoring simply does not exist at this time. The procedures and results to be presented next are based on the best available detection systems and it is recognized by the investigators and practitioners that they may all be invalid if hepatitis A virus turns out to have totally unique characteristics of resistance to treatment processes (14).

When raw domestic sewage is discharged into a stream it carries a tremendous load of human viruses. Estimates based on the number of detectable viruses usually run about 10,000 units per gram of feces. The virus particles remain associated with or become associated with solids (15) so the stream may "purify" itself in circumstances which permit sedimentation of the solids. However, the virus is not necessarily inactivated and may be spontaneously eluted if, for example, a heavy rainfall changes the concentration of ions in the overlay water (16). Silt from the Houston ship canal contained enteric viruses, including virulent poliovirus, even though the water above it was virus-free (17). Oysters collected in the same area also contained enteric viruses.

Oxidation ponds and lagoons can effect significant reduction in indicator bacteria without elimination of enteric viruses or, in fact, pathogenic enteric bacteria (18). Enteric viruses have been isolated in nearly every sample from effluent of a single pond or of two ponds operated in series no matter what the season of the year. Effluents from three or four ponds in series also contained enteric viruses in numbers which could not be predicted from the results of bacteriological tests. In oxidation ponds, depletion of reduced organic compounds, bacteriophages, and Bdellovibrio all act to reduce bacterial numbers.

None of these significantly affect animal viruses.

In aerated lagoons or activated sludge units both dense and dispersed sludge will adsorb a given amount of virus, about 90%, almost immediately. In an experimental unit, the same sludge continued to adsorb the same proportion of poliovirus throughout a continuous feed of virus for thirteen days. The adsorption was shown to be a dynamic process, with continuous adsorption and release, but an increasing amount of the released virus was non-infective. The mechanism of virus inactivation has not been explained, but it was shown to be due to a property of the sludge, not to the inate toxicity of the sewage (19).

Primary and secondary sewage treatment at best achieve a 90% reduction in virus load. Disinfection by chlorination is now being increasingly used as a final process before discharge. There are two major objections to this practice. First, because of the amount of ammonia present in sewage, much of the introduced chlorine is converted to chloramines, which are comparatively ineffective as viricidal agents (reviewed in 7). Second, chloramines have been shown to be toxic for some fish and for some of the microscopic forms on which they feed (20): a doubtful removal of virus at the price of another form of pollution. It is essential that alternative tertiary or advanced treatment be devised.

There are at least two operating advanced treatment plants today which have achieved the goal of returning waste water to usable form. One, at South Tahoe, California, pumps the effluent directly to a reservoir which is used as and meets all standards for a recreational body of water. The other, in Windhoek, South Africa, produces drinking water. Both use essentially the same process train and I will use these treatment sequences as a framework for discussion of various aspects of virus removal.

The first step is coagulation by the addition of chemicals to the secondary effluent. In studies using various dosages of aluminum sulfate, ferric chloride, ferric sulfate, and several polyelectrolyte flocculation aids, Drewry (21) found that optimal dosages for removal of viruses did not always correspond to the optimal dosage for other parameters such as turbidity or COD reduction. All three chemicals could remove 99% of virus. The flocculation aids had the effect of broadening the range of dosage for optimum virus removal so there was a better chance for concomitant optimum turbidity and COD reduction and less chance for missing the ideal dosage. The status of the virus in the floc is not known. It may not be inactivated, which means it might possibly be eluted in an infective state.

The coagulation process used at South Tahoe and Windhoek is the high lime method. The primary purposes are phosphorus removal, clarification, and preparation for ammonia stripping. Calcium hydroxide slurry is added until the pH of the waste water is greater than 11.0. An added benefit is a very effective inactivation of viruses with a reduction in numbers of 3-5 logs (22). Bacterial reduction is 99% and all gram negative bacteria are destroyed. The lime sludge contains no infective virus.

The next step in the treatment train is the removal of ammonia. This process has no direct effect on virus removal but plays an important role in subsequent disinfection with chlorine.

The waste water is then recarbonated with CO_2 to a pH of 9.5. The South African plan also includes addition of $FeSO_4$ and polyelectrolyte for removal of $CaCO_3$ and mixing with powered activated carbon. The clarified liquid shows a 5 log reduction in virus numbers. The floc contains active virus. Ten percent of virus deliberately added before this unit process could be eluted from the sediment (22).

Both plants follow with filtration, rapid sand filtration or mixed medium filtration. Apparently no actual studies have been done on virus removal during this step. If nothing else, the effective removal of organic substances during filtration enhances virus removal during the two subsequent processes.

The next step is disinfection. Both plants under discussion use chlorination and this very standard process is, nevertheless, still the subject of much controversy. Probably, the most accepted concept of the viricidal action of the chlorine compounds formed in water is that which ranks them in decreasing effectiveness as Cl_2, HOCl, OCl^-, dichloramine, monochloramine, and organic chloramines (reviewed in 7, 23). In order to achieve a disinfecting level of free chlorine or hypochlorous acid, enough chlorine must be added to satisfy the demand of nitrogen containing compounds in the water. In raw sewage or even secondary effluent this means a very large dose of chlorine must be added to reach a breakpoint.

Some workers now, however, feel that the viricidal effect of chlorine addition is due only to chlorine itself (24,25,26). The formation of chloramines is a slow process, requiring up to 90 minutes at 25°C and longer at lower temperatures (25). If the chlorine and receiving water are adequately mixed during this time, so that contact of the uncombined chlorine and every virus particle is assured, disinfection can be achieved with much lower chlorine doses than required for breakpoint chlorination. Various methods and kinds of equipment are now being tested for provision of optimum mixing (26).

An alternate method of disinfection is ozonation. Ozone at 15 mg/l is totally viricidal in 5 minutes (27). In addition it removes color, odor, and organic material. Ozonation is approximately 50% more expensive than chlorination and the ozone must be generated at the place of use. In Kentucky, "tack-on" ozonation units are being tried for tertiary treatment at small waste disposal plants or "package units". The secondary effluent is passed through a series of nine ozone chambers. Virus recovery was zero beyond the third chamber. Much of the solids in the waste water, including 78% of the phosphate accumulates in a froth at the top of the chambers. It was suggested (27) that the cost differential between ozonation and chlorination might be reduced because of this side benefit of phosphate removal. There is some increase of BOD in the treated water corresponding in time to the disappearance of bacteria and viruses. Pavoni (27) believes it is possible that the mechanism of ozone action is oxidation of microorganisms to the point of cell lysis and virus disruption, this leading to an increase in biodegradable-material.

The final step in the advanced waste water treatment plants is "polishing" the effluent by passing it through activated carbon columns.

Virus will be adsorbed on the columns but desorption will occur if the flow is continued too long (22). Organic matter competes with virus for sites on the column and, at the usual pH of effluent (8.5), viruses may be poorly adsorbed. Lowering the pH to 3.5-4.5 gives a net positive charge on poliovirus and greatly enhances its adsorption and retention (28).

To conclude, there are a few "tack-on" subjects which are relevent to wastewater disposal but which did not fit smoothly into the main discussion.

Oysters, mussels, and clams in polluted waters accumulate enteric viruses in their tissues (29) and, as is obvious from recent outbreaks, hepatitis A virus can also be found in them. The human enteric viruses do not multiply in the shellfish but are apparently protected from the inactivating effects of sea water, so their concentration in the shellfish may be greater than their concentration in overlay water (17). The use of shellfish as "sentinels" has been suggested because of their ability to concentrate viruses.

The use of wastewater for spray irrigation has presented some new problems. For example, what respiratory viruses may be present and what are the dangers of aerosol dissemination?

How well do the human viruses survive in soil? This question could be of importance not only in soils irrigated by wastewaters, but in soils to which sludge is applied as fertilizer, in soils receiving runoff from sanitary landfills. The answers are being sought, but at this point the problems far outnumber the solutions.

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