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Drug Dosage
The authors and the publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new and/or infrequently employed drug.

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Preface

On behalf of the participants, the editor is delighted to acknowledge the enthusiasm and support accorded this International Symposium by the Israeli National Council for Research and Development. Dr. *Y. Saphir*, Director of this host organization, when opening the conference, emphasized that 'their prime aim is to promote research and development for solving basic national problems, among which water is one of life's most essential needs which, when contaminated, becomes a vector of disease with enormous implications for public health'. Elaborating on the significance of this symposium, Dr. *Saphir* remarked that 'prevalent conditions in modern industrial societies have generated the need for substantial investments in research and in systems operation in order to avoid serious environmental and public health hazards. Distinguished scientists from various countries have been invited to deliberate and exchange ideas with Israeli counterparts on relevant problems. The synthesis of local and foreign experience facilitates the formulation of recommendations regarding directions for research and development to be pursued and promoted in the future. Specific contributions of individual participants in the symposium, when put together as "Proceedings", will undoubtedly have a broad general significance.'

Israel has provided a unique example of the philosophy of 'need-based research' as a national policy. The extreme shortage of water resources has led to maximum recycling of wastewater. Reuse of wastewater in agriculture and eventually for domestic purposes creates a potential risk of the transmission of virus disease by water, and this situation prompted the development of an active research program on viruses in water in Israel which has been sustained through nearly two decades and has generated valuable information.

These Proceedings draw attention to: (1) advances in the state of the art for detecting the culturable enteroviruses (polioviruses, coxsackieviruses, echoviruses, newly designated enterovirus types) and the common adenoviruses, and the need to reexamine their significance in waterborne virus disease; (2) the minimum infective dose of an enterovirus; (3) the recent thrust in the study of human enteric viruses that are difficult to cultivate, such as hepatitis A virus, rotavirus, and the Norwalk agent; (4) the natural history and control of poliomyelitis in Israel; (5) elucidation of the factors contributing to the replacement of a winter peak of hepatitis A incidence in Israel by a more equal distribution of these cases throughout the year; (6) evaluation of the exposure of children and adults to aerosolized viruses from wastewater used in sprinkler irrigation; (7) factors that account for virtually virus-free reclaimed water from groundwater recharge of wastewater effluents; (8) questionable safety of recreational use and the growing of shellfish in estuarine waters, where large numbers of viruses associated with solids can be transported from polluted to supposedly non-polluted areas.

In the past, the admonition that 'the presence of even a few enteric viruses in a large volume of water can and should be prevented' has been endorsed. For the future, the question was raised 'whether the additional effort and cost of rendering water totally virus-free is a justifiable expenditure for public health'. A related concern revolved about the difficulties of trying to set virus standards for drinking water with the present imprecise methods.

In this volume, thirty papers are grouped in three sections: a state-of-the-art review of viruses in the environment; the problem of enteric viruses in Israel; and international studies. The reports represent a comprehensive overview of recent research on enteric viruses in the environment. Because of limitations in space, for publication the presentations have had to be shortened by deleting details of methods, many references, and acknowledgements. Readers are encouraged to write to the authors if more details are desired than could be included in this volume.

Sincere appreciation and thanks are due also to the members of the organizing committee and to the chairmen of the sessions, for their effective planning and guidance, which contributed greatly toward making the Symposium a success.

The editorial assistance of my colleagues Dr. *V. C. Rao* and Ms. *Verle Rennick* is gratefully acknowledged.

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Contents

International Studies

Round Table Discussion

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Etiologic Agents and Their Potential for Causing Waterborne Virus Diseases

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Introduction

Well over 100 different viruses are excreted in human feces and urine, find their way into sewage, and become common water pollutants. They are listed in table I together with diseases that they cause. Enteric viruses are divided into several groups, based on morphologic, physical, chemical, and antigenic differences. The most commonly studied group in natural waters has been the Picornaviridae family, which includes poliovirus, coxsackievirus A and B, echovirus, and the high-numbered enteroviruses (types 68 to 71). They have been the most studied of the enteric viruses because of the ease with which they can be isolated from sewage and assayed in the laboratory.

Hepatitis type A virus has been proven beyond doubt to be responsible for waterborne epidemics traced to sewage contamination. The virus has proven to be a small RNA virus whose size and polypeptide composition place it in the enterovirus group, where it has been classified as type 72.

Adenoviruses are also found in the feces and have been isolated from domestic sewage. These are large, double-stranded DNA viruses that cause respiratory and eye infections and have been responsible for epidemics of eye infections among bathers in nonchlorinated swimming pools. Recently, three new noncultivable types of adenovirus have been recognized as a cause of acute gastroenteritis in children.

Reoviruses have also been isolated from sewage, but their role in human disease is not understood. They are large (60–70 nm in diameter), double-stranded RNA viruses.

Table I. Viruses present in human excreta

Virus group	Number of serotypes	Diseases caused
Enteroviruses:		
Poliovirus	3	paralysis, meningitis, fever
Echovirus	34	meningitis, respiratory disease, rash, fever
Coxsackievirus A	24	herpangina, respiratory disease, meningitis, fever
Coxsackievirus B	6	myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia
New enterovirus types 68–71	4	meningitis, encephalitis, respiratory disease, rash, acute hemorrhagic conjunctivitis, fever
Hepatitis A (enterovirus 72)	1	infectious hepatitis
Norwalk virus	2	epidemic vomiting and diarrhea, fever
Rotavirus	4	gastroenteritis, diarrhea
Reovirus	3	unknown
Parvovirus:		
adeno-associated virus	3	unknown
Adenovirus (feces and urine)	> 30	respiratory disease, conjunctivitis
Cytomegalovirus (urine only)	1	infectious mononucleosis, hepatitis, pneumonitis, immunologic deficiency syndrome
Papovavirus, SV40-like (urine only)	2	associated with progressive multifocal leukoencephalopathy and immunosuppression

Rotavirus, also a double-stranded RNA virus, is excreted in large numbers in the stools of children with diarrhea. It appears to be the major cause of childhood diarrhea, especially during the winter months in temperate climates, and is also associated with gastroenteritis in adults.

Norwalk-like agents are small (27-nm) viral particles that have been implicated in several waterborne disease outbreaks of gastroenteritis. They have not yet been grown in the laboratory, and their characterization is incomplete.

Members of other virus groups implicated in human viral diarrhea include coronavirus, calicivirus, astrovirus, and unclassified small agents. While these viruses are known to be excreted in the feces of humans, adequate methodology for their study has yet to be developed. Adeno-associated parvoviruses are excreted in the feces, but their pathogenic potential is unknown. In addition, papovavirus and cytomegalovirus are excreted in the urine and may also find their way into raw sewage.

Some enteric viruses are excreted in concentrations as high as 10^{10} per gram of feces, and more than 100,000 infectious virus particles per liter have been detected in raw sewage. The amount of virus present in sewage is highly variable, depending on factors such as the hygienic level of the population, the prevalence of infection in the community, and the time of year. In the USA, peak levels of enteroviruses occur in the late summer and early fall. Enteric viruses survive customary secondary sewage treatment and chlorination as commonly practiced, so that they can usually be isolated at all times of the year. The average concentration of enteroviruses in sewage appears to be about 100 per liter in the USA, but much higher concentrations may be found. Because of the widespread use of live poliovaccine, poliovirus is often present in the highest concentration. Poliovirus is also one of the easiest to grow by commonly used laboratory tissue culture techniques. When attempts are made to use techniques favoring the isolation of rotavirus, reovirus and adenovirus, large numbers may also be detected.

In other parts of the world much higher concentrations of virus have been observed in wastewater than in the USA. It appears that the average concentration of enteric viruses in sewage in less-developed countries of the world may be 100 times that observed in the USA, although these concentration differences may reflect to some extent the higher per capita water consumption in industrialized countries and consequently a greater dilution of virus in wastewater.

In most of the world, human wastes are discharged into natural waters, with little or no treatment. Thus, there is little reduction in the initial input of viruses. However, in the developed countries, a large part of sewage is processed by biological or physicochemical methods before discharge into receiving waters. During biological treatment (most often activated sludge), a 90% reduction in the initial concentration of enteroviruses may occur. Physicochemical treatment involving lime or the use of other coagulating agents causes a further reduction, but it must be borne in mind that most reported studies were conducted using laboratory-grown enteroviruses which appear to adsorb more readily to flocs than some other groups of enteric viruses, e.g., rotaviruses. In addition, the removal efficiency of enteroviruses varies somewhat among members of the group. While treatment processes can result in large reductions in the concentration of viruses present in raw sewage, substantial numbers usually remain. Disinfection of treated sewage by chlorine is sometimes practiced; while effective in the reduction of bacterial pathogens, viruses are not eliminated by this treatment because of their greater resistance, particularly in the presence of organics. In some studies the concentration of virus in sewage effluent has been greater than in influent, apparently because of solubilization of virus attached to solids and resistance to chlorination.

It has long been established that there is a significant risk of contracting infectious diseases from the ingestion of sewage-contaminated water. The spread of enteric bacterial disease by this route has been largely controlled by the widespread application of bacterial standards and modern treatments for drinking water and sewage. However, enteric viruses are less effectively removed than bacteria by many treatments, particularly by disinfection, and viruses survive longer than indicator bacteria in the environment. Since viruses may be detected in water free of fecal coliforms and streptococci, the adequacy of bacterial standards has been questioned in evaluating the sanitary quality of water as it relates to its potential for transmission of viral diseases.

Enteric Viruses in the Etiology of Waterborne Disease

Acute gastrointestinal and diarrheal illnesses continue to be the major waterborne diseases in the more developed countries, usually with no etiological agent being identified. Recently, seroepidemiological studies have identified Norwalk agents as the probable cause in several waterborne outbreaks of gastroenteritis. Rotaviruses have also been suspected. Hepatitis A virus (enterovirus type 72) is the only other viral agent clearly recognized as having caused waterborne disease outbreaks. Several very large outbreaks of drinking-water-transmitted hepatitis have been recognized in India. Outbreaks of swimming pool adenovirus pharyngoconjunctivitis also have been documented, in which improperly chlorinated and maintained swimming pools were involved. While waterborne transmission of poliovirus and other enteroviruses has been suspected, conclusive evidence has been lacking.

Waterborne outbreaks due to enteroviruses and other enteric viruses are not easily recognized and are difficult to document for several reasons: (i) Many of these viruses cause inapparent infections that are difficult to recognize as being waterborne. A person may contract a viral infection by coming in contact with contaminated water, and the virus may actively multiply in the alimentary tract without the person developing overt symptoms of the disease. The person may have only mild malaise for a few days or no symptoms at all, yet may act as an effective carrier and transmit the virus to others, who may then develop acute symptoms of the disease. (ii) Epidemiological techniques are not sufficiently sensitive to detect low-level transmission of virus diseases through water. As shown in table I, most enteric viruses cause such a broad spectrum of disease syndromes that scattered cases of acute illness would probably have symptoms too varied to be attributed to a single etiological agent.

Because of the inadequacy of surveillance systems and methods, only a small percentage of outbreaks would be expected to be reported or investigated. The importance of low-level transmission is difficult to document because of the occurrence of large numbers of asymptomatic or subclinical cases characteristic of the enteric viruses. However, its importance in maintaining a disease in a community should not be overlooked.

Low-level transmission of agents of viral gastroenteritis by water or food may play its most significant role in the spread of these agents between communities distant from each other. For example, viruses may be discharged by one community into a river which is used farther downstream as a water source by another community. Only a few individuals in the downstream community need contract the disease by bathing or water consumption to establish new foci of infection from which viruses can be spread by other routes (i.e., respiratory or fecal-oral).

In most cases it is very difficult to identify the point source of viruses that are detected at any distance downstream from their discharge, but sometimes it has been done and the results show that viruses can travel long distances from the source of contamination and still be infectious (fig. 1, table II).

The study of the survival of enteric viruses under natural conditions in a subarctic Alaskan river has contributed useful information. A site 317 km downstream from all sources of domestic pollution was monitored. Moreover, ice totally covered the river, effectively sealing it against possible extraneous viral contamination. The mean flow time through the

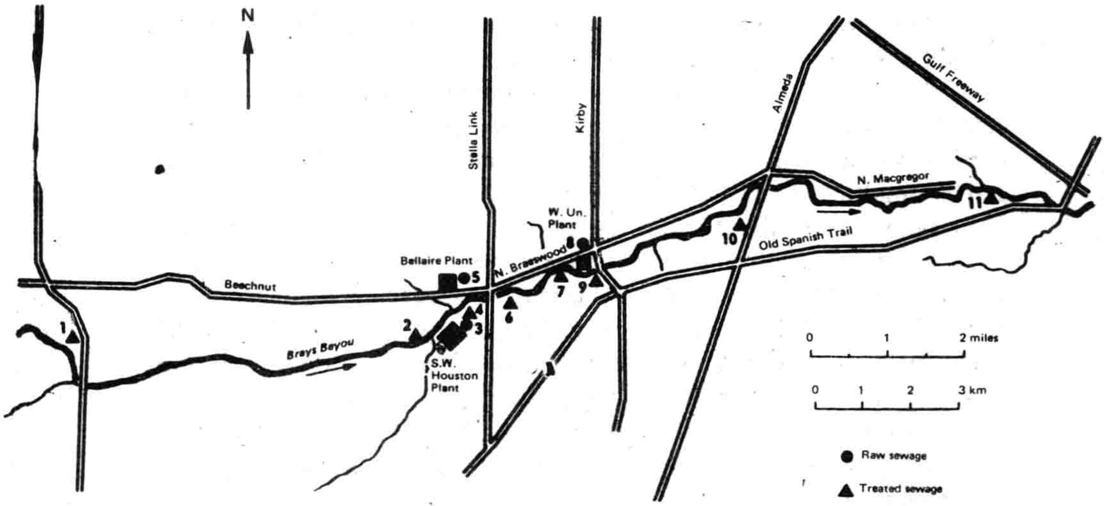

Fig. 1. Collection points 1 to 11 along the 16-km-section of Brays Bayou. At 8 km upstream from point 1 there was another residential sewage treatment plant. Collections at point 3, 5 and 8 were taken at the sewage treatment plants prior to chlorination. The flow rates at the collection stations and virus recoveries are listed in table II.

Table II. Survey of sewage plants and the stream receiving sewage effluents[a]

Collection point[b]	Flow rate gal/min	Total virus recovered from 1-gal samples PFU/gal	Calculated virus flow rate PFU/min
1. Stream	822	150	123,000
2. Stream	3,500	330	1,155,000
3. Plant[c]	9,700	620	6,014,000
4. Stream	11,750	210	2,467,500
5. Plant[c]	1,170	820	959,400
6. Stream	13,000	200	2,600,000
7. Stream	13,500	150	2,025,000
8. Plant[c]	1,050	290	304,000
9. Stream	14,500	132	1,914,000
10. Stream	15,250	130	1,982,500
11. River	ND[d]	110	–

[a] Taken from *Grinstein* et al. [1970].
[b] See figure 1.
[c] Collected from settling tank in plant, at the step preceding chlorination.
[d] ND = Not determined.

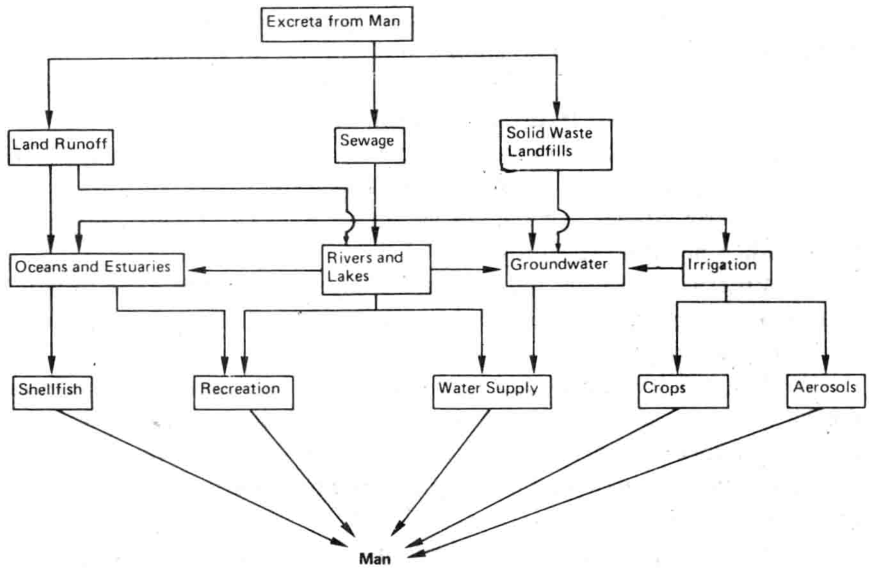

Fig. 2. Routes of enteric virus transmission [*Melnick* et al., 1978].

region was 7.1 days, during which time the initial virus population showed a relative survival rate of 34%. The ratio of enteric viruses to fecal indicator bacteria was not constant, clearly indicating that these bacteria were not a satisfactory measure of virus concentration.

Understanding environmental factors controlling enteric virus survival and transport in nature could be a key to defining the sudden occurrence of epidemics and maintenance of infections within a population. Environmental conditions can greatly affect the survival and transport of viruses in nature, and there are many routes by which excreted virus may find its way back to the human host (fig. 2).

Drinking Water

The public health significance of enteric viruses in drinking water is based on a number of factors, which are listed below.

(1) Pathogenic viruses are abundantly present in wastewater and can be found even after activated sludge and chlorine treatments.

(2) Viruses are concentrated in wastewater sludges that are ultimately used on land as fertilizers and soil conditioners.

(3) Viruses are present in surface waters, especially rivers, which many communities use as sources of potable water.

(4) Enteric viruses can remain viable for months in water and even longer when attached to solids.

(5) Enteric viruses are more resistant than indicator bacteria to inactivation by disinfectants, such as chlorine or ozone. They are particularly resistant when organics are present, as in sewage.

(6) Water is being recycled inadvertently as one community pollutes the water source of a second community. In addition, increased water demand is leading to direct recycling of wastewater for some water systems, but conventional water treatment is not designed to produce potable water from wastewater.

(7) Increasing numbers of communities are turning to land application of wastewater and sludges. Soils vary greatly in their capacity to adsorb viruses and thus to prevent groundwater contamination.

(8) Human infections can be caused by small doses of virus; 1 to 10 tissue culture doses of poliovirus or other enterovirus have proven infectious for humans.

(9) Contaminated water and shellfish harvested from contaminated water have been proven to cause hepatitis A outbreaks. Hepatitis A virus has recently been classified as enterovirus type 72.

(10) Waterborne outbreaks of nonbacterial gastroenteritis occur regularly. Norwalk virus has been incriminated by seroepidemiological studies, and rotaviruses have been isolated regularly from wastewater and at times from potable water.

(11) As methods improve and more investigations are made, human enteric viruses are being isolated from treated drinking water of large and small communities.

(12) Waterborne viral infections may occur without being detected. The significance of even low numbers of viruses in drinking water is not only that they may infect the persons in the original community polluting the water, but they may also serve to spread enteric viral disease from community to community. Many communities draw their drinking water from sources downstream to another community's sewage discharge. In the developing nations of the world, where sewage and drinking water