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Epidemic Viral Gastroenteritis

MARY KOLB ESTES, Ph.D.
DAVID YATES GRAHAM, M.D.
Houston, Texas

Epidemic viral gastroenteritis is a significant world wide problem. In developed countries, gastroenteritis accounts for significant morbidity and loss of time from work; in the Third World it is the leading cause of mortality among infants and children. Recent technologic advances have been associated with an explosion of research activity. Two virus groups, the Norwalk-like agents and the rotaviruses, are currently accepted as causative agents of viral gastroenteritis in man. The problem of viral gastroenteritis is reviewed both from a current and a historic perspective.

Viral gastroenteritis, more correctly termed acute enteritis or enteropathy, is a common cause of illness in man. In the United States, the illness is usually not of significant severity or duration to cause the patient to seek medical attention. Therefore, even widespread outbreaks remain unrecognized unless they are brought to the attention of the health authorities because of the large number of people involved. Viral gastroenteritis is a more significant problem in underdeveloped countries; estimates are that it is responsible for more than 500 million episodes of diarrhea and 5 to 18 million deaths annually [1].

A large number of viral agents have been implicated as causative factors of gastroenteritis. Many studies have consisted simply of screening stool specimens of diarrheal patients for viral agents; the frequency of positive cultures may or may not have been compared with results obtained in a control population. Such studies have accumulated data to foster epidemiologic hypotheses which require further testing; they have not provided definite evidence of causation. Current knowledge of the viral stool-shedding patterns is rudimentary. For example, in a recent study virus excretions in the stools of 27 babies were followed for one year [2]. The study revealed that babies frequently shed viruses and that the viral excretion patterns change rapidly. Fifteen per cent of the stools from healthy babies contained viruses, including rotaviruses, adenoviruses, astroviruses, caliciviruses and small unidentified viruses. During hospitalization, the proportion of infants who excreted viruses increased to 44 per cent, with many of the viruses appearing to be hospital-acquired. This study emphasized the current problems in interpreting data from studies of fecal viral excretion, including the need for concurrent strict control populations.

With these problems in mind, one can critically evaluate previous studies and carefully plan future studies to differentiate between diarrheal-associated viruses and proved enteritis-inducing agents.

From the Department of Virology and Epidemiology, and the Department of Medicine, Baylor College of Medicine, Houston, Texas. This study was supported in part by a grant from Vick Divisions Research and Development, Mount Vernon, New York. Requests for reprints should be addressed to Dr. David Yates Graham, Department of Medicine, Baylor College of Medicine, Houston, Texas 77030. Manuscript accepted January 17, 1979.

TABLE I Comparison of Proved Human Gastroenteritis Viruses

Parameter	Norwalk-like Agents	Rotaviruses
Clinical syndromes	Epidemic diarrhea All age groups	Sporadic seasonal gastroenteritis Infants and young children
Physical properties		
Diameter (nm)	27	60–70
Nucleic acid	?	Double-stranded segmented RNA
Acid-stable	+	+
Heat-stable	+	+
Ether-stable	+	+
Stable to 50°C in 1 M $MgCl_2$	?	−
Density in CsCl	1.38–1.41	1.36 double-shelled particles 1.38 single-shelled particles
Biologic properties		
Growth in tissue culture	−	±*
Experimental animal infection	± (chimpanzees)	+
Antigens	?	Type-specific and type-common antigens
Serotypes	2	4
Miscellaneous	−	Virion hemagglutinin Virion RNA-dependent RNA polymerase

* Bovine, porcine and simian isolates adapted to growth in vitro.

Proof of causation of infectious disease was codified by Robert Koch in 1890 [3]. Koch's postulates stated that an organism must be regularly isolated from patients with the illness and in a logical relationship to the pathologic changes observed. The isolated organism should be grown in pure culture and shown to be capable of causing the same illness in susceptible animals or human subjects. Finally, the organism should be reisolated from the subject with the experimentally-induced disease. Noting that many viruses cannot be cultured, Thomas Rivers [4] and later Robert Huebner [5] proposed modifications of Koch's postulates that would allow proof of causation of viral illnesses. They suggested that for viral diseases the specific virus must be regularly associated with the disease, that the illness must be transmissible to susceptible hosts from material known to be free from nonviral agents, and that control and immunologic studies be carried out to exclude that the virus was fortuitously present or picked up from the experimental host. Supporting immunologic critera are also important, including evidence that specific viral antibody is absent before infection and that it is produced by the infection; the absence or presence of antibody should correlate with disease susceptibility and protection, respectively. Of the many viruses identified in stools, only two groups have met the criteria as definite etiologic agents of epidemic gastroenteritis in human subjects: rotaviruses and the small 27 nm agents (Norwalk-like agents) (**Table I**).

History of Epidemic Gastroenteritis in the United States. Clinical investigations into the cause and epidemiology of viral gastroenteritis in the United States began in 1941. On October 14, 1943, at the 72nd Annual Meeting of the American Public Health Association, Light and Hodes [6] presented the results of two years of investigations which began with an epidemic of gastroenteritis occurring in the newborn in the Baltimore-Washington area in the fall of 1941. They were able to obtain multiple passages of the infectious agent (the Baltimore agent) by administering filtered stool to calves. Calves were found to be uniformly susceptible with an incubation period of two to five days. The illness in calves had a mortality rate of 13 per cent, and infection was followed by homologous immunity. Horizontal infection was evident, and strict isolation procedures were employed. The Baltimore agent is the only isolate from the early studies that was saved, and recent characterizations revealed that it is probably a rotavirus [7].

In 1945 Reimann, Hodges and Price [8] reported the results of an investigation of an epidemic occurring in Philadelphia between September and December 1943 and recurring in 1944. They also provided the first overview of the problem. They were the first to use volunteer subjects to passage their material. However, the results were inconclusive because an epidemic of viral enteritis was ongoing in the community, and no attempts were made to isolate the subjects.

On December 16, 1946, an epidemic of gastroenteritis began in the Marcy State Hospital near Utica, New York. Subsequently, Gordon and his associates [9,10] published a series of papers on the Marcy strain that included volunteer studies showing the transmission of a nonbacterial gastroenteritis.

At the same time, an outbreak of gastroenteritis occurred in a group of families being followed in Cleveland as part of a long-term study of illnesses. This group observed that nonbacterial gastroenteritis was the second most common disease in their families. The Family Study (FS) gastroenteritis agent was also passed in vol-

unteer subjects and compared with the Marcy strain [11]. Clear differences were found between the two isolates with respect to symptoms, incubation period, disease duration and immunity. Infection with each agent conferred homologous but not heterologous immunity. Japanese workers showed that infection with a Japanese agent, the Niigata strain, conferred protection against infection with the Marcy strain [12].

Work was largely abandoned during the 1950s and 1960s because of the inability to either propagate or to visualize the infectious agents. Advances in knowledge and methodology for the detection of fastidious and noncultivatable viral agents in the 1960s led to a renewal of interest in gastroenteritis viruses in the 1970s. The application of immune electron microscopy, the technique used to identify hepatitis A virus particles in stools, to diarrheal samples brought about the successful identification of two enteritis viruses in human subjects. The recent development of rapid and very sensitive tests (radioimmunoassay, immune adherence hemagglutination and enzyme-linked immunosorbent assays) which can detect symptomatic and asymptomatic infections should quickly expand our knowledge of the natural history and epidemiology of these diseases. The history of investigations in viral gastroenteritis is a good example of the fact that major scientific advances often require and parallel new technologic opportunities.

27 nm Particles (Norwalk-Like Agents). On October 30, 1968, an attack of viral gastroenteritis occurred in an elementary school in Norwalk, Ohio [13]. In this attack, 50 per cent of the teachers and students were affected within a 24-hour period with an illness characterized by nausea, vomiting and abdominal cramps. The symptoms lasted from 12 to 24 hours. No patient required hospitalization, and recovery was complete. Because of the suddenness of the attack and the large number of persons involved, Public Health authorities including the Center for Disease Control were asked to investigate the outbreak. No evidence of food or water spread was found. No pathogenic bacteria were discovered, and the symptomatic characteristics of the illness were different from common bacterial diarrheas.

Investigators at the National Institutes of Health began work with this "Norwalk agent." The disease was transmitted to volunteer subjects which provided enough infectious material (stool filtrates) for the performance of detailed clinical studies and preliminary laboratory analyses of the physical and biochemical properties of the agent [14,15]. Stool filtrates were characterized as being free from bacteria, fungi, bacteriophages and enterotoxin. Although no viral agent could be propagated in a variety of tissue culture systems or in laboratory animals (including primates), the studies in volunteer subjects demonstrated that the infectious agent would pass through a 60 μm filter but that it was retained by a 20 μm filter. It was also heat stable, resistant to ether and acid treatments, and produced homologous immunity following infection [15]. Subsequently, the agent was propagated briefly in culture of material from intestinal organs [16] and in 1972 Kapikian and co-workers [17] succeeded in identifying a 27 nm particle by immune electron microscopy of stools from infected volunteer subjects. In 1973, two groups described histologic studies of small bowel mucosa in volunteer subjects infected with the agent [18,19]. Histologic changes included abnormalities in the mucosal absorptive cells, mucosal inflammation, villus shortening and secondary crypt hypertrophy. In addition, there were decreases in specific activities of intestinal brush border enzymes. These changes were associated with malabsorption of the carbohydrates, D-xylose and lactose, and transient steatorrhea. The histologic findings became apparent several hours prior to the onset of symptoms and persisted for several days. Histologic abnormalities were also found in patients without symptoms, but severe lesions tended to be associated with the severe illnesses. Two other Norwalk-like agents have been obtained in outbreaks of gastroenteritis: the H agent in Honolulu in March 1971 and the MC agent, in an attack in Montgomery County, Maryland, in June 1971. Studies in volunteer subjects suggested that the Norwalk and Hawaii agents were antigenically dissimilar, whereas the Norwalk agent conferred immunity to challenge with the MC agent [20].

Rechallenge studies in volunteer subjects with the Norwalk agent revealed a pattern of susceptibility to re-infection with the same agent [21]. Six of 12 subjects became ill with the first challenge, and, when rechallenged many months later, the same six subjects became ill again. Subsequent rechallenge of five of the six "susceptible" subjects several weeks later revealed immunity in four of them. An increase in serum antibody titer was demonstrable in those patients who became ill but apparently offered no protection.

Work with these agents has been hampered by the lack of a reliable cultivation method in vitro. In recent studies the transmission of infection to chimpanzees has been reported but, unfortunately, no signs of clinical illness (such as vomiting or diarrhea) occurred [22]. The illness in chimpanzees was identified by use of a highly-sensitive solid phase microtiter radioimmunoassay [23] which detected both virus excretion in the stool and increase in serum antibody. In recent immunologic studies it has been demonstrated that serum antibody to the Norwalk agent is acquired gradually; by the fifth decade, one half of the adults tested had antibody [23,24]. Similar 27 nm viral agents have been under investigation in the United Kingdom and in Japan [25,26].

The 27 nm particles have been called "parvovirus-like agents," DNA viruses, based on their small size, density in cesium chloride of 1.38 to 1.41 g/ml, and stability to ether, acid and heat. However, this provisional designation may be a misnomer. Correct classification of these agents must await propagation of the agent(s) to

sufficient titers to allow biochemical characterization, including type of nucleic acid. The limited information available about 27 nm agents is also consistent with their classification as caliciviruses, RNA viruses which have recently been demonstrated to cause enteritis in animals [27]. Until a reproducible system is devised for the propagation of the 27 nm agents, future investigations will continue to progress at a slow rate.

Rotaviruses. In 1973, Bishop et al. [28] reported finding viral particles in biopsy specimens of duodenal mucosa from children with gastroenteritis in Melbourne, Australia. This same year Flewett and associates [29] in England described similar viral particles in diarrheal feces. It was soon recognized that the particles are morphologically similar to the epizootic diarrhea virus of infant mice and to the Nebraska calf diarrhea virus [30]. Rotaviruses are a major cause of diarrheal illnesses in young mammals of a wide variety of species.

These isolates have also been termed infantile gastroenteritis virus, reo-like virus and duovirus; however, the designation of "rotavirus" as a separate genus of the Reoviridae family has recently been accepted by the International Committee on Taxonomy of Viruses as their official name. The derivation of that terminology is based on the electron microscopic appearance of a wheel with radiating spokes. Rotaviruses have been established as enteritis viruses by isolation and purification from stools of subjects suffering from gastroenteritis, and by induction of disease and seroconversion in both animals and volunteer subjects with purified preparations.

Epidemiologic studies on the prevalence of rotavirus infections have shown these ubiquitous agents to be a major cause of gastroenteritis in children. In a typical study, Davidson et al. [31] found that 52 per cent of the cases of acute gastroenteritis in 378 children hospitalized in Australia were caused by rotavirus. An etiologic agent was identified in 75 per cent of these cases, and the remaining causes were shigella 1 per cent, Escherichia coli 2 per cent, enterovirus 2 per cent, adenovirus 7 per cent and salmonella 11 per cent. Similar findings have been repeated the world over. Rotavirus infections usually predominate during the winter season with an incubation period of two to four days. Symptomatic infections are most common in children aged six months to six years, and transmission of rotavirus gastroenteritis appears to be by the fecal-oral route.

Clinical features of rotavirus gastroenteritis in infants and children include diarrhea, vomiting, fever and abdominal pain [32]. In contrast to the clinical illness seen in hospitalized infants, adult contacts often become infected, as evidenced by seroconversion, but they suffer only mild symptoms and the virus is rarely detected in the stool [32,33]. The failure to identify virus in the stool may reflect the insensitive methods used for viral detection.

The ubiquitous nature of rotaviruses can also be demonstrated by measuring the prevalence of immunity in the population. By age six, 60 to 90 per cent of children have serum antibody titers [34,35]. The protective nature of the circulating antibodies remains unclear, since it is known that both human subjects and animals can become infected even when they possess detectable immunity [33,36]. Local immune factors, such as secretory immunoglobulin A or interferon, may therefore be important in protection against rotavirus infection. Alternatively, reinfection in the presence of circulating antibody could reflect the presence of multiple serotypes of virus [37]; at least four agents in human subjects have been characterized to date [38–41]. Asymptomatic infections are common in infants before the age of six months, the time during which protective antibody acquired passively by newborns should be present and active [42,43], and breast-fed babies excrete fewer particles/g feces than bottle-fed babies although both groups of babies become infected [42]. Rotavirus antibody has been detected in colostrum for up to nine months postpartum [44].

Other investigations have emphasized characterization of rotavirus particles and replication patterns in an attempt to understand the mechanism of infection and pathogenesis. Such studies have shown that the rotavirus agents in human subjects are very similar in physical properties to the diarrheal agents in animals recognized by veterinary researchers since 1969 [30]. Rotaviruses contain segmented double-stranded RNA genomes with the pattern of RNA segments varying according to the species of origin [45]. The RNA pattern is becoming increasingly important as a method of identifying species of origin of rotavirus isolates as laboratory contamination and cross-species infections occur.

The rotaviruses are double-shelled particles with an average diameter of 70 nm. The outer-shelled particle contains type-specific antigenic components, whereas the inner shell contains type-common antigenic determinants [46,47]. The outer shell presumably contains a hemagglutinin [48] and glycoproteins [49], but the role(s) of the various protein components in viral infectivity or virus neutralization remain(s) to be elucidated. The double-shelled particles exhibit a density of 1.36 g/ml in cesium chloride and have been referred to as smooth particles. These particles appear to be the infectious particles as determined by their ability to induce viral antigen detectable by immunofluorescence in cells in tissue culture [50]. Particles lacking the outer shell have a density of 1.38 g/ml in cesium chloride and have been referred to as rough particles. The single-shell particles contain an RNA-dependent RNA polymerase activity which can be assayed directly. Alternatively, the polymerase can be activated from the double-shell particles by treatment with chelating agents which reportedly remove the outer shell from the particles [51].

The rotaviruses appear to be stable entities. Their resistance to acid, like most enteric viruses, guarantees their survival after transversing the acidic environment of the stomach. In addition, these viruses are stable to

freezing, sonication, treatment with liquid solvents and heating (50°C). The rotaviruses, unlike the polioviruses, are not stable to heating in the presence of 1 M magnesium chloride, although they are stabilized in the presence of magnesium sulfate [52]. Such stabilization could be useful to prepare vaccines not requiring refrigeration for distribution in tropical climates.

Biologically, the rotaviruses are fastidious organisms. The agents from human subjects have not been successfully cultured in vitro in most laboratories, although Wyatt et al. [53] reported limited success in growing one isolate in human embryonic kidney cells for up to 14 passages. Rotavirus agents isolated from animals, including calves, pigs and monkeys, have been able to be cultured or adapted to growth in tissue culture in the laboratory. Success with cultivation and development of plaque assays for the calf and simian agents have required the use of proteolytic enzymes, such as pancreatin or trypsin [54,55]. The agents from the calf and monkey, now easily grown in tissue culture, are being used as model viruses to study rotavirus replication, and to enhance and develop methods for rotavirus detection. The inner coat of all known rotaviruses contains common antigens. This property has allowed the development of diagnostic procedures for infected human subjects using calf and simian viruses in place of the more fastidious agent isolated from human subjects.

Rotaviruses from many species, including human subjects, have been used successfully in experimental infections of colostrum-deprived gnotobiotic animals [56,67]. Such studies have suggested that cross infection between species may occur.

Our knowledge of the histopathology and pathophysiology of rotavirus infections has come from analyses of such infections in animals and from limited studies of mucosal biopsy specimens from infected children. The general pattern of infection involves virus penetration and infection of the differentiated enterocytes in the villi of the small intestine [58]. Rotaviruses multiply in the cytoplasm of these cells and damage the absorptive cells, resulting in damage to both the digestive and the absorptive functions. Available evidence suggests that such damaged cells are sloughed into the small intestine; lysis of the infected cells releases the virus into the intestine, resulting in the large quantities of virus detected in stools of infected subjects. These studies suggest that the diarrhea caused by rotavirus infection is due to malabsorption which also includes impaired glucose-sodium absorption. The highly differentiated absorptive villous cells are replaced by immature crypt cells that are not able to immediately compensate for the absorptive defect [59].

Treatment of Viral Gastroenteritis. Treatment of viral gastroenteritis is supportive. Death is associated with loss of electrolytes and water, leading to dehydration, acidosis and shock; it is not due to an irreversible effect of the causative agent. Due to the damage to the intestinal digestive and absorptive functions, initial therapy should include withdrawal of milk or lactose-containing products. Dehydration should be combatted with the oral replacement of fluids and electrolytes. Recent studies have suggested that oral therapy, using sugar and electrolyte solutions, is as effective as intravenous therapy in most infants and children.

Of great interest are two recent controlled double-blind studies that compared the use of sucrose-electrolyte solutions with glucose-electrolyte solutions; both sugar electrolyte solutions were found to be effective in the treatment of rotavirus diarrhea [60,61]. This is an important observation since sucrose is less expensive and more readily available than glucose, particularly in developing countries in which the mortality from rotavirus infection is a significant problem. In addition, it appeared that oral glucose-electrolyte solutions are effective therapy for diarrheal disease of both bacterial and viral etiology. Although limited knowledge exists, widely administered antidiarrheal agents, such as kaolin-pectin or Lomotil®, do not appear to be useful in the relief of viral diarrhea in children [62].

Areas for Further Research. Despite the tremendous amount of investigative work carried out in viral gastroenteritis, an understanding of the natural history and epidemiology of this disease is still lacking. Both rotaviruses and the Norwalk-like viral agents have been established as etiologic agents in some cases of viral gastroenteritis. The role and clinical significance of other viral agents that have been associated with diarrhea in man, including adenoviruses, enteroviruses, astroviruses, coronaviruses and caliciviruses, remain to be elucidated. The inability to cultivate rotaviruses and Norwalk-like viruses from human subjects has hampered progress in elucidating the natural history, virology and epidemiology of these illnesses. To date, no small animal model has been identified which would permit the study of the replication of these viruses in vivo.

Due to the inability to cultivate the viruses in vitro, there has been slow development of rapid, reliable and sensitive means for detecting the agents and comparing the antigenic variations of viral isolates or strains recovered from differing geographic areas. Four strains of rotavirus from human subjects and at least two strains of Norwalk-like viruses have been reported; future studies need to determine the clinical significance of these strain differences. It remains to be established whether avirulent as well as virulent strains exist, whether defective strains are produced and whether recombinant viruses occur. Recombinant viruses are a particular possibility if one considers that the segmented RNA genomes of the rotaviruses are similar to those of the influenza viruses.

The role of the immune response in viral gastroenteritis requires further analysis, including the possible protective effects of serum and local intestinal antibody, interferon and cellular immunity. In addition, the effect of host nutritional status is unknown.

Our knowledge of the epidemiology of viral gastroenteritis infections is still in its infancy. Most studies have focused on hospitalized children and adults; these studies may reflect a considerable bias in relatic ıship to the occurrence of infections in the population at arge, since most of the illnesses are of insignificant severity and duration for the patient to seek medical attention.

Transmission of both the Norwalk-like agent and rotaviruses appears to be by fecal-oral routes. The role of contaminated food and water in the transmission of gastroenteritis requires further examination, as does the effectiveness of our present sanitation procedures for eradication of the agents.

The role of rotaviruses in persistent and recurrent infections is unknown. It has been demonstrated that rotaviruses can establish persistent infections in cell culture [63], but the control mechanisms responsible for chronic infections in animals and/or cell lines have yet to be unraveled. Lastly, the hosts and reservoirs of these viruses remain to be established. Unfortunately, possible cross-species infections and the lack of reliable sensitive methods to detect rotavirus strain differences do not make these goals easily attainable. Finally, the possible role of these viral agents in the establishment and maintenance of chronic diarrheal disease warrants further investigation, both with respect to chronic diarrhea of childhood and the relationship to inflammatory bowel disease. Although prevention of gastroenteritis by vaccination is suggested in many publications, the primitive state of our knowledge of gastroenteritis viruses makes such discussions truly academic at the present time.

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