CHAPTER 11

Viral Gastroenteritis

Albert Z. Kapikian

1. Introduction

Viral gastroenteritis, with diarrhea or vomiting or both as its major clinical manifestation, affects a broad segment of the population throughout the world. In the developed countries, it is a major cause of morbidity in infants and young children, whereas in the developing countries, it is a major cause of both morbidity and mortality in this same age group.[33] In the Cleveland Family Study, which included some 25,000 illnesses over an approximate 10-year period, infectious gastroenteritis (considered nonbacterial) was the second most common disease experience, accounting for 16% of all illnesses, averaging 1.5 episodes per person per year.[126] It is remarkable that the frequency of episodes was found to be quite similar (1.2–1.9 per person/year) in surveys carried out 20 or 30 years later.[224,393,394] In addition, a winter survey of a sample of US physicians engaged in pediatric practice revealed that gastrointestinal (GI) disturbance was the second most common disease for which children were brought to the physicians' offices, being responsible for 9.5% of all visits.[7] Although deaths from diarrheal illnesses are not a major public health problem in the United States today, scientists at the Centers for Disease Control (CDC) estimated that between 1979 and 1984, 209,000 infants and young children were hospitalized with a diarrheal illness.[247] This resulted in 877,800 inpatient days (4.2 days per hospitalization). In addition, they estimated that between 1973 and 1983, there were an average of 504 deaths annually from diarrheal illness in children 1 month to 5 years of age and that this comprised 2% of the postneonatal deaths in infants 1 to < 12 months of age. In addition, 79% of these deaths occurred before 1 year of age.

Albert Z. Kapikian • Epidemiology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892.

On the global scale, the impact of diarrheal disease is staggering; World Health Organization (WHO) statistics reveal that such illnesses account for a large proportion of the total reported deaths in many countries.[324,615] An estimate of the total number of diarrheal episodes during a single year (1975) in children less than 5 years of age in Asia, Africa, and Latin America revealed that over 450 million episodes of diarrhea would occur, and of these 1–4% would be fatal, resulting in the deaths of 5–18 million infants and young children in this 1-year period.[459] In a recent report on a strategy for disease control in developing countries, it was estimated that in Africa, Asia, and Latin America in a 1-year period (1977–1978), there would be 3–5 billion cases of diarrhea and 5–10 million deaths; diarrheas were ranked number one in frequency in the categories of disease and mortality.[593] In addition, from selected studies it was estimated that in Latin America, Africa, and Asia (excluding China), 744 million to 1 billion diarrhea episodes and 4.6 million deaths from diarrhea occur annually in children under 5 years of age.[516]

Despite the great importance of this problem, studies failed to reveal an etiologic agent for the majority of diarrheal illnesses.[100,652] However, the discovery of the 27-nm Norwalk virus in 1972 and discovery of the 70-nm rotavirus in 1973 paved the way for an abundance of new information about viral gastroenteritis.[43,299] The Norwalk virus group has been associated with gastroenteritis outbreaks occurring in school, community, and family settings affecting school-aged children, adults, family contacts, and some young children as well.[215,287,306] The Norwalk virus is now officially classified as a calicivirus following the recent cloning and characterization of its genome.[111,269,270,363] The 70-nm rotaviruses are associated with 35–52% of acute diarrheal diseases of infants and young children requiring hospitalization in developed countries; they occur with equal frequency as etiologic

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agents of severe acute gastroenteritis in this same age group in developing countries as well.[41,286] Rotaviruses have consistently been shown to be the single most important etiologic agents of severe diarrheal illness worldwide. This chapter deals primarily with the rotaviruses and the Norwalk group of viruses. Other viral agents that play a role in these syndromes but to a lesser degree are discussed at the end of the chapter.

2. Historical Background

Diarrhea in humans has been documented since pre-Hippocratic times.[324] Discoveries made in the past century in the fields of bacteriology and parasitology resulted in the elucidation of the etiology of only a portion of diarrheal illnesses. However, it soon became apparent that despite the bacteriological and parasitic discoveries, a significant proportion of epidemic and infantile gastroenteritis could not be ascribed to any etiologic agent.[508] By exclusion, it was assumed that many of these infectious gastroenteritides were caused by viruses. In 1945, Reimann, Price, and Hodges[448] described the transmission of gastroenteric illness to volunteers following administration by the respiratory route of nebulized bacteria-free filtrates of throat washings or fecal suspensions from gastroenteritis patients. Gordon *et al.*,[195] in 1947, induced an afebrile diarrheal illness in volunteers by the oral administration of bacteria-free fecal filtrates and throat washings from gastroenteritis patients; this infectious inoculum was designated the Marcy strain, since it was derived from pooled diarrheal stools obtained from two patients in a gastroenteritis outbreak at Marcy State Hospital near Utica, New York.

In 1948, Kojima *et al.*[315] induced gastroenteric illness in volunteers following oral administration of bacteria-free fecal filtrates derived from diarrhea cases in the Niigata Prefecture and other districts in Japan; serial passage was achieved, and short-term immunity was demonstrated on challenge with a single strain. Yamamoto *et al.*,[632] in 1948, also induced diarrheal illness in volunteers (and cats as well) with bacteria-free fecal filtrates derived from an epidemic of gastroenteritis in the Gumma Prefecture. Later, in 1957, Fukumi *et al.*[177] reported on the relationship between the Niigata Prefecture strain (derived from a pool of stools of several patients with diarrhea as described above and shown to have been infectious in volunteers) and the Marcy strain. In cross-challenge studies, Niigata and Marcy strains were found to be related.

In 1953, Jordan, Gordon, and Dorrance[276] reported the induction of a febrile gastroenteric illness in volunteers following the oral administration of a bacteria-free fecal filtrate derived from a patient with gastroenteritis who was enrolled in the Cleveland Family Study (FS) cited in Section 1; the agent, which was designated the FS strain, was serially passaged in volunteers. Cross-challenge studies in volunteers revealed that the Marcy and FS strains were not antigenically related; in addition, the incubation period of the illness induced by the two strains as well as the clinical manifestations were somewhat different.[276]

Studies on the etiology of severe infantile gastroenteritis also failed to reveal an etiologic agent in the majority of instances. However, in 1943, Light and Hodes[342] were able to induce diarrhea in calves with a filterable agent derived from diarrheal illness during outbreaks of such illness in premature or full-term nurseries. A calf stool that had been lyophilized and stored for over 30 years was later examined by electron microscopy (EM) and found to contain rotavirus.[248] Whether this represented a true calf rotavirus or the human strain passaged in calves could not be determined conclusively; in further studies, the agent was not infectious when administered to a gnotobiotic calf[248] (R. G. Wyatt, unpublished data).

In 1972, Kapikian *et al.*,[299] employing immune electron microscopy (IEM), discovered 27-nm particles in stool material derived from a gastroenteritis outbreak in Norwalk, Ohio (Fig. 1A). This technique, which had actually been described in 1939 but not used to its fullest potential, enables the direct observation of antigen–antibody interaction by EM.[9,10,14,293] The 27-nm particles were visualized in a stool filtrate derived from a volunteer who had developed illness following administration of the Norwalk agent[299]; the particle-positive specimen had also induced illness in other volunteers on serial passage.[129] The particles were recognized following reaction of the known infectious stool filtrate with a volunteer's convalescent serum.[299] Serological evidence of infection with this particle was also demonstrated by IEM in certain experimentally and naturally infected individuals, and from these and other data it was postulated that the 27-nm particle was the etiologic agent of the Norwalk outbreak.[299] Particles morphologically similar to Norwalk virus—such as the Hawaii, Montgomery County, Taunton, and Snow Mountain agents—were later detected from patients in outbreaks of gastroenteritis by IEM or conventional EM.[80,81,133,551]

In 1973, Bishop *et al.*,[43,45] employing thin-section EM, discovered 70-nm particles in duodenal biopsies obtained from infants and young children hospitalized with acute gastroenteritis in Australia. Subsequently,

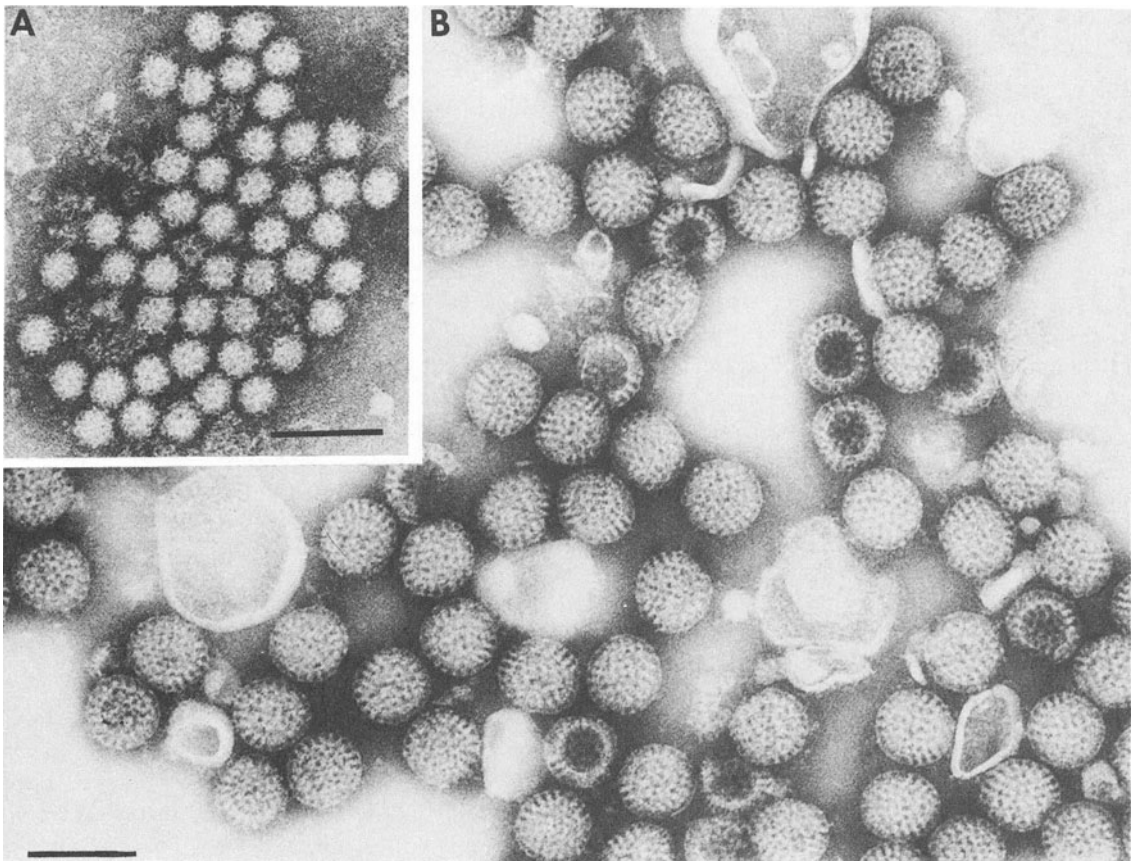


Figure 1. (A) A group of Norwalk virus particles observed after incubation of 0.8 ml of Norwalk stool filtrate (prepared from a stool of a volunteer administered the Norwalk agent) with 0.2 ml of a 1 : 5 dilution of a volunteer's prechallenge serum and further preparation for EM. The quantity of antibody on these particles was rated as 1+. Scale bar, 100 nm. From Kapikian *et al.*[299] (bar added). (B) Human rotavirus particles observed in a stool filtrate (prepared from a stool of an infant with gastroenteritis) after incubation with phosphate-buffered saline and further preparation for EM. The particles appear to have a double-shelled capsid. Occasional "empty" particles are seen. Scale bar, 100 nm. From Kapikian *et al.*[298]

these viruslike particles were found to be readily detectable in stool preparations by EM and became known by various names such as orbivirus, orbiviruslike, reovirus-like agent, duovirus, and infantile gastroenteritis virus, but now are officially designated as rotavirus.[160,163,297,370,436] In a relatively short time, laboratories from all over the world reported in rapid succession the presence of rotavirus in stool specimens from infants and young children with diarrheal illness, and it thus became apparent that this virus was indeed the long-sought major etiologic agent of diarrhea of infants and young children (Fig. 1B).

Two final notes of historical interest: In 1963, Adams and Kraft,[1] using thin-section EM to study intestinal tissue from mice infected with epizootic diarrhea of infant mice virus, described particles very similar to those first observed in 1973 in infants and young children in Australia. In 1969, Mebus *et al.*,[378] described the presence of reoviruslike particles in stools obtained from calves with a diarrheal illness. Later, both the mouse and calf viruses were found to be antigenically related to human rotavirus.[160,290,291] It is of interest that both the Norwalk virus and the rotavirus could have been discovered much sooner if the concept of "direct virology," using EM, had been applied to appropriate specimens.[304]

3. Methodology Involved in Epidemiologic Analysis

3.1. Sources of Mortality Data

Age-specific mortality data are available in the United States in the Vital Statistics Report prepared by the

National Center for Health Statistics (NCHS) of the Office of Health Research Statistics and Technology, Public Health Service, Hyattsville, Maryland. Specific sources from the NCHS include the National Hospital Discharge Survey (HDS) and the Multiple Causes of Death (MCD) mortality data.[246] The HDS represents a 0.5% sample of all discharges reported in the United States from selected hospitals. The MCD data includes all of the reported deaths in the United States. Since the clinical manifestations of viral diarrheas are not distinctive enough to permit differentiation from many other causes of diarrhea, and since the laboratory diagnosis of infection with viral gastroenteritis agents remains essentially a research tool, it is not yet possible to define the role of specific viruses in overall mortality from diarrhea.

On a worldwide scale, mortality data from diarrheal diseases are available in WHO and Pan American Health Organization publications. Vital statistics from around the world give the overall importance of diarrhea as a cause of death but, for the same reasons noted above, do not specify the role of the newly discovered viruses. Such information is inferred from data from epidemiologic studies. With the emergence of rotaviruses as a major cause of infantile diarrhea, it is generally acknowledged that this group of agents is of major importance as a cause of mortality from diarrheal diseases in the developing countries.

3.2. Sources of Morbidity Data

Since the clinical manifestations of viral gastroenteritis are indistinguishable from many other forms of gastroenteritis, it is not possible to obtain specific morbidity data without the aid of laboratory diagnosis, and as yet such diagnosis remains essentially a research tool. Most viral gastroenteritis morbidity data come from cross-sectional hospital-based studies involving (1) infants and young children admitted for diarrheal illness and (2) outbreaks of gastroenteritis. Such studies undoubtedly provide only a limited view of the total morbidity associated with these viruses, since they include only patients sick enough to come to the hospital or ill persons in selected outbreaks. However, limited morbidity data are available from several pediatric longitudinal studies and from various rotavirus vaccine efficacy field trials.

3.3. Serological Surveys for Rotaviruses and Norwalk Group of Viruses

Serological surveys have been carried out with the rotaviruses and the Norwalk agent to elucidate the prevalence of infection, the pattern of antibody acquisition by age, and the geographic distribution of these agents.[213,291,296,625,651] Rotavirus serology has relied heavily on the complement-fixation (CF) and enzyme-linked immunosorbent assay (ELISA) techniques.[290,291,651] However, with the successful cultivation of human rotaviruses, neutralization assays in tissue culture are now being performed.[490,574]

Until the development of second- and third-generation serological assays, large-scale surveys could not be carried out with Norwalk virus, since the only method available was IEM; this technique was not practical for such studies because it not only was very time-consuming but also required relatively large amounts of antigen, which was in short supply. However, the development of an immune adherence hemagglutination assay (IAHA), a radioimmunoassay-blocking test (RIA), and an ELISA-blocking test made it possible to perform serological surveys with Norwalk virus.[52,180,209,218,241,296] In addition, RIA-blocking and ELISA-blocking tests became available for the Snow Mountain agent as well as an ELISA-blocking test for the Hawaii virus.[349,557,558] A major drawback to serological studies with the Norwalk virus group of agents has been the need to rely on particle-positive stools from humans as the source of antigen. However, a major breakthrough occurred recently when the Norwalk virus genome was cloned, leading to the expression of its capsid protein in the form of viruslike particles in insect cells that were infected with a baculovirus recombinant (Fig. 2).[272] Because of this, a ready source of Norwalk virus antigen is available for the first time.

3.4. Laboratory Methods

3.4.1. Norwalk Group of Viruses

3.4.1a. Antigen Detection. Since this group of viruses has not yet been cultivated in any *in vitro* system, EM remains a mainstay for their recognition from stool specimens. Immune EM entails the reaction of antibody (such as that present in the patient's convalescent serum or in pooled immune serum globulin) with virus particles that may be present in the patient's stool.[282,304] Following centrifugation, the pellet (which contains the antigen–antibody complex) is prepared for examination by negative-stain EM. Antibodies directed against the particle are seen on the surface of the particle, and under appropriate conditions, antibodies induce aggregation of the particles. However, aggregation per se is not indicative of the presence of antibody, since nonspecific aggregation may occur. The presence of antibodies on the particle with or

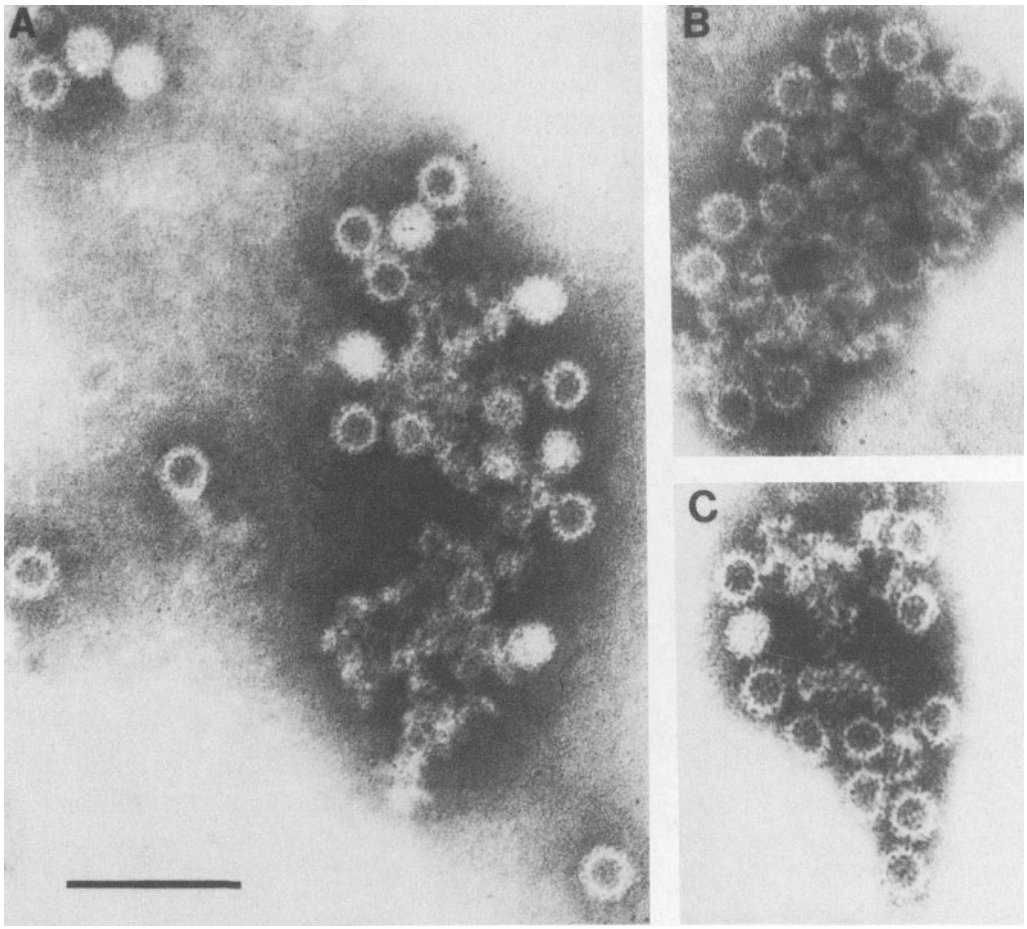


Figure 2. 27 nm viruslike particles observed by direct electron microscopy in 100 microliters of a 1 : 100 dilution of the baculovirus-expressed recombinant NV capsid protein after staining with 3% phosphotungstic acid. The viruslike particles were predominantly empty, readily seen in most fields, and similar to native NV, except that some had a double-shelled capsid appearance, a characteristic not reported with the native NV. Bar, 100 nm (for panels A, B, and C). From Green *et al.*[205]

without aggregation enables its differentiation from nonspecific matter. The specificity of the reaction must be determined in additional IEM experiments, since stools contain a large amount of particulate matter that may cause considerable confusion. Thus, a serological test as outlined in Section 3.4.1b must be carried out with the putative particle as antigen and paired acute (or pre-) and postinfection sera as the source of antibody to determine whether an increase in antibody to the particle occurred. Such paired sera may be from the patient, another individual in the outbreak, or an individual with a response to a known agent. Such a study should routinely be done under code. The direct examination of stool material without addition of serum may also be carried out if sufficient antigen is present; however, identification or determination of the significance of such a particle should be carried out by IEM as outlined above.[282,304]

An RIA and an ELISA for detection of the Norwalk virus have been developed[52,180,209,218,241] that are more efficient than IEM. The test is essentially a research tool, since suitable reagents are not generally available. Recently, the availability of antibody to the recombinant Norwalk virus has led to the development of an ELISA for detection of the Norwalk virus.[272] Norwalk virus can also now be detected by reverse transcription and polymerase chain reaction methodology.[18,122,271] An IAHA has also been developed for the Norwalk agent,[296] but it is not efficient for its detection in clinical specimens. An RIA and an ELISA are available for the Snow Mountain agent and an ELISA has been developed for the Hawaii

virus,[134,349,557] but EM and IEM remain the only methods for the detection and IEM the only method for the identification of the other members or putative members of the group.

The Norwalk group of agents do not produce illness in any experimental animal.[57,129,130,304,619,626] However, the Norwalk virus infects chimpanzees by the alimentary route as indicated by shedding of antigen and a serological response.[218,619]

3.4.1b. Serological Studies. As noted above, IEM remains a mainstay for studying this group of agents. In this technique, the stool material that contains the particle is incubated with a standard dilution of an acute, or pre-, and a postillness serum specimen, and the amount of antibody coating the particle is scored on a $0–4^+$ scale.[292,293,299,304] An example of a seroresponse to the Norwalk virus by a volunteer who developed illness following oral administration of Norwalk virus is shown in Fig. 3. The difference in the amount of antibody coating the Norwalk virus following its incubation with the volunteer's prechallenge serum or postchallenge serum is clearly evident. Since numerous spherical particles are detected in stool by EM, it is essential to establish the significance of these objects by IEM employing appropriate paired sera. After a viruslike particle has been detected, a seroresponse should be demonstrated as an initial step in associating this particle with infection.

The development of an RIA- and an ELISA-blocking test for measurement of Norwalk virus antibody has greatly facilitated epidemiologic study of this agent.[52,180,213–215] These assays are as efficient as IEM for detecting a seroresponse and are much more practical, since they are much less time-consuming and also require much less antigen and antibody. In addition, an IAHA for detection

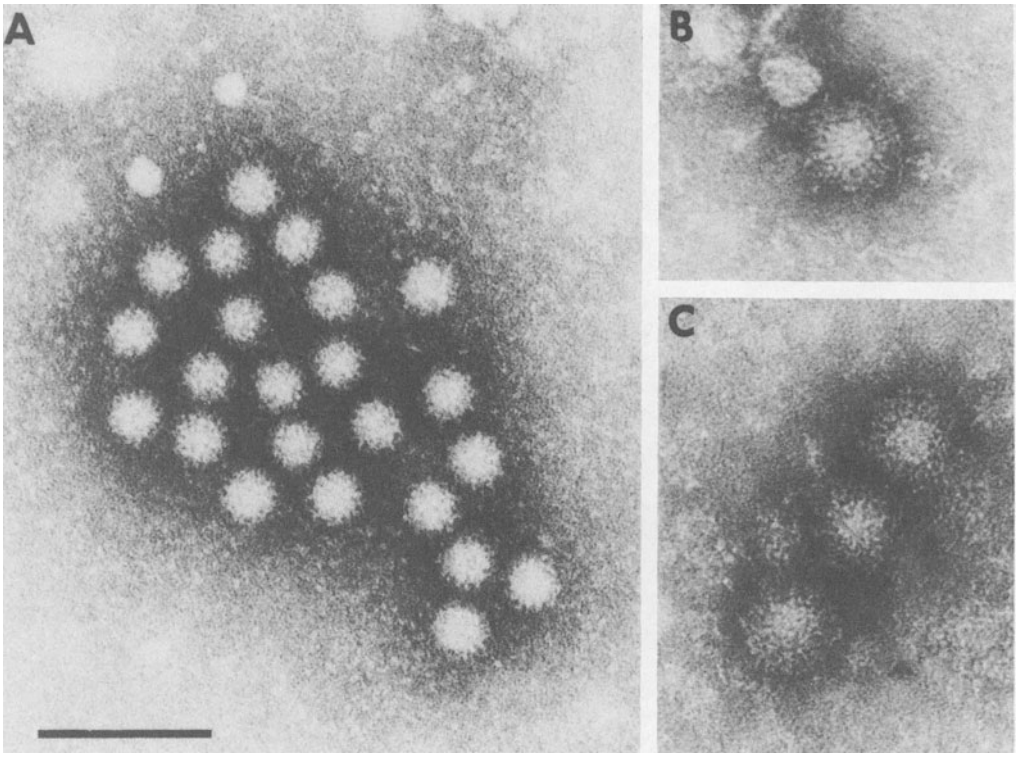


Figure 3. (A) An aggregate observed after incubation of 0.8 ml of Norwalk (8FIIa) stool filtrate with 0.2 ml of a 1:5 dilution of a volunteer's prechallenge serum and further preparation for electron microscopy.[293] This volunteer developed gastroenteritis following challenge with a second-passage Norwalk filtrate that had been heated for 30 min at 60°C.[129] The quantity of antibody on the particles in this aggregate was rated 1–2–2+, and this prechallenge serum was given an overall rating of 1–2+. (B) A single particle and (C) three single particles observed after incubating 0.8 ml of the Norwalk (8FIIa) stool filtrate with 0.2 ml of a dilution of the volunteer's postchallenge convalescent serum and further preparation for EM. These particles are very heavily coated with antibody. The quantity of antibody on these particles was rated 4+, and the serum was given an overall rating of 4+ also.[293] The difference in the quantity of antibody coating the particles in the prechallenge and postchallenge sera of this volunteer is clearly evident. The bar = 100 nm and applies to A, B, and C. From Kapikian *et al.*[293]

of Norwalk virus antibody has been developed.[296] It is not quite as efficient as either IEM or RIA but is, of course, more practical than IEM. The RIA, ELISA, and IAHA are essentially research tools because of the paucity of appropriate Norwalk antigen, which until recently has relied on particle-positive stools as the source of antigen. However, as noted earlier, the availability of baculovirus recombinant-expressed Norwalk capsid protein represents a major breakthrough in the study of these viruses. With this antigen as a precoat, a direct ELISA has been shown to be as specific, sensitive, and efficient as previously described methods for detection of serological evidence of Norwalk virus infection.[205,270,392] RIA- and ELISA-blocking tests have been developed for the Snow Mountain virus and an ELISA-blocking test has been developed for the Hawaii virus.[134,349,557]

3.4.2. Rotavirus

3.4.2a. Antigen Detection. Important progress has finally been made in the cultivation of the fastidious human rotaviruses directly in tissue culture from clinical specimens.[490,574] This was accomplished by pretreatment of the specimen with trypsin (10 μg/ml), incorporation of trypsin into the maintenance medium, use of roller tube cultures of MA104 cells, and incubation at 37°C. Virus can be isolated successfully from over 75% of fecal samples known to be rotavirus positive by other tests. Primary African green or cynomolgus monkey kidney cell cultures are even more efficient than MA104 cells for virus isolation.[236,404,598] However, such methods are not practical for most laboratories since tissue cultures are expensive and, in addition, require a sophisticated laboratory with experienced personnel. In the early studies, EM was the mainstay for detection of human rotavirus in stool specimens since these agents could not be propagated in cell cultures directly from clinical specimens. Although IEM was also employed, the addition of antibody was not essential, since in contrast to the Norwalk group, rotaviruses have a quite distinct morphological appearance, as shown in Fig. 1B.[298] Various simple and more readily available methods for rotavirus detection have been developed as practical alternatives to EM for diagnosis and for research epidemiologic studies. Table 1 shows numerous methods that have been described for rotavirus detection and presents a rating for each on a 1–4+ scale, with 1+ indicating a low degree of efficiency or practicality and 4+ a high degree. Though EM and IEM are very efficient, they are not practical, but nevertheless they remain the "supreme court" of rotavirus detection, since questionable results by any of the assays can usually be resolved by examination of the specimen by EM. In our laboratory, the method of choice at present is the confirmatory ELISA, since it is practical and efficient, does not require sophisticated equipment[62,303,304,642] and has a "built-in" control for nonspecific reactions. This is essential because fecal samples may bind nonspecifically to the precoat, which is comprised of rotavirus specific antibody.[94] Without a rotavirus-negative serum as the precoat in a companion well it is not possible to determine if the binding is specific.[441,446,560] Recently, the polymerase chain reaction (PCR) was applied to the detection of rotavirus and shown to be exquisitely sensitive.[631] Commercial kits are now available for the ELISA, latex agglutination (LA), and reverse passive hemagglutination assays.

The selection of technique will depend on the investigator's capabilities and experience and on the availability of appropriate reagents. Regardless of the methods employed, it is striking that most of the rotavirus detection methods do not require *in vitro* cultivation but rather employ "direct virology," a simple concept that as so often occurs in medical research could have been employed years ago but has been applied only relatively recently.[304]

3.4.2b. Serological Studies. Numerous assays have been described for detection of rotavirus antibody. Since in early studies the human rotaviruses could not be grown in cell cultures, the detection of serological responses relied on IEM, utilizing human rotavirus-positive stools as antigen.[298] However, this time-consuming method was soon superseded by the development of a CF test in which particle-rich human stools were used as antigen.[290,291,298] This method was limited by the paucity of human stools containing sufficient particles for the CF assay. It was soon discovered, however, that animal and human rotaviruses shared a common CF antigen, and thus animal strains could be used as substitute CF antigens for detection of infection with human rotavirus.[290,291] This antigenic relationship was of special importance, since certain animal rotaviruses such as a few calf strains, simian strain SA-11, and the "O" agent (from intestinal washings of sheep and cattle) had been propagated efficiently in cell cultures, and thus the quantity of CF antigen was virtually unlimited.[351,352,377,510] With the successful cultivation of human rotaviruses, neutralizing antibodies can now be measured by plaque reduction or by inhibition of cytopathic effect or virus yield in roller tube cultures.[151,236,260,301,302,574,575,624]

A variety of other serological assays have been developed to detect serological evidence of rotavirus infection, such as immunofluorescence (IF), IAHA, hemagglutination inhibition (HI), and ELISA. A summary of the relative efficiency and practicality of serological methods

Table 1. Efficiency and Practicality of Methods Available for Detection of Human Rotaviruses from Stool Specimens[a]

Method	Efficiency[b]	Practicality for large-scale epidemiologic studies (assuming 4+ efficiency)[b]
Electron microscopy (EM)[38,44,62,127,157,298,385,410,417,533]	4+	1+
Immune electron microscopy (IEM)[62,127,297,298,617]	4+	1+
Immunosorbent EM[417]	4+	1+
Complement-fixation (CF) (conventional)[193,317,385,496,520]	1+	4+
Modified CF[656]	3–4+	2–3+
Human fetal intestinal organ culture [with immunofluorescence (IF)][628]	1+	0
Counterimmunoelectro-osmophoresis[202,384,417,520,521,563]	3–4+	4+
Fluorescent virus precipitin test[173,434,647]	4+	1+
Cell culture (cytopathic effect)[398,620]	1+	2–3+
Cell culture (with IF)[6,38,70,398,443,620]	1+	1+
Cell culture (with EM)[443,620]	1+	1+
Centrifugation onto cell culture (with IF)[21,72,396,554,556]	3–4+	1+
Cell culture (with trypsin, roller tubes, 37°C)[8,176,236,489,490,574,575,624]	3–4+	2+
Rescue in cell culture by genetic reassortment[208,217,621]	3+	1+
Gel diffusion[612]	1+	4+
Smears (with IF)[606]	1+	4+
Radioimmunoassay (RIA)[53,109,278,383,633]	4+	3–4+
Enzyme-liked immunosorbent assay (ELISA)[38,62,304,549,638,642,644,645,648]	4+	4+
Immune adherence hemagglutination assay (IAHA)[364,368]	3+	2–3+
Reverse passive hemagglutination assay (RPHA)[480,481]	3–4+	4+
RNA electrophoresis patterns in gels[149,183,252,316]	3+	1+
RNA electrophoresis patterns in silver-stained gels[240,444]	4+	1+
Enzyme-linked fluorescence assay (ELFA)[643]	4+	3+
Ultrasensitive enzymatic radioimmunoassay (USERIA)[235]	4+	3+
Self-contained enzymic membrane immunoassay (SCEMIA)[637]	4+	4+
Solid-phase aggregation of coupled erythrocytes (SPACE)[58]	3–4+	3–4+
Agglutination of coated latex beads (LA)[479,483,580]	3–4+	4+
3′ Terminal labeling of extracted RNA[97]	4+	1+
Dot hybridization[145,164]	4+	1+
Polymerase chain reaction (PCR)[631]	4+	3–4+

[a]From Kapikian *et al.*,[304] with additions.
[b]On a scale of 1 to 4+, where 1+ indicates a low degree of efficiency or practicality and 4+ indicates a high degree of efficiency or practicality.

for rotavirus is presented in Table 2. The ELISA-blocking and -binding assays are more efficient than CF for detecting rotavirus infection in infants less than 6 months of age and also in adults.[646,649] The CF and ELISA methods are comparable in efficiency for infants and young children 6–24 months of age; IF is almost as efficient as ELISA for rotavirus antibody detection.[646] ELISA appears to be the most efficient of the available methods; however, it is not quite as practical as CF in laboratories where the latter is used routinely for other agents. CF may be employed with confidence if its limits of efficiency are recognized and alternate tests are employed when needed. The ELISA has an additional advantage over CF in that the former permits the measurement of immunoglobulin classes.[373,649,650] In addition, early diagnosis may be made by ELISA, since a specific IgM response may appear as early as 5 or 6 days after onset of illness.[649] Of extreme importance in evaluating serological responses in the under-6-months age group of infants who possess naturally acquired maternal IgG antibodies was the development of an efficient and sensitive ELISA IgA antibody test.[121,344,345] This made it possible to determine if prior rotavirus exposure had occurred because IgA does not cross the placenta. The ELISA IgA has also been used to evaluate the level of coproantibodies as well as local salivary antibodies.[4,268,345] In addition, fecal IgA antibody levels appear to correlate with duodenal IgA antibody levels.[103]

There is no substitute as yet for neutralization assays when serotype-specific antibody is being measured. In this regard, the plaque reduction neutralization assay is

Table 2. Efficiency and Practicality of Methods Available for Detecting Serological Evidence of Human Rotavirus Infection[a]

Method	Efficiency[b]	Practicality for large-scale epidemiologic studies (assuming 4+ efficiency)[b]
Immune electron microscopy (IEM)[57,159,297,298,317]	4+	<1+
Complement-fixation (CF)[57,138,147,229,290,291,297,298,354]	3–4+	4+
Immunofluorescence (IF)[116,147,160,310,398,424,623,628]	4+	1+
Gel diffusion[612]	Not known	2+
Counterimmunoelectro-osmophoresis[101,384]	Variable	2+
Neutralization of calf rotavirus in cell culture[160,285,301,302,366,612]	2+	1+
Neutralization of human rotavirus or reassortant in cell culture tubes[301,302,621]	4+	1+
Plaque reduction neutralization of human rotavirus or reassortant in cell culture[301,302,621]	4+	1+
Radioimmunoassay (RIA)[53,488]	Not known	3+
Enzyme-linked immunosorbent assay (ELISA)[509,634,646,649–651]	4+	4+
ELISA-based neutralization assay[312a]	4+	2+
Inhibition (neutralization) of fluorescent foci[72,147,553,554,623]	4+	1+
Immune adherence hemagglutination assay (IAHA)[262,365,368]	3–4+	4+
Hemagglutination-inhibition (HI)[154,354,482,506,522]	1+	4+

[a]From Kapikian *et al.*,[304] with additions and modifications.
[b]On a scale of 1 to 4+, where 1+ indicates a low degree of efficiency or practicality and 4+ indicates a high degree of efficiency or practicality.

more sensitive than tube neutralization for detection of antibody, although the latter is somewhat more efficient for detecting a seroresponse (A. Z. Kapikian, unpublished data).

An important addition to dissecting the immune response was the development of a competition-solid-phase immunoassay that measures epitope-specific immune responses to individual rotavirus serotypes.[204,207,361,504] The test serum is used as the blocking reagent, whereas individual monoclonal antibodies serve as the detecting reagent.

4. Biological Characteristics

4.1. Norwalk Group of Viruses

This group of agents is composed of several viruses that: (1) were detected in the stool of patients with gastroenteritis; (2) have not been propagated *in vitro*; (3) are about 27 nm in diameter and do not have a distinctive morphological appearance by EM; (4) have a buoyant density in CsCl of 1.33–1.41 g/cm^3; and (5) until recently, had not been characterized regarding the type of nucleic acid in the genome.[295,299,304,551,626] However, in an important breakthrough, two members of the group, first the Norwalk virus and then the Southampton virus (a Norwalk-like virus) were cloned and found to possess a positive-sense single-stranded RNA genome.[269,328,363] The Norwalk virus represents the prototype strain of this group of agents.[299]

Some of the biochemical or biophysical characteristics of the Norwalk virus have been elucidated in volunteer studies in which the ability of a treated stool filtrate to induce illness was determined. The Norwalk virus was found to be acid stable (pH 2.7 for 3 hr at room temperature), relative heat stable, and ether stable (20% ether at 4°C for 24 hr).[129]

The Norwalk group of viruses has been characterized antigenically by cross-challenge studies and by IEM or solid-phase (SP) IEM using antisera from infected individuals. Thus, four distinct serotypes are recognized: Norwalk, Hawaii, Snow Mountain, and Taunton viruses, which are numbered 1, 2, 3, or 4 based on historical precedence (Table 3).[328,339,340] By carrying out reciprocal IEM studies on viruslike particles and paired sera obtained from various outbreaks of gastroenteritis in Japan, investigators identified nine antigenic patterns (designated S1–9).[422]

Examination of paired sera from volunteers who had developed illness following challenge with the Norwalk, Hawaii, or Snow Mountain viruses by ELISA demonstrated that this assay could not discriminate serotypically among these strains.[348] This is important, epidemiologically, because it is likely that with shared antigens, the ELISA will not enable a specific etiology to be established. In contrast, a relatively high degree of specificity has been demonstrated by IEM and SPIEM techniques.[328,339,422]

Table 3. Characterisitics of the Norwalk and Norwalk-like Viruses[a,b]

Virus	Size (nm)	Buoyant density in cesium chloride (g/cm^1)	Growth in cell culture	Administration[c] of virus induces illness		Particles detected by	Serological studies by	Antigenic relationships [suggested serotype number[328]][d]
				Humans	Animal(s)			
Norwalk[18,122,129,130,180,218,241,271,295,299,349,618]	27 × 32[e]	1.38–1.41	No	Yes	No	IEM, RIA, IAHA, ELISA, PCR	IEM, RIA, IAHA, ELISA	Distinct [1]
Hawaii[131,299,551,557,618]	26 × 29[e]	1.37–1.39	No	Yes	No	IEM, ELISA	IEM, ELISA	Distinct [2]
Montgomery County[299,551,618]	27 × 32[e]	1.37–1.41	No	Yes	No	IEM	IEM	Related to Norwalk virus
Snow Mountain[67,134,371]	25–26	1.33–1.34	No	Yes	NT	IEM, RIA, ELISA	IEM, RIA, ELISA	Distinct [3]
Taunton[80,81,339]	32–34	1.36–1.41	No	NT	NT	EM	SPIEM	Distinct [4]

[a]From Kapikian.[282]

[b]Also designated small round structured viruses (SRSVs). Reference numbers listed in parentheses. IEM, immune electron microscopy; RIA, radioimmunoassay; IAHA, immune adherence hemagglutination assay; ELISA, enzyme-linked immunosorbent assay; SPIEM, solid-phase immune electron microscopy; PCR, polymerase chain reaction; NT, not tested.

[c]By alimentary route.

[d]By IEM, SPIEM, or virus challenge studies or a combination. There are two other serotyping schemes: one describes four serological groups of these viruses by SPIEM: (1)"SRSV UK1" = Taunton virus; (2) "SRSV UK2" = Norwalk virus; (3) "SRSV UK3" = Hawaii virus; and (4) "SRSV UK4?" = Snow mountain virus = SRSV Japan 9.[340] Another describes nine antigenic patterns of SRSV from Japan designated SRSV S1–9.[422]

[e]Shortest × longest diameter.

The classification of the Norwalk virus is of special interest because this fastidious agent was initially considered to resemble a parvovirus on the basis of certain properties.[129] However, when it was found later to possess a single structural protein of about 65,000 Da and subsequently, when it was shown that patients with documented calicivirus infections developed serological responses to the Norwalk virus, it seemed almost certain that the Norwalk virus was a calicivirus.[75,107,212,215,282,492,493] This was in spite of the lack of knowledge regarding its nucleic acid content.

Recently, molecular biological studies established that the Norwalk virus was indeed a calicivirus, when it was cloned and sequenced and found to have a genome organization similar to that of various established caliciviruses.[270,272,288,328] It possesses a positive-sense polyadenylated single-stranded RNA of 7642 nucleotides (excluding the poly A tail) that was predicted to encode three open reading frames (ORFs). The second ORF was expressed in insect cells that had been inoculated with a baculovirus recombinant containing ORF2; as noted earlier, empty particles were formed by self-assembly of the expressed protein (Fig. 2).[272] The Southampton virus, another Norwalk-like virus morphologically, which was also cloned, sequenced, and expressed, is also classified as a calicivirus on the basis of its genome organization.[328]

It should also be noted that an earlier attempt at a tentative interim classification of small round fecal viruses based on morphological appearance by EM, two broad groups of viruses were distinguished: featureless or structured, as shown in Table 4.[80] The featureless group included the picornaviruses and the parvoviruslike agents. (The latter included the cockle, Wollan, Ditchling, and Parramatta viruses, each of which had been detected in the feces of individuals with gastroenteritis.) The structured group included the small round structured viruses (SRSVs) composed of the Norwalk, Hawaii, Montgomery County, and Taunton viruses. Other structured viruses included the classical caliciviruses (i.e., those with the characteristic deep surface hollows that are not observed in the Norwalk virus group), astroviruses, minireoviruses, and the Otofuke, Sapporo, and Osaka agents.[386,421,523,545]

Evidence for the etiologic role of the Norwalk group of agents in gastroenteritis differs for each member. In one study, the Norwalk agent was shown to induce illness in 30 (58%) of 52 volunteers[618]; serological evidence of infection has been demonstrated in most volunteers who developed illness as well as in certain individuals who developed illness during the original outbreak.[218,296,299] Further evidence of an etiologic association was the demonstration in volunteers of a close temporal relationship between virus shedding and illness, with maximal shedding at the onset of experimental illness.[550] Only short-term immunity characteristically occurs in volunteers who develop illness after initial challenge[129,426,618]; prechallenge serum antibody titers did not correlate with susceptibility to illness.[426] Volunteers not only developed illness following administration of stool filtrates containing the Hawaii, Montgomery County, or Snow Mountain agents,[397,405] but also demonstrated seroresponses to the challenge virus by IEM.[397,618]

4.2. Rotaviruses

Human rotavirus has been detected in stools of about 35–52% of infants and young children hospitalized with acute gastroenteritis and much less often in older children and adults with this disease.[61,63,114,215,297,304,317,321,341] Rotaviruses have also been found in stools of numerous animals.[473] They have been associated with a diarrheal illness in the bovine calf,[66,377,378,610] infant mouse,[399] piglet,[333,376,453,473,611] foal,[158,569] lamb,[514] young rabbit,[73,435,442,555] monkey,[351,532] newborn deer,[567] newborn antelope,[447] young chimpanzee,[16] young gorilla,[16] young turkey,[32,374,375] chicken,[275,375] young goat,[499] young kitten,[511] young dog, and buffalo calf[401,460,461,473]; pneumoenteric illness has been found in the newborn impala,[153] newborn addax,[153] and newborn gazelle.[153] The offal ("O") agent was derived from mixed intestinal washings from abattoir waste.[352] Until the relatively recent breakthrough that enables the cultivation of most rotaviruses, as described earlier, only human rotavirus Wa, the calf, piglet, and monkey isolates, and the "O" agent had been successfully propagated efficiently in cell culture. With the exception of the SA-11 strain of simian rotavirus, canine rotavirus, and the "O" agent, each of the others has been associated with naturally occurring diarrheal (or pneumoenteric, as already noted) illness in newborns of each respective group.

As shown in Fig. 1B, rotaviruses have a distinctive morphological appearance. Complete particles possess a double-shelled capsid and measure about 70 nm in diameter; single-shelled particles measure about 55 nm in diameter, and within this shell is a third layer, the core, which contains the 11 segments of double-stranded RNA and has a diameter of about 37 nm.[286,371,439a] Cryoelectron microscopic studies demonstrate the presence of 60 spikes about 10–12 nm in length that protrude from the outer capsid.[439a,440] The term rotavirus comes from the Latin word *rota*, meaning wheel, and was suggested because the sharply defined circular outline of the outer capsid gives the appearance of the rim of a wheel placed on short

Table 4. Comparison of Human Caliciviruses with Other Small, Round Particles Shed in Feces by Patients with Acute Gastroenteritis[a]

Virus group	Size (nm)	Characterisitics of certain strains in indicated category				Growth in cell culture	Important as cause of:	
		Morphology (previous interim classification)	Nucleic acid and genome organization	Viral proteins	Density in CsCl g/cm^3		Severe infantile gastroenteritis	Epidemic gastroenteritis of children and adults
Caliciviruses								
Norwalk group[212,215,295,299,337]	26–32	Spherical, without sharply defined edge; suggestion of surface hollows (structured : SRSV[b])	RNA, calicivirus genome	1 structural protein (58–62kDa)	1.33–1.41	No	No	Yes
UK 1-4 and Japanese virus (also designated as HuCV strains)[77,106,210,327,548]	≈32–35	Prominent surface hollows and may have six-pointed surface star with central hollow (structured : calicivirus)	RNA	1 structural protein (62 kDa)	1.37–1.38	Abortive replication	No	No
Possible caliviruses								
Otofuke, Sapporo, and Osaka strains[313,421,545]	35–40	Surface projections (structured : SRSV)	Not determined	Not determined	Not determined	No	No	No
Astrovirus[210,326,327]	27–34	Spherical; five or six pointed star without a central hollow (in ~ 10%); triangular surface hollows; smooth edge (structured : astrovirus)	RNA	Reports vary (90-kDa precursor cleaved to 31-,39-,20-kDa proteins)	1.33–1.42	Yes	No	No
Parvoviruslike viruses: "W,"[12,98,293] Cockle,[11,13,565] Ditchling,[12] Parramatta[92]	23–26	Spherical; smooth outer edge; smooth surface [featureless : probable (parvovirus)]	DNA (cockle virus)	Not determined	1.38–1.40	No	No	No
Small round viruses (SRVs)[11,40,161,314]	23–26	Spherical; smooth outer edge; smooth surface (featureless : SRV)	Not determined	Not determined	Not determined	No	No	No

[a]From Kapikian *et al.*[287]
[b]SRSV, small round structured virus.

spokes radiating from a wide hub.[155,160,163] Rotaviruses resemble orbiviruses and reoviruses morphologically but differ in their fine structure.[625] Rotaviruses have a density of 1.36 g/cm^3 in cesium chloride and are ether stable but acid labile.[286]

The rotavirus genome is comprised of 11 segments of double-stranded RNA.[371] The migration patterns of the RNA segments of rotaviruses as determined by polyacrylamide-gel electrophoresis (PAGE) are of importance not only in the biophysical characterization of these agents but also as epidemiologic probes; they have served as one of the methods of differentiating human and animal rotaviruses as well as various human rotavirus strains.[452] The term "electropherotype" was applied to this method of distinguishing strains.[152] The distinctive RNA migration pattern of rotaviruses has also been used for detection and identification of rotavirus strains from clinical specimens.[149]

Each of the 11 rotavirus genes has now been sequenced.[371] As shown schematically in Fig. 4, the outermost surface of the virion is composed of two proteins, VP7 and VP4. VP4 takes the form of 60 surface projections (spikes) extending about 12 nm from the outer surface (VP1) of the capsid.[439a,440] VP6, which represents over 51% of the virion, makes up the inner layer of the double capsid.[371] Within the inner capsid is the core, which has been described as a third shell, comprised of VP2.[371,439a] The core encloses the genome as well as the viral proteins VP1 and VP3, designated recently as subcore proteins.[439a] Each of the six structural proteins is encoded by a single gene. In addition to the six structural proteins, rotaviruses possess five nonstructural proteins that are found only in infected cells and not in the mature virion.[371] Double-shelled ("complete" or "smooth") particles have a density of 1.36 g/cm^3 in CsCl and a sedimentation coefficient of 520–530 S.[371] Single-shelled (rough) particles have a density of 1.38 g/cm^3, whereas "empty" particles that have been penetrated by negative stain have a density of approximately 1.29–1.30 g/cm^3.[371] Core particles have a density of 1.44 g/cm^3 in CsCl.[36,371]

Since rotaviruses share certain properties with the reoviruses and orbiviruses and yet are distinct serologically and in certain biophysical aspects, they have been officially classified as a new genus in the family *Reoviridae*.[370] This family now contains nine genera: orthoreovirus (sigla from respiratory enteric orphan), orbivirus, rotavirus, Coltivirus (sigla from Colorado tick fever), phytoreovirus, Fijivirus, Cypovirus (sigla from cytoplasmic polyhedrosis), aquareovirus, and oryzavirus. Certain members of the first three genera infect humans, whereas the phytoreoviruses and Fijiviruses infect plants, the cytoplasmic polyhedrosis viruses infect insects, and the aquareoviruses infect fish.[370,477,478] Antigenically, rotaviruses are distinct from reoviruses by CF, IEM, and neutralization or RIA, from selected orbiviruses by CF, and from bluetongue virus (an orbivirus) by IEM.[116,278,290,291,298]

Rotaviruses have three major antigenic specificities: group, subgroup, and serotype.[170,289,371] Currently, seven groups (A–G) have been identified.[64] In diagnostic as-

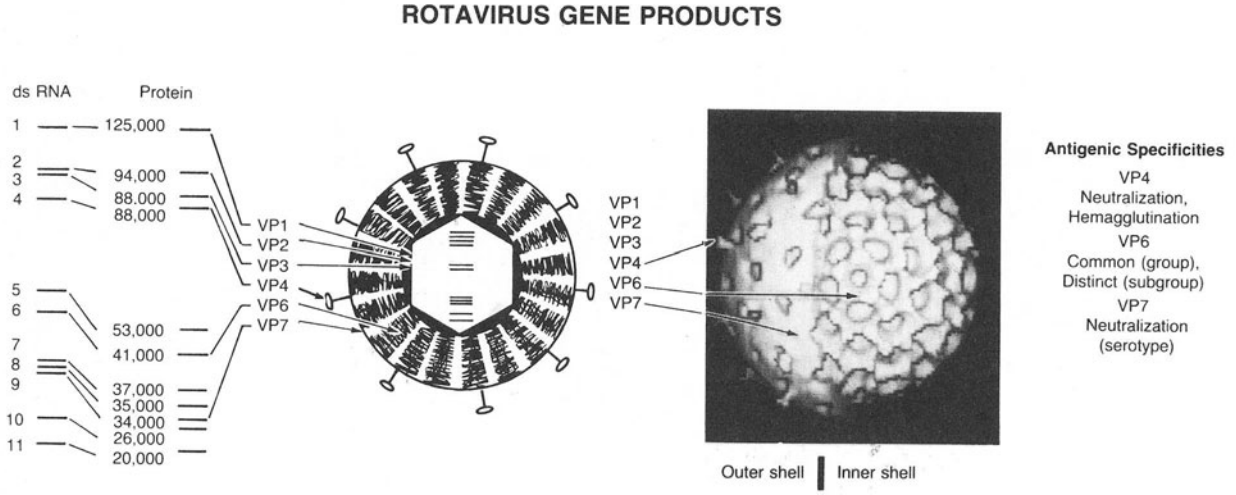


Figure 4. The figure on the left is a schematic representation of the rotavirus double-shelled particle. The figure on the right (from Prasad *et al.*[440]) shows surface representations of the three-dimensional structures of a double-shelled particle (on the left half) and a particle (on the right half) in which most, if not all, of the outer shell and a small portion of the inner shell mass have been removed. The apparent molecular weights derived from sodium dodecyl sulfate PAGE for the SA-11 strain are shown for individual proteins.[371] From Kapikian and Chanock.[286]

says, VP6 (encoded by the sixth gene) is the predominant group antigen. Most epidemiologically important rotaviruses that infect humans and animals share group A specificity. VP6 also mediates subgroup specificity, which continues to be an important epidemiologic marker, especially when serotyping of strains is not feasible. Most group A rotaviruses belong to either subgroup I or II.[19,253] The group A rotaviruses are further divided into serotypes according to the specificity of VP7 (encoded by gene 7, 8, or 9 depending on the strain) and VP4 (encoded by gene 4).[27,165,253,258,277,390] VP7 and VP4 each induce neutralizing antibodies and the separation into serotypes is made by neutralization assay in cell culture.[253,258] At present, 14 VP7 serotypes (also termed G serotypes because VP7 is a glycoprotein) have been described in humans or animals as shown in Table 5.[253] The sharing of VP7 serotype specificity among various human and animal rotaviruses is readily apparent.

A serotyping system for VP4 specificity has recently been suggested. At present, seven VP4 serotypes (termed "P" serotype because VP4 is protease sensitive) and four subtypes have been described on the basis of neutralization as shown in Table 6.[253,500] The genetic homology of the various VP4s has also led to a classification system based on genotype, but the "P" serotype designation is reserved for VP4 neutralization specificity. It should be noted that alphabet letters designate group, Arabic numbers serotype, and Roman numerals subgroup. In addition, a binary system of rotavirus serotype classification is in place to indicate the role of VP4 and VP7 neutralization specificities.[257,258]

As noted above, the rotaviruses that share the group antigen are classified as group A rotaviruses, whereas the others that do not share this antigen are designated as non-group-A rotaviruses.[64] These non-group-A rotaviruses are also known as pararotaviruses, rotaviruslike viruses, novel rotaviruses, antigenically distinct rotaviruses, and adult diarrhea rotaviruses.[64,262] They have been recovered from humans, calves, pigs, lambs, rats, ferrets, and birds.[64,594] The group B rotaviruses have been responsible for large outbreaks of gastroenteritis in China, predominantly in adults.[64,90,262,594] However, the group B rotaviruses have been detected only rarely outside of China.

The human rotaviruses were considered to be rather fastidious agents, defying efficient propagation in any cell- or organ-culture system. A few strains had been cultivated to a limited extent with only a small percentage of cells exhibiting evidence of infection.[153,620,628] However, human rotavirus Wa, a VP7:1, VP4:1A virus, was

Table 5. Serotypic and Genotypic Classification of Group A Rotavirus VP7 and Subgroup Specificities[a]

VP7 (G) serotype[b]	Human rotavirus strains (subgroup)	Animal rotavirus strains (subgroup) [species]
1	Wa, KU, D, M37, RV-4, WI79, K8 (II)	C60, C86 (I) [pig]; T449 (I) [cow]
2	DS-1, S2, KUN, RV-5, 1076 (I)	C134 (I) [pig]
3	P, MO, YO, RV-3, Ito, Nemoto, WI78, McN (II); AU-1, AU228, Ro1845, HCR3 (I); 0264 (I and II)	SA11 (I) [vervet monkey]; MMU18006 (I) [rhesus monkey]; CU-1 K9, RS-15 (I) [dog]; TAKA, Cat2, Cat97, FRV-1 (I) [cat]; H-2 (not I or II), FI-14 (I and II) [horse]; CRW-8, C176 (I) [pig]; R-2 (II), Ala, C11 (I) [rabbit]; Eb (I), EW (not I or II) [mouse][c]
4	ST3, ST4, VA70, Hosokawa, Hochi, 57M (II)	Gottfried, SB-1A (II), BEN-144 (?), SB-2 (I) [pig]
5	IAL28 (II)	OSU, EE, TRF-41 (I) [pig]; H-1 (I) [horse]
6	PA151, PA169 (I)	NCDV, UK, RF, WC3, Q17, OK, ID (I), B641, C486 (?) [cow]
7	None	Ch2 (not I or II) [chicken]; Ty1[d] (not I or II) [turkey]; PO-13 (I) [pigeon]; 993/83 (not I or II) [cow]
8	69M, B37, HAL1271, HAL1166 (I), PA171 (II)	678, J2538, A5 (I) [cow]
9	WI61, F45, 116E (II), Mc323 (I)	ISU-64 (I) [pig]
10	A64 (II), Mc35, I321 (I)	B223, V1005, KK3, 61A, Cr (I) [cow]; Lp14 (?) [sheep]
11	None	YM, A253.1 (I) [pig]
12	L26 (I)	None
13	None	L338 (I) [horse]
14	None	F123 (I) [horse]

[a]Adapted from Hoshino and Kapikian,[253] with additions from Gerna *et al.*,[189] Gouvea *et al.*,[199] and Isegawa *et al.*[267]

[b]VP7 (G) serotype as determined by reciprocal cross-neutralization; in addition, it should be noted that VP7 genotype as determined by comparative amino acid sequence analysis and/or nucleic acid hybridization has been used widely as a means of distinguishing among rotavirus strains and where evaluated, has correlated with serotyping differences by neutralization assay. Thus, genotyping is now frequently used as a proxy method for neutralization for VP7 (G) serotyping.[191,206]

[c]Human rotaviruses with neither subgroup I or II specificity have also been reported but without serotype designation.

[d]Recent amino acid sequence anaysis of the VP7-encoding gene of Ty1 suggests that it may not belong to this serotype.

Table 6. Serotypic and Genotypic Classification of Group A Rotavirus VP4[a]

VP4(P) serotype[b]	VP4 genotype[c]	Human rotavirus strains (G serotype)	Animal rotavirus strains (G serotype) [species]
1A	8	KU, Wa (1); P, YO, MO (3); VA70, Hochi, Hosokawa (4); WI61, F45 (9)	None
1B	4	DS-1, S2, RV-5[d] (2); L26[d] (12)	None
2A	6	M37 (1); 1076 (2); McN, RV-3[d] (3); ST3 (4)	None
2B	6	None	Gottfried (4) [pig]
3A	9	K8 (1); AU-1[d] (3); PA151[d] (6)	FRV-1[d]; Cat2[d] (3) [cat]
3B	14	Mc35 (10)	None
4	10	57M[d] (4); 69M (8)	None
5A	3	HCR3[d], Ro1845 (3)	CU-1, K9 (3) [dog], Cat 97(3) [cat]
5B	3	None	MMU18006 (3) [rhesus monkey]
6	1	None	NCDV, C486, J2538 (6); A5[d] (8) [cow]; SA11-4fM (3) [vervet monkey]
7	5	None	UK, B641, IND[d], OK[d] (6); 61A[d] (10) [cow]
8	11	116E[d] (9); I321[d] (10)	B223, B-11, A44[d], KK3[d], Cr[d] (10) [cow]
9	7	None	CRW-8 Ben-307 (3); BMI-1, SB-1A (4); OSU, TFR-41 (5); YM, A253.1 (11) [pig]; H1[d] (5) [horse]
10	16	None	Eb (3) [mouse]
11	18	PA169 (6); HAL1166 (8)[d]	None
?	2	None	SA11 (3) [vervet monkey]
?	12	None	H-2, FI-14[d], FI-23[d] (3) [horse]
?	13	None	MDR-13 (3/5) [pig]
?	15	None	Lp14 (10) [sheep]
?	17	None	993/83 (7)[d] [cow]
?	19	None	L338 (13) [horse]

[a]Adapted from Hoshino and Kapikian,[253] with additions from Gerna *et al.*,[189] Gorziglia *et al.*,[197] Hardy *et al.*,[234] Isegawa *et al.*,[267] Nagakomi *et al.*,[405] Sereno and Gorziglia,[500] and Taniguchi *et al.*[542]

[b]VP4(P) serotype as determined by reciprocal or one-way cross-neutralization.

[c]VP4 genotype as determined by comparative amino acid sequence analysis and/or nucleic acid hybridization (from Estes and Cohen,[150] with additions).

[d]Not tested by neutralization; relationship suggested by amino acid sequence and/or nucleic acid hybridization analyses.

adapted to grow efficiently in primary African green monkey kidney (AGMK) cells following 11 serial passages in gnotobiotic piglets.[622,623] Pretreatment of this porcine-grown human rotavirus strain with trypsin was required for optimal growth in AGMK cells; low-speed centrifugation of the virus inoculum onto cell cultures was also employed.

Noncultivatable human rotaviruses were successfully rescued following mixed infection of cell cultures with noncultivatable human rotavirus and cultivatable bovine rotavirus, and application of various selective pressures.[208] The cultivatable reassortants had mixed genotypes but also had the neutralization specificity of human rotavirus. Such arduous methods are no longer required to grow human rotaviruses in cell culture directly from clinical specimens since efficient methods are now available, as described in Section 3.4.2.

Experimentally, human rotavirus induces a diarrheal illness in various newborn animals including gnotobiotic calves, gnotobiotic and conventional piglets, rhesus monkeys, gnotobiotic lambs, and suckling mice[31,473]; in addition, subclinical infections were induced in newborn puppy dogs.[472,566] In studies in China, a severe diarrheal illness was induced experimentally in nonhuman primates, the *Tupaia belangeri yunalis*, following administration of a human rotavirus by the alimentary route.[425] Particle-positive stools from calves have been an important source of human rotavirus for biophysical and serological studies following experimental infection.

Firm evidence exists for the association of rotavirus with gastrointestinal illness. The virus has been consistently detected significantly more often in stools from patients 6–24 months old with gastroenteritis than in those without gastroenteritis[63,114] in both hospitalized

patients and outpatients. Serological evidence of rotavirus infection has also been observed significantly more often in hospitalized gastroenteritis patients than in hospitalized "controls."[297] The virus is detectable predominantly during the acute phase of illness.[114] In addition, in early studies illness was induced in volunteers with a stool filtrate containing a VP7 serotype 1 strain of human rotavirus.[301,302]

5. Descriptive Epidemiology

5.1. Norwalk Group of Viruses

5.1.1. Incidence and Prevalence Data. Specific incidence data for the Norwalk group in the United States are not available. An estimate of the importance of this group of agents is suggested from various sources. Infectious gastroenteritis was the second most common disease experience in the Cleveland Family Study over an approximate 10-year period,[126] and the Norwalk group was probably associated with some of these illnesses, especially in adults. About one third of all outbreaks of nonbacterial gastroenteritis studied are associated with Norwalk virus infection,[214,215] and in a recent estimate, 65% of nonbacterial gastroenteritis outbreaks were provisionally associated with the Norwalk or a related virus.[306]

It is probable that other members of the group are also responsible for a portion of these illnesses, but appropriate tests are not available for the entire group. In children in developed countries, the Norwalk group is probably not an important cause of severe gastroenteritis. Thus, 27-nm virus particles were present in fewer than 2% of infants and young children hospitalized with diarrhea at the Children's Hospital, Washington, DC, a value not significantly different from that observed in controls,[63] nor was serological evidence of Norwalk virus infection detected in selected diarrhea patients from this study.[296] Similar findings with regard to virus detection were made in Japan in a cross-sectional study of pediatric patients hospitalized with diarrheal illness.[321]

In a prospective study of 28 families over a 2-year period, each of 14 families experienced one outbreak of nonbacterial gastroenteritis in which no enteropathogen could be identified by conventional assay; two were associated with the Norwalk virus.[438] None of 28 infants enrolled at birth developed serological evidence of Norwalk virus infection in the first 2 years of life.

In studies in which the recombinant Norwalk virus was used as antigen, Norwalk virus infections were detected in 49% infants and young children studied retrospectively over a 2-year period in a longitudinal study in Finland.[338] This was an unexpected finding because in other studies in developed countries the Norwalk virus did not appear to readily infect individuals in this age group. It should be noted, however, that illnesses could not be associated with the responses.

Incidence data are available from several longitudinal studies outside the United States. In Bangladesh, the incidence of Norwalk virus infection in children less than 5 years of age as determined by a significant RIA serum antibody rise was 29 per 100 children per year; in addition, it was estimated that 1–2% of the diarrheal episodes in these children (who have 5.6 such episodes per year) were caused by Norwalk virus.[49] Moreover, only 1 of 31 children less than 10 years of age who underwent treatment for dehydrating diarrhea and who did not have rotavirus or bacterial pathogens in their stools had serological evidence of Norwalk virus infection.[49] In a similar study in the San Blas Islands in Panama, 35% of the children under 5 years of age developed a seroresponse to Norwalk virus, and it was suggested that the Norwalk virus infection was associated with mild gastroenteritis in this age group.[466]

Finally, in a longitudinal study of infants and children in three northern communities in Canada, the incidence of Norwalk infection was highest (0.15 infection per child per year) in neonates in the only community with relatively unsafe water supplies.[227]

Prevalence data for Norwalk virus infections are available for various parts of the world. By IAHA, the acquisition of antibody to Norwalk virus and rotavirus was compared in infants and young children in the metropolitan Washington, DC, area, young adults at the University of Maryland, and adults in the metropolitan Washington, DC, area.[296] As shown in Fig. 5, the pattern of antibody acquisition differed markedly for these two viruses. There was a gradual acquisition of Norwalk antibody beginning slowly in childhood and accelerating in the adult period, so that by the fifth decade of life, 50% of the adults possessed Norwalk antibody. In contrast, rotavirus antibody was acquired early in life so that by the 36th month of age, over 90% had such antibody. The gradual acquisition of Norwalk antibody is similar to that observed with hepatitis A virus and certain rhinovirus serotypes in comparable populations.[230,535,536] This pattern of antibody acquisition in a major metropolitan area of a developed country suggests that Norwalk virus is not an important cause of gastroenteritis in infants and young children but rather is associated most often with such illness in older persons. A comparison of the prevalence of IAHA Norwalk and rotavirus antibody in a welfare institution for homeless but otherwise normal children

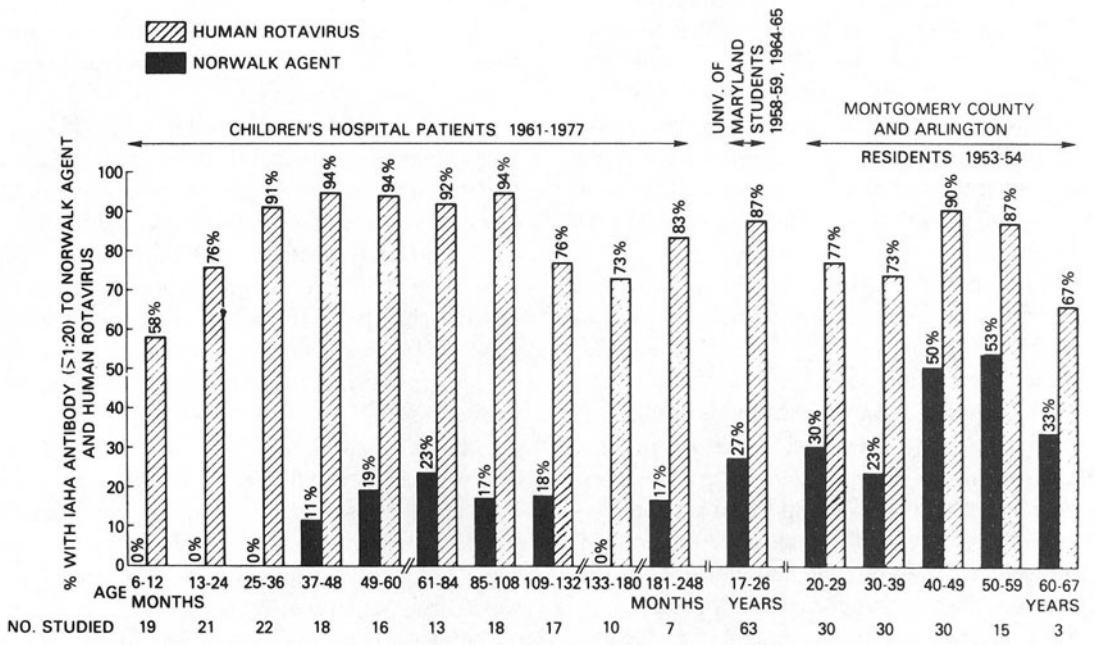


Figure 5. Prevalence of antibody to Norwalk agent and rotavirus by IAHA in three groups. From Kapikian *et al.*[296]

yielded a pattern similar to that just described.[296] In a small study, IAHA antibody to Norwalk agent was also detected in infants, children, and adults in Bangladesh, but the Norwalk antibody prevalence was markedly less than that of rotavirus.[296]

The prevalence of Norwalk antibody was studied in individuals from various parts of the world with the RIA-blocking assay.[213] As shown in Fig. 6, the prevalence rates in adults in the United States and in certain European and less developed countries were similar, with at least a majority of individuals from each country possessing such antibody. An exception was a highly isolated Ecuadorian Indian tribe in Gabaro in which none of the adults studied had evidence of prior Norwalk infection. This was in

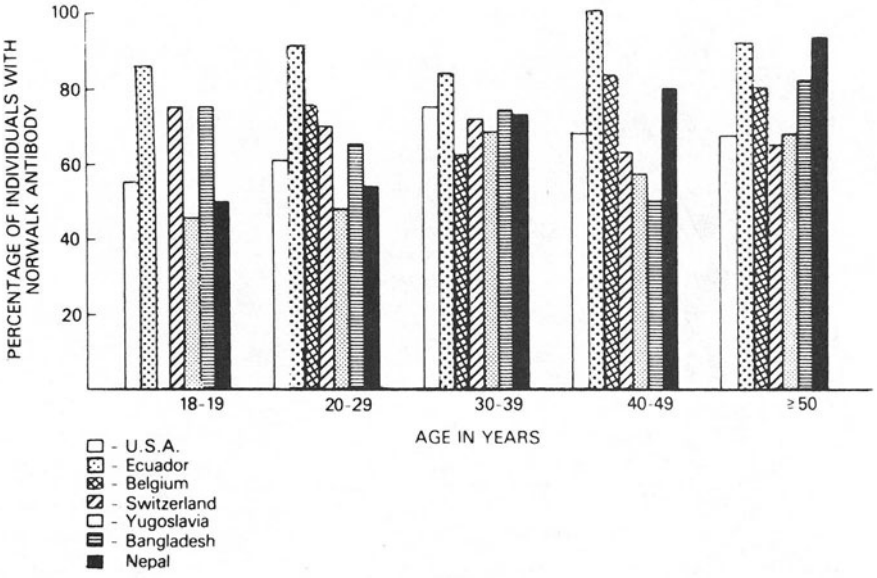


Figure 6. Prevalence by age of serum antibody to Norwalk virus in healthy adults from various parts of the world. Note that no specimens were tested in the 18- to 19-year age group from Belgium. From Greenberg *et al.*[213]

marked contrast to three other less isolated Ecuadorian villages, where approximately 90% had Norwalk antibody. The prevalence of Norwalk antibody in adult male and female homosexuals in the United States was approximately equal (57% and 65%, respectively) and not appreciably different from that in adult blood donors in the United States studied by RIA or in adults studied by IAHA as described above.[213]

Children from the United States, Taiwan, and Yugoslavia acquired antibody more slowly than did children from less well-developed countries such as Ecuador, Bangladesh, Thailand, the Philippines, and Panama.[49,110,137,213,466] This is shown graphically for some of these countries in Fig. 7. The high antibody prevalence in the pediatric age group in Bangladesh and Ecuador (not Gabaro) was unexpected and indicates that the Norwalk or an antigenically related virus infects early in life in at least certain areas of these less-developed countries. Its importance as an etiologic agent of clinical gastroenteritis in this age group remains to be determined; however, as noted above, it does not appear to be an important cause of severe infantile diarrhea. Prevalence data are not available for the other members of the Norwalk group, since suitable serological assays have yet to be developed or have only recently been developed.

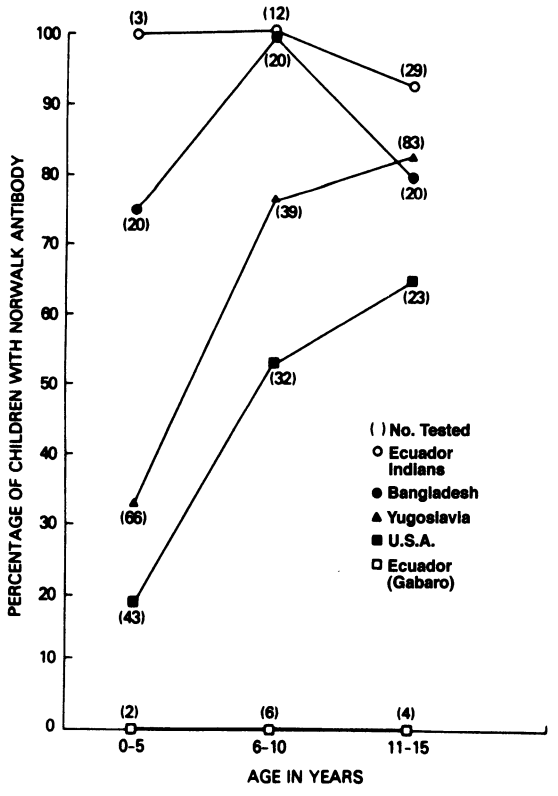


Figure 7. Age-related prevalence of serum antibody to Norwalk virus in children from various countries. From Greenberg *et al.*[213]

5.1.2. Epidemic Behavior. The Norwalk group of agents is associated with epidemic viral gastroenteritis that occurs in family, school, group, institutional, or community-wide outbreaks affecting adults, school-aged children, family contacts, and some young children as well.[215,305,306,312] Although the term "winter vomiting disease" has been applied to certain outbreaks of epidemic viral gastroenteritis, a clear-cut seasonality does not appear to occur, at least for Norwalk virus-associated outbreaks.[214,215,653]

In the Norwalk outbreak, which occurred in an elementary school, and the Snow Mountain outbreak, which occurred at a resort camp, the primary attack rate was 50 or 55%, respectively, whereas the secondary attack rate was 32 or 11%, respectively.[2,397] The incubation period was short, with an average of 48 hr in secondary cases in the Norwalk, Ohio, outbreak.[2] The Hawaii, Montgomery County, and Southampton viruses were obtained in family outbreaks.[328,551,618] The Taunton agent was derived from a hospital outbreak of gastroenteritis in patients and staff in England; the agent was detected in the stools of 9 (47%) of 19 of the patients.[80,81]

In a systematic study of selected paired sera from 70 outbreaks of nonbacterial gastroenteritis employing the RIA-blocking assay, 24 (34%) appeared related to Norwalk virus.[215] Of the 24 outbreaks, four occurred in each of four settings: recreational camps, cruise ships, contaminated drinking or swimming water, and a community or family; three involved elementary or college students; two, nursing homes; one, shellfish; and two others, adults in other settings. The Norwalk-related outbreaks occurred in both the cooler and the warmer months. The association of such a high percentage of nonbacterial gastroenteritis outbreaks with a single member of this group of viruses was unexpected.

In a review of 74 outbreaks of nonbacterial gastroenteritis studied by the CDC from 1976 to 1980 (including most of the 70 noted above), 42% were associated with Norwalk virus infection; an additional 23% were provisionally associated with the Norwalk virus or a related agent, and the remainder were not related to Norwalk virus infection.[306] The overall importance of the Norwalk virus in unselected outbreaks of gastroenteritis was estimated by a review of 642 gastroenteritis outbreaks reported to the CDC from 1975 to 1981.[305] Fifty-four (9.9%) of 558 such outbreaks for which sufficient data were available to enable a provisional etiologic diagnosis based on clinical and epidemiologic characteristics resembled outbreaks previously linked by laboratory methods to

Norwalk virus. The provisional role of Norwalk virus in various outbreaks was as follows: 23% of 96 waterborne outbreaks, 4% of 430 food-borne outbreaks, 6 of 9 nursing home outbreaks, 5 of 18 cruise ship outbreaks, and 3 of 5 summer camp outbreaks.

This group of viruses is also frequently implicated as the cause of various food-borne outbreaks of gastroenteritis. They have been cited as the etiologic agent following the ingestion of a myriad of foods such as frosting, salad, celery, melon, potato salad, vermicelli, consomme, fruit salad, coleslaw, tossed salad, cold foods, sandwiches, lettuce, cold cooked ham, oysters, and commercial ice.[141,282] In this regard, in Minnesota, Norwalk and Norwalk-like viruses are associated with food-borne outbreaks of gastroenteritis more frequently (35%) than those caused by any single bacterial agent.[325] The vehicle in these outbreaks was often considered to be a salad item. In Japan, 26 of 38 outbreaks considered nonbacterial were associated with a Norwalk or Norwalk-like particle with the major vehicle of transmission (92%) considered to be oysters.[422]

The Norwalk virus or related viruses have emerged as important causes of gastroenteritis in military personnel in several different parts of the world, as serological evidence of Norwalk virus infection was detected in 10% of U.S. military personnel who developed a diarrheal illness while deployed to South America or West Africa; the most frequently detected pathogens (17%) were the enterotoxigenic *Escherichia coli*.[57a] Norwalk virus infections was demonstrated in 1 of 10 outbreaks of diarrhea among Israeli military personnel.[99]

The impact of diarrheal disease in military personnel was demonstrated more recently in Operation Desert Shield in which 57% of 2002 men who were surveyed by questionnaire and who spent an average of at least 2 months in Saudi Arabia developed at least one episode of diarrhea.[263] Bacterial enteropathogens were identified in the stools of almost one half of 432 individuals with gastroenteritis. Vomiting was reported infrequently as a primary symptom. However, 9 (82%) of 11 military personnel who developed vomiting as a major symptom (two with vomiting and diarrhea, and nine with vomiting alone) demonstrated serological evidence of Norwalk virus infection.

A subset of 404 individuals in a cohort of 883 military personnel who were deployed to Saudi Arabia and Kuwait were evaluated for the incidence of Norwalk virus infection; paired sera obtained prior to or after the 5-month interval of deployment were examined by ELISA using the recombinant Norwalk virus protein as antigen.[264] The impact of gastroenteritis in the military was demonstrated again, since 61% of the entire cohort developed at least one episode of gastroenteritis during the 5-month period. In the subset of 404 troops, serological evidence of Norwalk virus infection was demonstrated in 14.5% of those with vomiting alone or with vomiting and diarrhea, in 6.5% of those with diarrhea alone, and in 3% of the troops without diarrhea or vomiting. Overall, the adjusted incidence of Norwalk virus infection in the entire cohort over the 5-month interval of deployment was 6.2%.

In addition, gastroenteritis disrupted the activities of Navy personnel at sea aboard an aircraft carrier as 25 (19%) of 130 crew members surveyed developed vomiting and/or diarrhea.[502] Norwalk virus infection played a prominent role as 13 (52%) of the 25 individuals developed serological evidence of Norwalk virus infection; a significantly fewer number (24% of 105) without illness had such a response.

The role of Norwalk virus infection in outbreaks of diarrheal illness in families was evaluated during a 1-year prospective study of 28 families that were enrolled at the time of birth of an infant.[438] Fourteen of the families experienced one outbreak of diarrheal disease that could not be associated with a bacterial enteropathogen. Two of those outbreaks were associated serologically with Norwalk virus infection. None of the 28 infants and young children in the study developed serological evidence of Norwalk virus infection.

5.1.3. Geographic Distribution. Norwalk virus appears to have a worldwide distribution because antibody has been detected in populations in the United States, Belgium, Switzerland, Yugoslavia, Bangladesh, Nepal, Japan, Ecuador, Indonesia, Australia, Panama, Thailand, Taiwan, and the Philippines.[5,49,110,137,213–215,218,220,296] The only population studied that lacked detectable antibody was the very isolated Gabaro Ecuadorian Indians, who also lacked antibody to hepatitis B virus (anti-HBc negative).[213] In contrast, they were found to have serum antibody to rotavirus, respiratory syncytial virus, and hepatitis A virus.[213] Gastroenteritis outbreaks associated with Norwalk virus infection have been reported in at least 16 states in the United States.[306]

5.1.4. Temporal Distribution. In developed countries, illness with the Norwalk group of agents was believed to occur predominantly in outbreaks during the cooler months of the year, from the fall through the spring seasons. However, recent studies reveal that Norwalk virus outbreaks occur throughout the year in the United States.[213,214,306] Of 34 outbreaks, 32% occurred in the spring, 29% in the summer, 21% in the fall, and 18% in the winter.[306] The temporal distribution in tropical countries is not known, although in Bangladesh, Norwalk virus infections occurred most often during the cool, dry periods.[49]

5.1.5. Age. During outbreaks, the peak incidence is observed in school-aged children and adults who are in close contact in various group settings.[215] However, close contact is not always essential, since outbreaks have occurred after ingestion of contaminated water or various types of food as noted previously. In the United States, antibody-prevalence data indicate that the Norwalk virus is not an important cause of gastroenteritis in infants and young children, nor has it played an important role in diarrheal illness of early life serious enough to require hospitalization.[63,296,297] Its overall importance in the developing countries is not known. As noted previously, a recent study in which 49% of infants and young children in Finland developed serological evidence of Norwalk virus infection that could not be associated with illness over a 2-year period raises important new questions regarding the natural history of Norwalk virus infection.[338]

5.1.6. Sex, Race, Occupation. There is no evidence of differential susceptibility to this group of agents on the basis of sex, race, or occupation.

5.1.7. Occurrence in Different Settings. Illnesses associated with the Norwalk group of agents tend to occur in sharp outbreaks in families, schools, institutions, or communities and to affect adults, school-aged children, and family contacts as well as some young children. Overall, this epidemic characteristic is one of the main epidemiologic features that differentiates the Norwalk group from the rotaviruses, since the latter are characteristically associated with sporadic gastroenteritis of infants and young children and only infrequently affect older age groups.[286]

5.1.8. Socioeconomic Status. In developed countries, there is no evidence of differential susceptibility to the Norwalk group on the basis of socioeconomic standing. However, the greater prevalence of Norwalk antibody in the pediatric age group in developing countries in comparison to that in developed countries may reflect a role of crowding or other socioeconomic factors in facilitating the spread of this agent.

5.1.8a. In Traveler's Diarrhea. Norwalk virus does not play an important role in the etiology of traveler's diarrhea. Its estimated importance in several studies ranged from 0 to 15%.[47,139]

5.1.8b. In Human Immunodeficiency Virus (HIV) Positive Patients. Norwalk virus infection has been observed in patients who are HIV positive. However, the role of Norwalk virus in the etiology of gastroenteritis in each individual does not appear to be any greater than that observed in non-HIV-infected individuals.[112,279]

5.1.9. Other Factors. The influence of factors such as malnutrition on susceptibility to infection with the Norwalk group is not known. It has been suggested that genetic factors may play a role in determining susceptibility or resistance to infection with Norwalk virus.[426] These factors are discussed in greater detail in Section 7.1.3.

5.2. Rotaviruses

5.2.1. Incidence and Prevalence Data. Rotaviruses have emerged as the major etiologic agents of serious diarrheal disease in infants and children under 2 years of age in practically all areas of the world where this disease has been studied etiologically.[41] The illness rate among family contacts of patients with rotavirus gastroenteritis is low, although subclinical infections in contacts occur frequently.[297,310,537,564] In the metropolitan Washington, DC, area, the pattern of acquisition of rotavirus antibody contrasted sharply to that of the Norwalk agent.[296] By the end of the third year of life, over 90% of infants and young children had acquired rotavirus antibody, a pattern similar to that observed for respiratory syncytial and parainfluenza 3 viruses.[296,309,427] A high prevalence of antibody was maintained into adulthood, probably as a result of frequent reinfection with these agents. In other studies, the acquisition of rotavirus antibody has followed a similar pattern.[69,147,229,291,651]

Cross-sectional epidemiologic studies of hospitalized infants and young children with diarrhea have provided the most striking data regarding the importance of rotaviruses. In a study of patients admitted with a diarrheal illness to Children's Hospital National Medical Center in Washington, DC, from January 1974 to July 1982, 34.5% of 1537 patients shed rotavirus in a stool or rectal swab specimen (Fig. 8).[61] The major role of rotaviruses in diarrheal illnesses requiring hospitalization has also been observed in many other countries of the world, such as Australia, Canada, England, and Japan.[71,114,318,321,385,386] For example, in an Australian study lasting 1 year, 52% of 378 patients admitted with gastroenteritis shed rotavirus.[114] In a Japanese study from December 1974 to June 1981, rotavirus was detected in stools of 45% of 1910 infants and young children hospitalized with diarrhea.[321] A characteristic temporal pattern of rotavirus infections has been observed in temperate climates and is discussed in Sections 5.2.2 and 5.2.4. Sequential infections with human rotavirus have been documented, but it appears that the second infection results in a milder illness.[41]

Although rotaviruses have been implicated in cross-sectional studies as a major cause of gastroenteritis requiring hospitalization of infants and young children, the rates of hospitalization were not known, because of difficulty in

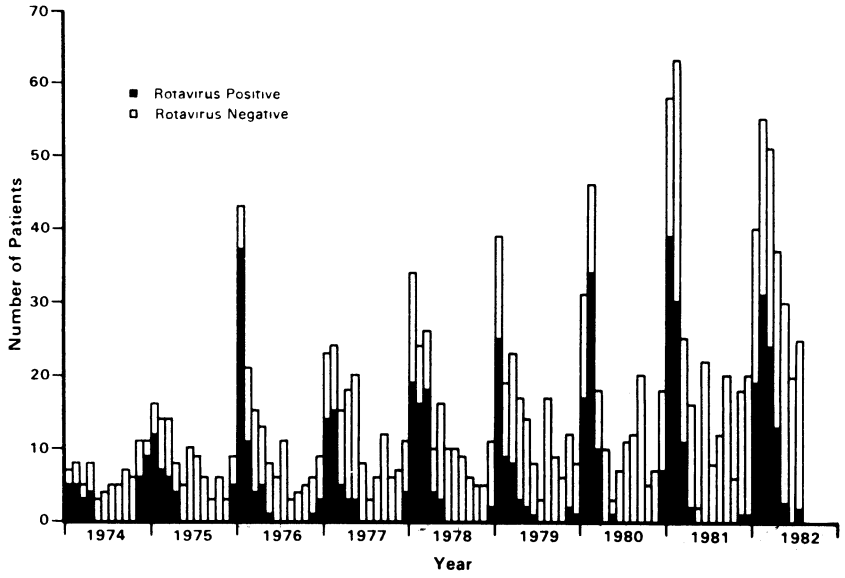


Figure 8. Temporal distribution of rotaviruses detected in stools from infants and young children hospitalized with gastroenteritis at Children's Hospital National Medical Center, Washington, DC, January 1974 (partial)–July 1982. From Brandt *et al.*[61]

estimating the population base of the hospitalized patients. However, the incidence of hospitalization was estimated in the Washington, DC, area because (1) medical care for the population under study was provided by Group Health Association, Inc., and (2) almost all pediatric hospitalizations of the group occurred at the Children's Hospital National Medical Center, which had an ongoing study of the etiology of gastroenteritis.[456] Between January 1977 and March 1979, which included three periods of rotavirus prevalence, about 29,000 patients less than 15 years of age were under surveillance each or part of a year. Thirty-one of the 38 children hospitalized were under 2 years of age. Thirty of the children were studied microbiologically, 19 (63%) of them shed rotavirus, and one developed only serological evidence of infection. From this analysis, it was calculated that 1 in 272 (3.7/1000 per epidemic year) individuals less than 1 year of age and 1 in 451 (2.2/1000 per epidemic year) 12–24 months of age were hospitalized for rotavirus gastroenteritis; this rate dropped precipitously in the 25- to 60-month age group (1 in 5519), and such illness was not observed after 5 years of age. In total, rotavirus infection was associated with 62% of the pediatric hospitalizations for gastroenteritis in this population over a period of 2¼ years. Other agents played a minor role in comparison to the rotaviruses. In the 12- to 24-month age group, 1 of every 3.4 children on the average made an outpatient visit to the clinic for gastroenteritis during each year of the study, and in the less-than-12-month age group, the visits decreased to 1 of every 7.8 infants. The incidence declined sharply in the 25-month to 15-year age group.[456]

The importance of rotaviruses in various other settings in developed countries has also been estimated. In a family study during a 6-year period in Tecumseh, Michigan, the incidence of enteric illnesses was 1.2 per person per year, with the youngest age groups experiencing the highest rates.[393,394] Overall, rotaviruses were detected in stools of 3.8% of those with gastroenteritis symptoms, but the highest rate (10.4%) was in children under 2 years of age. In another study, rotaviruses accounted for a major portion of diarrheal illnesses seen in two private practices and in a hospital in the same locality in Michigan.[323] In both practices, rotavirus was shed by 16% of the children with diarrhea who did not require hospitalization, whereas in the hospital study 32% of the children admitted with diarrhea were rotavirus positive. The frequency of rotavirus diarrhea during two combined rotavirus peak periods in the solo practice and group practice was 30 and 36%, respectively, whereas in the hospital, rotavirus accounted for 48% of the admissions for diarrhea. The incidence of rotavirus diarrhea in the solo practice was highest in infants less than 1 year of age, with an estimate of 0.15 episodes per person per year, whereas in the 1- to 2-year-old age group it was 0.5 such episodes per year. It was of interest that 11% of the patients under 2 years of age with rotavirus diarrhea and only 4% with nonrotavirus diarrhea were hospitalized. Moreover, from the two practices, 38% of the patients with diarrhea who were hospitalized were infected with rotavirus. Extrapolation of this figure to the entire United States indicated that 80,000 infants and young children would be hospitalized annually with rotavirus diarrhea.[265] In Winnipeg, Canada, in a

prospective study of diarrheal disease in a cohort of neonates, the most frequently detected enteric pathogens were the rotaviruses, which accounted for 23% of such illness.[228] Over 10% of the rotavirus infections were reinfections, and most were asymptomatic.

In a prospective family study in Virginia, 51% of the families experienced rotavirus infection during the 29-month surveillance period.[458] The 12–23 age group experienced the highest incidence of rotavirus gastroenteritis (40 per 100 person-years) with lower rates in the 6–11 month and 24–35 month age groups (12 per 100 and 13 per 100 person-years, respectively), whereas adults experienced the lowest rate (5 per 100 person-years). The figures from this study were extrapolated nationwide to obtain an indication of the incidence of rotavirus gastroenteritis in the United States annually with the following estimates for the various age groups: 0–11 months, 41,000; 12–23 months, 1,474,000; 24–35 months, 475,000; and 36–60 months, 573,000.[458] It was of interest that: (1) 88% of rotavirus infections were symptomatic in the 36-month and under age group; (2) 1 of 9 ill children who were seen by a physician were hospitalized; and (3) only 0.2% of nonill individuals were rotavirus positive. Demonstration of rotavirus infection in ill individuals at a significantly higher rate than in nonill individuals is the rule in most studies of infants and young children beyond the neonatal period,[63] although exceptions to this pattern have been described.[86]

Information regarding hospital admissions in the United States was also generated by extrapolation from the mean annual number of cases and probable cases of rotavirus gastroenteritis resulting in admission to a children's hospital in a Texas county over a 6-year period. In this analysis, the annual incidence of hospitalization for rotavirus gastroenteritis was estimated to be 11.1 and 5.8 per 1000 children in the first and second years of life, respectively, with a further decline with increasing age.[359] Moreover, it was estimated that 1 of 46 children would require hospitalization for rotavirus gastroenteritis by 18 years of age (the upper age limit for admission) with practically the entire risk accruing during the first 5 years of life. Thus, for the United States, it was estimated that presumptive rotavirus gastroenteritis would result in 110,000 hospitalizations and 583,000 hospital days, annually.

The importance of rotavirus infection as a cause of severe gastroenteritis was demonstrated in a prospective study of acute diarrhea in a cohort of 336 Finnish children from birth to approximately 2½ years of age.[464] Although more than half (55%) of the study group did not develop diarrhea, 26% had one episode and 19% had two or more episodes during the study period (for a total of 248 episodes). With regard to etiology, rotaviruses were the most frequently detected pathogen (26%), followed by adenovirus (4%) and bacterial pathogens (4%); in the remainder, a pathogenic agent could not be identified. The incidence of diarrhea was greatest in the 7–12 month age group (0.24/child per year) and lowest in the 0–6 month (0.09/child per year) and 25–32 month (0.06/child per year) age groups. In addition, rotavirus diarrhea was detected only once in any child with a documented rotavirus-positive episode. The disproportionate influence of rotavirus infections on severe diarrhea was demonstrated when 237 of the 248 diarrheal episodes were tabulated according to a severity score ranging from 0–20: 54% (128 episodes) were considered mild [rated 1–6 (usually not requiring treatment for diarrhea)]; 28% (66 episodes) were classified as moderately severe [rated 7–10 (requiring medical attention but manageable on an outpatient basis)]; and 18% (43 episodes) were considered to be severe [rated $\geq$ 11 (possibly requiring hospitalization)]. With regard to etiology and severity of illness: (1) 75% of the 128 mildest cases failed to yield an etiologic agent (rotavirus was detected in 4%); (2) 41% of the 66 moderately severe cases were associated with rotavirus [3% with adenovirus, 6% with enteropathogenic *E. coli* (EPEC), and 50% were etiologically unresolved]; whereas (3) 75% of the 43 severe cases were associated with rotavirus (5% with adenovirus and 21% were etiologically unresolved). The mean severity scores according to etiology were: rotavirus 11.0 $\pm$ 3.7, adenovirus 6.3 $\pm$ 4.2, bacteria 6.6 $\pm$ 3.5, and unresolved etiology 5.3 $\pm$ 3.1. The difference in severity score between the rotavirus-associated illnesses and the other groups was highly significant ($P < 0.0001$). It should be noted that while only about one third of the cases had an etiologic diagnosis (in contrast to almost twice that number in a previously described hospital-based study in Finland), the cases in which a specific etiology was found were generally more severe. Thus, in cases with a score $\geq$ 7, rotavirus was diagnosed in 54% with an overall diagnostic rate of 63%, whereas 75% of the milder cases ($\leq$6) failed to yield a diagnosis.[464]

Acute diarrheal disease occurs frequently in day-care centers, especially in centers caring for children who are not toilet trained,[525] with the rates being highest in the first 4 weeks of attendance. Only 21% of the episodes studied yielded a pathogen, but rotaviruses were detected in 18% of the total 513 episodes.

Rotaviruses are of extreme importance as etiologic agents of diarrheal illness in developing countries. By the end of the third year of life, over 90% of infants and young children acquire rotavirus antibodies, a pattern of infection quite similar to that in developed countries, but the consequences of such infections are markedly different in these settings.[39,49,55,137,147,229,266,291]

The relative role of rotaviruses and bacterial agents in the etiology of gastroenteritis was reported for 6352 patients for a 1-year period, February 1978 to January 1979, at the Matlab Treatment Center in Bangladesh.[51] This study showed that 46% of the patients under 2 years of age shed rotavirus and that 28% shed toxigenic *E. coli*. In the 2-year-and-over age group, bacterial agents were detected more frequently than rotaviruses.

Prospective longitudinal studies in developing countries consistently demonstrate the major role rotaviruses play in the etiology of diarrhea of infants and young children. In a village-based study in northeastern Brazil in which mortality during the first 5 years of life was greater than 14%, diarrhea was listed as either the primary or an associated cause of death in over 52% of the deaths.[225] In selected specimens from diarrheal episodes, enterotoxigenic *E. coli* (ETEC) (21%) and rotavirus (19%) were the most frequently detected pathogens. The highest incidence of diarrhea occurred in the poorest families and reached almost ten per child per year in the 6- to 11-month-old age group.

In Santa Maria Cauque, Guatemala, in 45 infants and young children studied over a 3-year period, the incidence of diarrhea was 7.9 per person per year, with rotavirus associated with 10% of the episodes.[357] The incidence of rotavirus diarrhea was 0.8 per child per year (and of rotavirus infections 1.2 per child per year). One child had only one rotavirus infection, whereas the remainder experienced two to seven rotavirus infections each over the 3-year period.[357] In a sample of 24 of the 45 infants and young children, dehydration occurred 14 times more often in those with rotavirus diarrhea than in those with diarrhea of bacterial, parasitic, or unknown etiology.[630]

In two longitudinal studies in Bangladesh, the disproportionate role of rotavirus as a cause of severe dehydrating diarrhea in comparison to its role in diarrheal illnesses overall was clearly shown. In one study of 197 children 2 to 60 months of age in two contiguous villages, diarrhea was the second most common illness but was the most frequent cause of admission to a treatment center.[48] The incidence of diarrhea was highest in children 2–11 months of age (more than seven per child per year); ETEC was the most frequently detected sole pathogen (27% of episodes), *Shigella* was second (12.8%), and rotaviruses were next (3.8%). However, although rotaviruses were associated with fewer than 4% of diarrheal episodes, they were linked with 39% of episodes with significant dehydration.

In the other village study in Bangladesh, the incidence of diarrhea was highest (>4 per child per year) in the 9- to 11-month age group, decreasing gradually with increasing age to three episodes per child per year for the next 4 years of life, to 0.2 episodes per child per year for children 10 years of age or older.[50] Once again, the ETECs were the most frequently detected pathogens in all age groups (23% of all diarrhea episodes), *Shigella* second (11%), and rotavirus third (5%). Rotaviruses were detected only in children less than 2 years of age and accounted for 11% of the diarrheal episodes in this age group. The peak incidence of rotavirus diarrhea was in the 6- to 12-month age group (0.5 per person per year). It is noteworthy that in children under 2 years of age, the incidence of rotavirus diarrhea was only half that of ETEC diarrhea, but one in six rotavirus diarrheal episodes necessitated a visit to the treatment center, whereas only 1 of 15 ETEC diarrheas required such visits. Furthermore, in children less than 2 years of age who had dehydrating diarrheal episodes, 46% were linked to rotavirus, 24% to ETEC, and 7% to *Shigella*. Moreover, it was estimated that (1) if fluid replacement therapy was not available, (2) if dehydration reached a level of 7.5% or more, (3) if 50% of the children did not survive this level of dehydration, then (4) there would be 6.5 deaths per 1000 children less than 2 years of age per year, of which 44% would be rotavirus associated.

In a study in Cairo, Egypt, of 145 infants and young children under 18 months of age with fatal or potentially fatal diarrhea, the most frequently detected agents that were considered etiologically important were rotavirus (33%), heat-stable ETEC (20%), heat-labile ETEC (11%), EPEC (8%), and *Salmonella* (5%).[507] In contrast, rotaviruses were associated with only 5% of outpatient diarrhea in infants under 1 year of age in a rural Egyptian area.[507,654]

Finally, the influence of age on the severity of symptoms was demonstrated in a study of infants ≤ 12 months of age with acute diarrhea who were brought to an emergency room of a municipal hospital in urban São Paulo.[194] The most frequently detected pathogen was the enteroadherence factor (EAF)-positive classic EPEC (26%), followed by rotavirus (14%), *Salmonella* species (8%), ETEC (7%), and *Shigella* species (5%). The frequency of rotavirus illness was relatively low in the first 3 months of life but increased in the next quartile; however, in the 6- to up to 9-month age group, it almost equaled the frequency of detection of EAF-positive classic EPEC and in the 9- to 12-month age group, it exceeded it. Dehydration occurred most often in the EAF-positive EPEC group (71%) and less frequently in rotavirus (39%) infections.

5.2.2. Rotavirus Subgroups and Serotypes. The epidemiology of rotavirus infection and illness with regard to serotypic diversity is now known primarily because of the development of monoclonal antibodies that recognize the VP7 of most clinical isolates and to a lesser

extent because of the widespread application of molecular biological techniques.[253] Prior to this, a rotavirus strain could only be serotyped by techniques not readily adaptable to large-scale epidemiologic surveys, such as neutralization of immunofluorescent foci, plaque reduction neutralization, neutralization of cytopathic effect or virus yield in roller tube tissue culture, or solid-phase immune electron microscopy.[286] Because of this, subgroup specificity became a surrogate for serotyping as among human rotaviruses subgroup I strains almost always belonged to serotype 2, whereas subgroup II strains almost always included strains of serotypes 1, 3, and 4.[253] Overall, in various parts of the world, subgroup II rotaviruses were detected more frequently than subgroup I strains.[15,404,408,527,562,601,651] The molecular epidemiology of rotavirus strains has also been evaluated extensively by determining the electropherotype by gel electrophoresis of the segmented genomic dsRNA of rotavirus.[452] This method does not indicate the serotype but permits study of the variability of strains by PAGE.

However, with the development and availability of VP7 monoclonal antibodies with specificity for individual serotypes, studies of the natural history of human rotavirus serotypes became a reality,[37,104,238,543] because (1) these antibodies could be used to serotype rotavirus strains directly in stool material by ELISA, and (2) there was complete concordance between the serotyping ELISA and neutralization or SPIEM.

The distribution of rotavirus VP7 serotypes was evaluated by ELISA in rotavirus-positive stool specimens obtained from children with acute gastroenteritis over a 6-year period in various countries of Europe, North and South America, Africa, and Asia.[29] This study demonstrated that: (1) 95% of 907 rotavirus-positive stool specimens that were VP7 positive belonged to serotypes 1, 2, 3, or 4; (2) rotavirus type 1 was found most often (54%), followed by serotype 2 (18%), serotype 3 (12%), or serotype 4 (11%); (3) no single serotype was detected exclusively in one location; (4) each of the four serotypes were detected worldwide; (5) the distribution of serotypes differed year by year and in different countries during the same years; and (6) the distribution of serotypes detected in developed and developing countries did not differ.

This general pattern was observed in other studies as well. For example, (1) in a study of hospitalized children in Texas during an 11-year period and in the north central United States during a 9-year period, serotype 1 was predominant overall in Texas, but serotypes 1, 3, and 4 were each predominant in 1 or more years, whereas in the north central United States, serotype 1 was not only predominant overall, but it was the only serotype that predominated each year[360]; (2) in southeastern New England over nine winter seasons, serotype 1 strains were predominant in seven of nine seasons, and overall accounted for 63% of the typed strains[30]; (3) type 1 strains were predominant overall in various other locations including Australia, Venezuela (short-term), Italy, the Central African Republic, Thailand, Japan, Norway, Finland, West Germany, Switzerland, and Israel, with serotype 2, 3, or 4 predominating intermittently[15,37,46,113,171,182,185,187,201,407,439,505]; (4) in a summary of 28 studies in which over 5419 specimens obtained between 1979 and 1989 from North and South America, Europe, Asia, and Africa were serotyped, the most prevalent serotype was VP7:1 (61%), whereas serotypes 2, 3, and 4 were evenly distributed (10–16%), except that serotype 4 was not detected in Africa[614]; and (5) in a study in London of children admitted to two children's hospitals during a 6-year period, serotype 1 accounted for 60% of the cases, serotype 4 for 24%, serotype 2 for 11%, and serotype 3 for 3%.[416] Moreover, the electrophoretic profiles of 611 strains demonstrated at least 108 different profiles with continuous variation observed throughout the 6-year period. Strikingly, once an electropherotype had disappeared from the population, it never recurred. None of the electrophoretic profiles resembled those of group B or C rotaviruses. No strain appeared to be endemic in these two hospitals.

In a few other locations, the distribution of the four serotypes deviated from this pattern: (1) in an 11-year survey in Venezuela, serotype 3 strains were the most prevalent (25%), followed by serotype 1 (20%), serotype 4 (15%), or serotype 2 (10%)[600]; (2) in Malaysia in specimens obtained discontinuously over a 12-year period, serotype 4 was the predominant strain (71%) with serotype 1 a distant second (15%)[445]; and (3) in Bangladesh (by neutralization assay) over a 2-year period, serotype 3 strains were predominant.[597]

The distribution of serotypes in two day-care centers for infants and young children over three rotavirus seasons was similar to that observed concurrently in hospitalized children: serotype 1 strains accounted for about two thirds and serotype 3 strains for approximately one third of the typed strains.[423] Although it is not possible to determine serotype from the migration pattern of the 11 RNA segments by PAGE or by subgroup analysis, the latter methods are important epidemiologic tools for monitoring the circulation of rotavirus strains.[15,452] Data regarding variability in severity of illness in association with a specific serotype has been inconsistent. For example, in Bangladesh, VP7 serotypes 2 or 3 were associated with more severe dehydrating illness than serotype 1 or 4, but the differences were not of major clinical impor-

tance,[34] and in Australia differences in virulence between different rotavirus VP7 serotypes were not observed.[23]

Analysis of the genotype or serotype of clinical specimens according to VP4 specificity is gaining impetus with the use of PCR techniques as well as monoclonal antibodies.[34,102,226,484,500] In studies from various parts of the world, P1A serotypes occur most often.[34,102,226] This was not surprising, because VP7 serotype 1, which is the most frequently detected VP7 serotype, shares VP4 P1A specificity with two other epidemiologically important VP7 serotypes (i.e., 3 and 4). For example, analysis by a PCR-based assay of the VP4 genotype of 89 electrophoretically distinct rotavirus strains that represented each of the electropherotypes detected in 675 rotavirus-positive stool specimens obtained over a 10-year period from Japanese infants with rotavirus diarrhea: (1) the Wa and DS-1 alleles occurred most frequently overall (83.1% and 15.6%, respectively); (2) the M37 and AU-1 alleles were found in none and in 1.3% of the strains, respectively; and (3) the Wa allele was predominant in 7 of the 8 years in which an adequate number of specimens were available, whereas in the most recent year surveyed, 1990–1991, the DS-1 allele was predominant.[226]

5.2.3. Epidemic and Endemic Behavior in Pediatric and Adult Groups. Unlike the situation with the Norwalk group, true outbreaks of rotavirus gastroenteritis occur infrequently. In the temperate climates, rotavirus infections demonstrate a consistent temporal pattern similar to that observed in studies at Children's Hospital, Washington, DC, during 1974–1982 (Fig. 8), which was characterized by a large number of hospitalizations in infants and young children for gastroenteritis during the cooler months of each year.[61,63,71,114,297,318,385] There is also a suggestion of a regional seasonal peak from west to east in the United States.[332] Rotaviruses are also associated with milder bouts of gastroenteritis not requiring hospitalization. For example, in the Children's Hospital study, 22% of 200 outpatients with gastroenteritis studied from November 1975 through June 1978 shed rotavirus.[63] Community outbreaks of rotavirus illness occur uncommonly, since most adults appear to be immune, most likely by virtue of previous rotavirus infection(s). However, subclinical rotavirus infections occur quite commonly in adults.[297,310,537,564] In one study, 22 (55%) of 40 adult contacts of patients hospitalized with gastroenteritis that was associated with rotavirus infection had serological evidence of rotavirus infection at or about the time of their child's admission, whereas only 4 (17%) of 24 control adults whose children also had gastroenteritis but were not infected with rotavirus were found to be infected with this agent.[310] Only 3 of the 26 adult contacts with rotavirus infection gave a history of an associated gastroenteritis illness. It appears that older siblings or parents might be a source of rotavirus infection for young persons. The frequency of rotavirus infection in contacts also demonstrates the highly contagious nature of rotavirus infection.[142,310,537,564] Intrafamilial spread of rotavirus infection and illness from infected infants and young children to adults has been described at several locations.[219,458]

Although community outbreaks of rotavirus gastroenteritis are uncommon, one unusual outbreak involving not only children and mothers in a play group but also fathers and grandparents was described[455]: all nine children 15 months through 5 years of age, three of five mothers who shared play group activities, four of five fathers, and each of two grandparents developed gastroenteritis. The incubation period was 24–48 hr. The index cases were most likely non-play group siblings who had been cared for (about 48 hr prior to the play group meeting) by the mother in whose home the play group met. The suspected index cases had gastroenteritis, the mother who took care of the index cases developed diarrhea 24 hr after the play group met, and her daughter had onset of diarrhea just before the play group met and vomited during the play group meeting. In all, 18 of 21 persons developed gastroenteritis, and evidence of rotavirus infection was demonstrated in 10 of 11 persons tested for virus in stools, or for a serological response, or both. This unusual outbreak further attests to the contagiousness of rotavirus infection.

Although subclinical infections are the most common outcome of rotavirus infection in adults, rotavirus gastroenteritis has been observed in various groups of adults in various settings in scattered parts of the world.[41,286] Moreover, a high attack rate was observed in several outbreaks in geriatric groups with some fatalities.[108,233,261,353,526]

It should be noted that in China large outbreaks of a severe form of gastroenteritis, in adults predominantly, have been associated with group B rotaviruses.[262,594] The clinical manifestations may be marked by choleralike, watery diarrhea that has been associated with a few deaths in the elderly.[262] As noted earlier, the group B rotaviruses do not share the common group antigen and have been detected almost exclusively only in China.

5.2.4. Geographic Distribution. Rotavirus infection has been detected in virtually all parts of the world.[41,286] In developed countries, rotaviruses have emerged as the major etiologic agents of diarrheal illnesses severe enough to warrant hospitalization. In temperate climates, the pattern has been similar to that described for Washington, DC.[61] In less-developed areas, rotaviruses have been shown to be major etiologic agents

of severe diarrheal illness in infants and young children[41,225,286,507,630]; however, it appears that toxigenic *E. coli* also plays an important role in underdeveloped countries.[412] In practically every area of the world studied, rotaviruses have exhibited an important role in acute gastrointestinal disease of the young.

5.2.5. Temporal Distribution. In developed countries in the temperate climates, rotavirus infections display a characteristic temporal pattern that peaks in the cooler months of the year.[61,63,71,114,297,385] The pattern for Washington, DC, for an 8-year period, was shown in Fig. 8.[61] A pattern of spread from the western to eastern United States has also been described.[332]

During the months of January and February, 168 (67%) of 250 and 127 (58%) of 219 hospitalized children, respectively, who had diarrhea were rotavirus positive, whereas none of the 256 diarrhea patients studied during the July–October interval shed rotavirus.[61] A similar pattern was observed in outpatients with diarrhea. In a similar study of hospitalized children in Japan over a 6-year period, rotaviruses were shed in the stools by 521 (66%) of 785 diarrhea patients during the cooler months (December, January, February) and by 56% of 549 such patients admitted during the spring (March, April, May).[321] In addition, only 5.6% of 576 children admitted with diarrhea in the summer or autumn months were rotavirus positive. The reason for the seasonal pattern of infection is not known, but it has been suggested that weather-related low relative humidity in the home might facilitate the survival of rotaviruses in the environment,[59] which along with indoor crowding[59] would affect the epidemiology of rotavirus infection.[59,386] This has not been a consistent observation in all settings.[321,391]

The striking seasonal pattern of rotavirus infections described above is not observed in all situations, since a significant number of rotavirus infections has been observed throughout the year in South Africa, during the summer in Taiwan, during the "small rains" in Ethiopia, during most months in the tropical climates but with peak periods during the slightly cooler months, during the summer in a newborn nursery in England, in all seasons in a newborn nursery in Australia, and in the autumn on a United States Indian reservation.[20,138,148,243,350,355,402,495,496,529,556,591] In both nursery studies, most rotavirus-positive infants were symptom-free, a finding that has yet to be explained satisfactorily. Studies of the frequency of rotavirus infections in relation to the amount of rainfall or to relative humidity have led to variable results.[59,321,355,391,591]

5.2.6. Age. In studies from various parts of the world, infants and young children (usually 6 months to 2 years of age) experience the highest frequency of rotavirus gastroenteritis that requires hospitalization[63,71,114,318]; infants under 6 months of age have the next highest frequency.[63,105] An unexplained paradox in the epidemiology of rotavirus infection is the low rate of clinical illness in neonates who shed rotavirus.[20,402] In one study, breast-fed infants shed rotavirus significantly less frequently than those who were not breast-fed; however, the effect of breast-feeding on illness could not be determined because most of the rotavirus infections in both the breast-fed and bottle-fed neonates were subclinical.[556] Recent studies indicate that the gene that encodes the outer capsid protein VP4 of neonatal rotavirus strains that persist in newborn nurseries is highly conserved and differs from that of strains recovered from symptomatic infections.[167,196]

Rotavirus gastroenteritis has been reported in older children and adults, who, as noted earlier, may be important in the transmission of infection to infants and young children.[56,57,297,310,564] Rotavirus gastroenteritis has also been observed in geriatric settings as noted earlier.

5.2.7. Sex. A somewhat larger number of males than females (M : F = 1.2 : 1.0) were hospitalized for rotavirus gastroenteritis in the Children's Hospital of Washington, DC, study of 1974–1978.[63] A higher frequency of males who were hospitalized with acute rotavirus gastroenteritis was also observed in a Canadian study.[386]

5.2.8. Race and Socioeconomic Status. In the Children's Hospital study, 1974–1978, the age distribution of patients admitted to the hospital for gastroenteritis of any etiology was quite different among black and nonblack patients: 59% of all black patients admitted for gastroenteritis were less than 6 months of age.[63] Also, DC residents and Medicaid recipients who were hospitalized with rotavirus infection tended to be younger than non-DC residents and non-Medicaid recipients. In addition, there was a tendency for rotavirus illness to occur earlier in the course of the outbreak in black patients and in DC residents. Transmission of rotavirus might be facilitated by crowding and poor sanitation, and this may explain the earlier appearance of rotavirus infection in DC than in the suburbs, in black patients as compared to nonblack patients, and in Medicaid recipients as compared to non-Medicaid recipients.[63]

Malnutrition appears to be an important factor in increasing the susceptibility of an infant or young child to develop severe clinical manifestations following rotavirus infection.[68] It has been suggested that repeated diarrheal infections may be a prelude to the development of malnutrition by various mechanisms including damage to the intestinal mucosa so that absorptive cells are compromised over an extended period.[356,358] The deleterious effect of malnutrition on the severity of rotavirus infection has been reproduced in the mouse model.[451]

5.2.9. Occurrence in Different Settings. Rotavirus gastroenteritis occurs predominantly in infants and young children with infection occurring by the 36th month of age in almost all children residing in a family setting.[55,147,229,296] Family contacts are also frequently infected with rotavirus, but usually subclinically.[296,310,537,564] Rotavirus infections have also been observed for extended periods in newborn nurseries almost exclusively as subclinical infections.[20,42,93] An exception to this pattern was described in Italy when outbreaks of gastroenteritis were documented in newborn nurseries.[184] In addition, nosocomial rotavirus infections occur commonly.[386,465] In one study, 10 (17%) of the 60 children admitted to the hospital without diarrhea (but during a period of rotavirus prevalence) developed diarrheal illness associated with rotavirus infection while hospitalized.[465] In another hospital study, over a 1-year period, about one of every five rotavirus infections appeared to be hospital acquired.[386] Outbreaks of rotavirus gastroenteritis have been observed in premature infants, in school-aged children, in a home play group setting, in a military group, in nursing homes for the elderly and in geriatric units with a few reported deaths, in adults in South America, and in an isolated South Pacific Island, but characteristically, rotavirus illness occurs sporadically and not in widespread community outbreaks as does the Norwalk group.[41,286] Rotavirus illness is not common beyond the first few years of life. However, as noted earlier, large outbreaks of gastroenteritis have been observed in China, predominantly in adults, associated with group B rotaviruses.[90,262,594]

6. Mechanisms and Route of Transmission

6.1. Norwalk Group of Viruses

Infection with the Norwalk group of agents is most likely spread from person to person by the fecal–oral route. Volunteer studies have established that the Norwalk, Hawaii, and Snow Mountain agents can be transmitted via the oral route, i.e., following the ingestion of stool suspensions containing these infectious agents.[129,130,133,335,397,618] A $10^{-4.7}$ dilution (the highest dilution tested) of a Norwalk virus stool suspension known to be infectious induced illness in volunteers (R. Dolin *et al.*, unpublished data). It is unlikely that this group of agents is transmitted by the respiratory route. Nasopharyngeal washings obtained from a volunteer with experimentally induced Norwalk illness failed to induce illness in three volunteers.[129] However, Norwalk virus has been detected in vomitus[216]; transmission of this group of agents via aerosols generated during projectile vomiting has been suggested as an important mode of spread of these viruses, especially in the hospital setting.[79]

The explosive nature of some of these outbreaks in which large numbers of individuals develop illness in a cluster within 24–48 hr has suggested that a common-source exposure should also be considered in certain outbreaks. Indeed, in a review of 38 outbreaks of gastroenteritis associated with Norwalk virus, 31 (82%) were considered to have originated from a common source of infection.[306] In the Colorado outbreak associated with the Snow Mountain agent, 61% of the 418 cases had onset of illness on a single day.[397]

Epidemiologic analysis revealed that the attack rate increased with consumption of water or ice-containing beverages and that the water supply of the camp was not only inadequately chlorinated but also contaminated by a leaking septic tank; it was therefore suggested that a waterborne agent was responsible for the outbreak. In the Norwalk, Ohio, outbreak, 50% of the students and teachers of an elementary school developed gastroenteritis; it was striking that such illnesses occurred in a 2-day period.[2] Although a common-source exposure was suspected, this could not be established. However, secondary cases among family contacts were observed, and the Norwalk particle was derived from a rectal swab of one such secondary case. Ingestion of contaminated seafood such as oysters was described in early studies as a source of infection with these agents.[220,403] However, outbreaks of Norwalk gastroenteritis have now been associated with the ingestion of many varieties of contaminated food as described earlier, such as cake frosting and salad, as well as contaminated drinking water or swimming in a contaminated lake.[306]

6.2. Rotaviruses

Rotaviruses are also transmitted by the fecal–oral route. Volunteer studies have clearly demonstrated that oral administration of rotavirus-positive stool material can induce a diarrheal illness.[301,302] The rapid acquisition of rotavirus antibody in the first few years of life in all populations studied regardless of hygienic conditions has led to the suggestion that rotaviruses might also be transmitted by the respiratory route.[55,147,229,291,296] Throat gargles obtained from volunteers with an experimentally induced rotavirus diarrheal illness failed to yield rotavirus.[301] However, there are scattered reports of rotavirus antigen being detected in respiratory tract secretions, but most other studies indicate that this is not the usual mode of transmission.[286,655] Although a common-source exposure to rotavirus, such as a contaminated water supply, has been suggested, it is unlikely that such exposure plays a major role in its worldwide occurrence.

The source of infection for the young infant who is not normally in contact with other infants and young children with gastroenteritis is not known with certainty. However, a substantial proportion of parents of rotavirus-infected infants and young children were infected with rotavirus at or about the time of their child's illness; most of these adult infections were subclinical.[297,310,537,564] Thus, an older sibling or family member who is undergoing subclinical rotavirus infection may be the source of infection for the infant or young child with whom he has contact. The highly contagious nature of rotavirus infection may be in part related to the rotavirus' high degree of stability, as demonstrated by the retention of infectivity of calf rotavirus-positive feces that had been kept at room temperature for 7 months.[159] It is likely that human rotavirus is also quite stable and may remain viable in the environment unless destroyed by careful disinfection. The persistence of rotavirus infections in certain newborn nurseries and the frequency of nosocomial rotavirus infection in hospitals provide additional evidence for this possibility.[437] The ability of rotaviruses to persist on different surfaces under various conditions likely contributes to their efficient spread. For example, by PCR, rotavirus was detected in day-care centers on moist surfaces such as water fountains, water-play tables, toilet handles, and telephone receivers, as well as on toy balls, high chair seats, and diaper pail handles.[604] In addition, human volunteers who licked dried preparations of a human rotavirus on a petri dish readily became infected.[595] Infection was prevented if the dried virus was sprayed with a disinfectant. Effective disinfection of contaminated material and care in handwashing may be important measures in containing rotavirus infection, especially in a hospital or day-care setting.[310,322,465,476,512,524,539,540,583,595]

The role, if any, of animals in transmitting rotaviruses to humans is not known. Human rotavirus induces a diarrheal illness in various newborn animals under experimental conditions,[473] and certain naturally occurring animal rotavirus strains may infect humans as whole virions or as reassortants following reassortment with human rotavirus strains.[406,542] However, this type of animal-to-human transmission does not appear to be of clinical or epidemiological relevance.[467]

7. Pathogenesis and Immunity

7.1. Norwalk Group of Viruses

7.1.1. Incubation Period. The incubation period as estimated from various Norwalk outbreaks is 24–48 hr with a range of 4–77 hr.[2,133,306,397] The incubation period in Norwalk virus volunteer studies ranged from 10 to 51 hr, and the illness usually lasted less than 48 hr.[54,129,130,618] The shedding of Norwalk virus by IEM coincided with the onset of illness and usually could not be detected after 72 hr following onset.[550] The incubation period in volunteer studies with the Snow Mountain agent ranged from 19 to 41 hr, with a mean of 27 hr.[133]

7.1.2. Pathogenesis. By light microscopy, biopsies of the proximal small intestine of volunteers with Norwalk or Hawaii virus-induced illness show broadening and blunting of villi, with the mucosa itself being intact histologically; mononuclear cell infiltration and cytoplasmic vacuolization are also observed.[131,497,498] Transmission electron microscopy of the proximal small intestine showed intact epitehlial cells with shortening of microvilli.[3,131,497,498] The extent of the small-intestinal involvement is not known, since studies have included only the proximal small intestine. Histological lesions were not observed in the gastric fundus and antrum or the colonic mucosa following challenge with the Norwalk agent.[605] Brush-border small-intestinal enzyme levels (including alkaline phosphatase, sucrase, and trehalase) were decreased during illness; adenylate cyclase activity was not elevated.[3,54,335] In addition, following Norwalk virus challenge, volunteers experienced marked delays in gastric emptying, which may be the cause of the nausea and vomiting frequently associated with this illness.[380]

7.1.3. Immunity. Volunteer studies with the Norwalk virus have raised rather perplexing questions about the mechanism of immunity. It appears that two forms of clinical immunity exist: one is short term and the other long term.[129,426,618] The former seems to be serotype-specific. For example, volunteers who become ill following administration of Norwalk virus are characteristically resistant to challenge with this virus 6–14 weeks later; in contrast, they are not resistant to challenge with the Hawaii virus nor are Hawaii-virus-infected volunteers resistant to subsequent challenge with Norwalk virus.[129,618]

The situation with regard to long-term immunity was found to be quite different, as indicated when 12 volunteers were challenged with the Norwalk virus on two separate occasions 27–42 months apart, and four were rechallenged 4–8 weeks after the second challenge.[426] Of these 12 volunteers, six developed illness following both the initial challenge and the rechallenge 27–42 months later. In contrast, six volunteers failed to develop illness after the initial challenge or after rechallenge 31–34 months later. Of the six volunteers who developed illness after each of the two sequential challenges, four were challenged a third time 4–8 weeks after the second

challenge and only one became ill. Serological studies carried out to clarify this unusual pattern of susceptibility and resistance to Norwalk virus failed to reveal a consistent relationship between the presence or absence of antibody and the subsequent occurrence of illness following challenge. Thus, the presence or absence of serum IEM antibody did not correlate with resistance or susceptibility. It is difficult to explain these findings on the grounds that local intestinal IgA antibody is of prime importance in long-term resistance, since this supposes the existence of two cohorts of individuals, one able and the other unable to sustain the production of local antibody essential for long-term resistance. It has been suggested that other factors that are genetically determined may influence susceptibility to Norwalk infection. For example, there may be a genetically determined specific receptor essential for entry of the Norwalk virus into epithelial cells of the small intestine.[426]

Further evidence for the possible role of nonimmunologic factors in resistance to Norwalk illness was observed when the prechallenge serum and local jejunal antibody levels in 23 volunteers were studied by the RIA-blocking technique.[215] Neither the geometric mean Norwalk antibody titer in serum nor that in jejunal fluid correlated with resistance to illness after challenge. Paradoxically, the prechallenge geometric mean Norwalk antibody titer of jejunal fluid was significantly greater, and such antibody in serum tended to be greater in volunteers who became ill after challenge than in those who did not become ill.[215] A similar paradoxical relationship between prechallenge serum antibody titer and lack of resistance to Norwalk illness in volunteers was reported in another study in which antibody was also measured by RIA.[52]

A more recent study has confirmed the paradoxical effect of serum antibody on protection. In a sequential challenge study in volunteers with the same Norwalk virus inoculum used in the original studies described above, preexisting serum antibody did not confer protection against challenge.[273] Moreover, certain volunteers who had low levels of serum antibody were resistant to challenge. After repeated exposure, however, antibody correlated with protection. In another report, examination of prechallenge sera by EIA failed to show significant correlation between the presence of homotypic serum antibody of $\geq 1:100$ and resistance or susceptibility to challenge with Norwalk, Hawaii, or Snow Mountain virus.[348] Similar observations were made in the natural setting: (1) preexisting serum antibody to Norwalk virus failed to protect medical students from the United States, Puerto Rico, and Mexico against Norwalk virus infection or illness, and (2) the occurrence of Norwalk virus infection or illness did not correlate with acute phase serum antibody after a common-source exposure of teenagers to a contaminated water supply.[24,274]

7.2. Rotaviruses

7.2.1. Incubation Period. From clinical studies, the incubation period of rotavirus diarrheal illness is estimated to be less than 48 hr.[114] In volunteer studies in which four adults developed a diarrheal illness after oral administration of an untitered stool filtrate containing rotavirus, the incubation period ranged from 1 to 4 days. Virus shedding began the second, third, or fourth day after inoculation and lasted a total of at least 6 days.[301,302]

7.2.2. Pathogenesis.[395] Limited studies of biopsies of the proximal small intestine of a few infants and children hospitalized with rotavirus illness show shortening of the villi, mononuclear cell infiltration in the lamina propria, distended cisternae of the endoplasmic reticulum, mitochondrial swelling, and sparse, irregular microvilli.[251,534] Impaired D-xylose absorption was also observed.[372] In addition, some patients had depressed disaccharidase levels (maltase, sucrase, and lactase).[43]

The pathogenesis of a human rotavirus VP7 serotype 1 strain was studied experimentally in newborn gnotobiotic colostrum-deprived calves that developed illness following intraduodenal administration of this virus.[379,624] Morphological changes in the small intestine proceeded in a cephalocaudad direction: within 2 hr of experimentally induced diarrhea, morphological changes such as denuding of villi and flattening of epithelial cells were observed in the upper small intestine, but rotavirus antigens were not detected by IF; at this time, the lower small intestine was intact, but abundant rotaviral antigens were observed by IF in swollen epithelial cells.[379] Moreover, 7 hr after onset of diarrhea, the lower small intestine demonstrated morphological changes such as denuded villi that were similar to those observed in the upper small intestine earlier; rotaviral antigens also could not be detected by IF. The intestine appeared relatively normal 48 hr after onset of diarrhea. When diarrhea was induced in piglets by human rotavirus, certain functional alterations were observed in the villous epithelial cells of the small intestine: glucose-coupled Na^+ transport was impaired, sucrase activity diminished, and thymidine kinase activity increased, and in contrast, adenylate cyclase and cyclic AMP were not stimulated.[115,178]

More recently, the absorption of the macromolecule human α-lactalbumin during and after acute gastroenteritis in predominantly rotavirus-positive children less than 3

years of age was studied.[249] A significantly greater absorption of proteins was documented 5–8 weeks after the acute phase when compared with the acute phase or controls. Studies in malnourished or normal suckling mice infected with a murine rotavirus showed that malnutrition was associated with more severe symptoms and greater mucosal damage.[570] In addition, although both malnourished and normal mice experienced increased permeability to macromolecules as measured by uptake of ovalbumin, this effect was increased in the malnourished animals.

7.2.3. Immunity. The correlates of immunity to rotavirus infection and illness have not been clearly elucidated. Results from various studies have yielded inconsistent or conflicting conclusions. However, highlights from various epidemiologic and experimental studies in animals and humans have indicated that antibodies in the intestine or in serum (as a surrogate marker for intestinal antibodies) are important factors in immunity to rotavirus illness).[286,362,559]

The observation was made that newborn calves frequently develop rotavirus diarrhea despite a high level of circulating rotavirus antibody acquired from ingestion of colostrum.[613] This was confirmed experimentally in calves challenged with calf rotavirus and additionally it was shown that antibody in the lumen of the small intestine was of prime importance in protection.[65,613] Similar studies in gnotobiotic lambs examining the relative role of local and systemic rotavirus antibody concluded that antibody in the lumen of the small intestine was the determinant of protection against rotavirus challenge.[513–515] From these and other studies in animals, it appears that antibody in the lumen of the intestine is of prime importance in resistance to rotavirus illness in animals.

The mechanisms of immunity were also studied in 18 volunteers who were administered a human rotavirus VP7 serotype 1 strain orally.[301,302] Five of the 18 individuals shed rotavirus and four of the five developed a diarrheal illness. Examination of the relationship of a moderately high level of prechallenge rotavirus antibody in serum measured by neutralization (to the homotypic or a heterotypic VP7 serotype 2 human rotavirus) to the development of diarrheal illness indicated that such antibody was associated with resistance to the development of illness. The role of local intestinal rotavirus antibody was not clear-cut and needs further evaluation. Two volunteers who developed illness following initial challenge were rechallenged with the same inoculum 19 months later; neither developed a diarrheal illness, although one had mild clinical manifestations.

Recently, the prechallenge sera from these volunteers were reexamined by an epitope-blocking assay that measured antibody to several defined epitopes of VP7 and VP4.[204] A significant protective effect against illness and shedding was associated with the presence of a prechallenge serum antibody titer of $\geq 1:20$ that blocked the binding of a serotype 1 VP7-specific monoclonal antibody that mapped to amino acid 94 in a major antigenic site of serotype 1. A similar protective effect was found for antibodies that blocked binding to a serotype 3 VP7-specific epitope that maps to amino acid 94 on a major antigenic site of serotype 3. Thus, this study not only confirmed the association of protection with serum antibody but also identified a specific epitope associated with such protection. However, in other volunteer studies, the role of serum antibodies has not been consistent.[35,596]

Reinfections with rotavirus occur commonly in adult contacts of patients with rotavirus illness; however, most of these reinfections are subclinical.[297,310,537,564] Sequential rotavirus infections and illnesses have been observed in infants and young children.[172,457,630,651] However, the second infection usually causes a milder illness, indicating that immunity resulted from the initial exposure.[41]

The role of antibodies was also examined in a longitudinal study of healthy 1- to 24-month-old children residents in a "nursery."[91] A serum-neutralizing antibody titer of 1:128 against VP7 serotype 3 human rotavirus appeared to be protective against gastroenteritis caused by this serotype, whereas levels of 1:64 or less did not afford significant homotypic protection.

Immunity to a specific serotype appears to be induced after infection with the first homotypic strains. Studies with live attenuated rotavirus vaccines demonstrated that adults develop a significantly greater number of heterotypic serum antibody responses following vaccination with a monovalent strain than do infants less than 6 months of age by epitope-blocking assay.[207] The broadening of the antibody response may explain the epidemiologic observation that a second rotavirus infection regardless of serotype is characteristically less severe than the initial infection.

One of the perplexing areas regarding immunity is the unexplained relative sparing of neonates from rotavirus illness despite frequent infections.[20,93,556] It is suggested that the high levels of transplacental antibodies contribute to this effect. In one study of newborn babies, rotavirus infections occurred significantly less often in breast-fed infants than in bottle-fed infants.[93] The effect of breast-feeding on illness could not be determined, since most of the infections in the breast-fed and bottle-fed infants were subclinical.[20,93,556] The role of breast-feeding on rotavirus infection needs additional study because available data from several studies do not enable a definite conclusion. However, it appears from current data

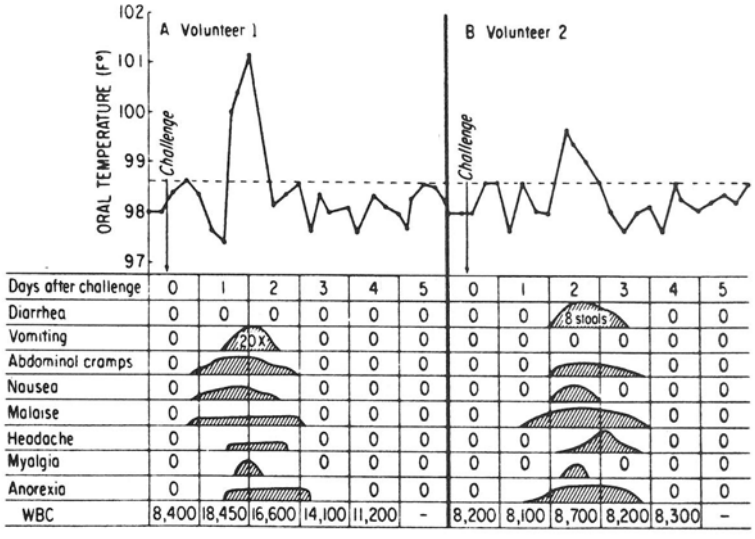
that the effect of breast-feeding on the prevention of rotavirus diarrhea will likely be modest.[192] Whether high levels of circulating rotavirus antibody acquired transplacentally play a role in resistance to disease during early life is not known. However, rotavirus illnesses are observed with moderate frequency in infants less than 6 months of age but beyond the neonatal period, a time when passively acquired circulating antibody is still present but not at as high a level as in neonates.[63,297]

In addition, however, such neonatal subclinical rotavirus infections induced significant protection against severe rotavirus diarrhea for up to 3 years later, but not against rotavirus infection and only limited protection against mild illness.[42] Mechanisms other than neutralizing antibodies such as cytotoxic T lymphocytes may also be important in the immune process.[418] The mechanism of immunity to rotavirus disease in humans needs further study, especially relating to the role of intestinal IgA antibody. Moreover, it was recently suggested that serum IgA antibody to rotavirus reflects the intestinal immune status to rotavirus.[245]

8. Patterns of Host Response

8.1. Norwalk Group of Viruses

8.1.1. Clinical Features. Clinical manifestations observed in the original Norwalk outbreak from which the Norwalk particle was derived demonstrate the key features of this infection. Six hundred and four individuals were considered to be primary or secondary cases: 85% had nausea, 84% vomiting, 62% abdominal cramps, 57% lethargy, 44% diarrhea, 32% fever, and 5% chills.[2] The duration of signs or symptoms was 12–24 hr; none of the affected individuals was hospitalized. These clinical findings are similar but not identical to those observed in a report describing the findings in 31 of 52 volunteers who developed definite or probable illness following administration of the Norwalk virus.[618] Of the 31 volunteers, 45% had fever (≥ 99.4°F), 81% diarrhea, 65% vomiting, 68% abdominal discomfort, 90% anorexia, 81% headache, and 58% myalgias; clinical manifestations usually lasted 24–48 hr. The diarrheal stools characteristically do not contain gross blood, mucus, or white blood cells.[128] Of 16 volunteers who became ill following Norwalk or Hawaii virus challenge, 14 developed a transient lymphopenia.[132] The illness observed in volunteers was generally mild and self-limited, although one volunteer who vomited about 20 times within a 24-hr period required parenteral fluids.[54,129,130,618] A graphic summary of signs and symptoms of illness observed in two volunteers who developed illness following administration of the Norwalk agent is shown in Fig. 9.[130] The difference in clinical manifestations in these two volunteers who received the same inoculum is striking, since one vomited but did not have diarrhea and the other developed diarrhea but not vomiting. Shedding of Norwalk virus by volunteers as determined by IEM was maximal around the onset of illness and was rarely detected after 3 days following onset.[550] A valid estimate of the ratio of subclinical-to-clinical Norwalk virus infections has not been made. However, serologically proven infection without definite gastroenteric illness has been observed in volunteers challenged with the Norwalk virus[52] (A. Z. Kapikian, unpublished



Days after challenge	0	1	2	3	4	5	0	1	2	3	4	5
Diarrhea	0	0	0	0	0	0	0	0			0	0
Vomiting	0			0	0	0	0	0	0	0	0	0
Abdominal cramps	0			0	0	0	0	0			0	0
Nausea	0			0	0	0	0	0		0	0	0
Malaise	0			0	0	0	0				0	0
Headache	0			0	0	0	0	0			0	0
Myalgia	0			0	0	0	0	0		0	0	0
Anorexia	0				0	0	0				0	0
WBC	8,400	18,450	16,600	14,100	11,200	-	8,200	8,100	8,700	8,200	8,300	-

Figure 9. Response of two volunteers to oral administration of stool filtrate derived from a volunteer who received original Norwalk rectal-swab specimen. The height of the curve is directly proportional to the severity of the sign or symptom. Volunteer 1 had severe vomiting without diarrhea, while volunteer 2 had diarrhea without vomiting, although both received the same Norwalk agent inoculum. From Dolin *et al.*[130]

data). Although the illnesses are usually self-limited, exceptions to this pattern were noted as three middle-aged individuals were hospitalized for severe dehydration during two outbreaks, two elderly debilitated patients died during the course of the illness in a nursing home outbreak, and three patients required intravenous fluids in a nursing home outbreak.[306]

8.1.2. Diagnosis. A specific diagnosis of infection with the Norwalk group is not possible from a patient's clinical presentation. There is no simple available assay for detection of these agents, which have yet to be grown in cell culture or in a suitable animal model. IEM remains the mainstay for detection and identification of the various members of the group as a whole.[292,293,299,304] Various tests have been developed for detection of certain members of this group: an RIA and ELISA (which are even more sensitive than IEM) for detection of Norwalk virus[52,180,218,239,241,349]; an RIA and an ELISA for the Snow Mountain agent[134,349]; and an ELISA for the Hawaii virus.[557] Direct EM examination of negatively stained stool material with or without prior concentration may also be attempted, but this is not usually satisfactory because of the relatively small amounts of virus shed.[62] Although EM examination of stools is a simple and relatively rapid procedure, caution must be used in interpreting the significance of "particles" observed, because stools contain a myriad of small objects that have no relationship to the illness being studied.[292] It is for this reason that carefully controlled IEM studies with appropriate paired sera should be carried out (under code) to determine the significance of the particles. Ideally, a patient's paired sera should show an IEM antibody increase with the observed particle as antigen (see Fig. 3); in addition, if paired sera are not available, careful IEM studies with γ-globulin, paired sera from other individuals in the same outbreak or from other similar outbreaks, or antisera to morphologically similar agents should be studied to determine the significance of the particle in question. Simple aggregation of particles by a serum should not be taken as evidence of a specific response, since certain particles, such as Norwalk virus, aggregate spontaneously without the addition of serum. These nonspecific aggregates may appear to be lightly coated with antibody. Thus, it is essential to quantify the antibody (on a scale of 0–4+) coating the particles even if they are aggregated. If there is any question about the significance of aggregation, the concentration of antigen or antibody should be varied. Such maneuvers should affect both the size of the aggregates and the amount of antibody coating the particles as the reaction proceeds from antigen excess to antibody excess.[292,304] Awareness of the specificity of aggregation is essential, because some stools contain groups of 22-nm "objects" that appear in "aggregate" form with little or no "antibody" on them and that have had no known relationship to the illness being studied.[292,304] These aggregates generally appear similar with regard to size and "antibody" coating when examined with paired acute or convalescent sera.

IEM is the only way to detect serological evidence of infection for a potential new member of the group. Serological diagnosis of Norwalk infection can be made by IEM, but the RIA- and ELISA-blocking tests are more efficient and practical.[52,180,218,241] RIA- and ELISA-blocking tests are available for the Snow Mountain virus and an ELISA-blocking test has been developed for the Hawaii virus.[134,349,557] However, as noted earlier, a major breakthrough in the study of these viruses occurred when the recombinant Norwalk viruslike particle (outer capsid) became available.[272] This recombinant is used as the antigen in a newly developed direct ELISA for detection of a seroresponse.[205,272] It is considered to be as specific, sensitive, and efficient as the native Norwalk virus present in stool that was the source of antigen previously. The availability of an unlimited source of recombinant antigen has already facilitated seroepidemiologic studies of this virus. An immune adherence hemagglutination assay had also proved useful for detecting serological evidence of Norwalk virus infection, but the requirement for a relatively large amount of antigen was a distinct disadvantage for its general use because the source of antigen was a particle-positive stool.[296] The assays for these 27-nm viruses are currently limited to research laboratories because reagents are not yet generally available.

8.2. Rotaviruses

8.2.1. Clinical Features. The three major clinical manifestations observed in infants and young children hospitalized with rotavirus gastroenteritis are vomiting, diarrhea, and dehydration. Signs and symptoms in 72 patients hospitalized with rotavirus diarrhea were compared with those of 78 patients hospitalized with a non-rotavirus diarrheal illness (Table 7).[454] The rotavirus group experienced significantly more vomiting and dehydration. The dehydration was isotonic in 95% of the patients in the rotavirus-infected group and in 77% of the rotavirus-negative group. As determined from history and hospital records, the mean duration of vomiting was also longer in the rotavirus-infected group (2.6 vs. 0.9 days). Diarrhea began later and lasted longer than vomiting in the rotavirus group (mean duration of diarrhea vs. vomiting, 5 days vs. 2.6 days). Once the patient was hospitalized, diarrhea continued for an average of 2.6 days (range 1–9 days) in the rotavirus group and 3.8 days

Table 7. Clinical Characteristics of 150 Children Hospitalized with Acute Gastroenteritis[a]

Clinical finding	Percentage having each clinical finding	
	Rotavirus infection detected (72 patients)	Rotavirus infection not detected (78 patients)
Vomiting	96[b]	58[b]
Fever (°C)		
37.9–39	46	29
> 39	31	33
Total	77	61
Dehydration	83[c]	40[c]
Hypertonic	5	16
Isotonic	95	77
Hypotonic	0	6
Irritability	47	40
Lethargy	36	27
Pharyngeal erythema	49	32
Tonsillar exudate	3	3
Rhinitis	26	22
Red tympanic membrane with loss of landmarks	19	9
Rhonchi or wheezing	8	8
Palpable cervical lymph nodes	18	9

[a]Adapted from Rodriguez *et al.*[454]
[b]$P < 0.01$.
[c]$P < 0.01$.

(range 1–16 days) in the nonrotavirus group. The duration of hospitalization ranged from 2 to 14 days (mean 4 days) for the rotavirus group. The greatest frequency of rotavirus diarrhea was in the 6- to 24-month age group.

Notable laboratory findings were related to the degree of dehydration.[454] Elevated BUN (>18 mg/dl) and urine specific gravity (>1.025) were observed in 58 and 71%, respectively, of the rotavirus group, frequencies significantly greater than those observed in the nonrotavirus group.

Deaths have been reported in infants and young children with rotavirus illness.[76,114,381,382,385,386] In a study in Canada, 21 deaths were reported between May 1972 and March 1977 in infants and young children with a rotavirus illness. Ten of the 21 children were dead on arrival at the hospital, and ten of the others were moribund and could not be successfully resuscitated on arrival.[76] One child was already in the hospital when he acquired the disease; this patient had congestive cardiomyopathy that contributed to his death. With the exception of this patient and one other, all of the children had been healthy previously. They ranged in age from 4 to 30 months, with a mean of 11 months. Deaths occurred 1 to 3 days after onset of symptoms. The major factor causing death in these children was dehydration and electrolyte imbalance in 16, aspiration of vomitus in 3, and in the remaining 2, seizures were a contributing factor. The rapid course of the disease is evidenced by the fact that the parents of 16 of the 20 children brought to the hospital had had some contact with a physician during the course of the illness.[76]

Rotaviruses can cause a chronic symptomatic infection in immunodeficient children.[267a,429,491,608] In addition, they may cause serious sequelae that can be life threatening in individuals immunosuppressed for bone marrow suppression.[635] Rotavirus does not appear to have an important role in the etiology of diarrhea in HIV-infected adult patients.[221]

Rotavirus infections have also been associated temporally on rare occasions with other conditions that occurred as isolated cases or single outbreaks such as intussusception,[319,320,400,413] self-limited gastrointestinal bleeding,[120,530,531,547] Henoch–Schoenlein purpura,[120] Reye's syndrome,[475] encephalitis,[475,578] aseptic meningitis,[607] hemolytic uremic syndrome,[616] disseminated intravascular coagulation,[616] elevated serum transaminase levels,[135,537] sudden infant death syndrome,[641] exanthem subitum,[474] Kawasaki syndrome,[144,369] necrotizing enterocolitis and hemorrhagic gastroenteritis in special-care nurseries.[119,231,308,462,463] pneumatosis intestinalis,[308] chronic diarrhea,[156,537] abortion,[74,156] febrile or afebrile convulsions,[341,393,419,581] hyperphosphatasemia,[250] pneumonia,[487] hepatic abscess,[222] acute laryn-

gitis,[414] colitis,[174] benign acute myositis,[237] pancreatitis,[415] and hyperechoic lesions in the basal ganglia.[599] Whether the rare temporal association of these conditions with these ubiquitous viruses is coincidental or significant remains to be determined. However, a primary etiologic relationship appears extremely unlikely, except in the severe illnesses in immunocompromised patients and in some cases of necrotizing enterocolitis and hemorrhagic gastroenteritis in neonates and pneumatosis intestinalis in infancy. Growth of rotavirus has also been described in tissue cultures inoculated with filtrates prepared from intestinal tissue of patients with Crohn's disease, but this observation could not be confirmed.[285,603]

In the volunteer studies in which the VP7 serotype 1 strain was administered orally to volunteers, four developed a diarrheal illness that began 2 to 4 days after inoculation.[301,302] Two of the four volunteers with diarrhea also vomited, one the day after inoculation (2 days before the onset of diarrhea) and the other 3 days after inoculation (the day of onset of diarrhea). The average duration of diarrhea was 2.5 days, with a range of 1 to 4 days. The number of diarrheal stools per illness ranged from 1 to 24, with one volunteer having a maximum of 11 in 1 day. Thus, under experimental as well as natural conditions, adults can develop a rotaviral diarrheal illness. However, subclinical rotavirus infection in adults occurs much more frequently, as demonstrated in one study in which 22 of 50 adult family contacts of pediatric patients hospitalized with rotavirus gastroenteritis themselves developed serological evidence of rotavirus infection at or about the time of their children's hospitalization[310]; however, only three infected parents had a gastroenteric illness at or about the time of their children's illnesses.

8.2.2. Diagnosis. As with the Norwalk group, a specific diagnosis of infection with human rotavirus cannot be made by clinical presentation. Even though rotavirus infections follow a predictable seasonal pattern of high prevalence during the cooler months in temperate climates, a laboratory diagnosis is essential, because other agents may also cause gastroenteritis even during these periods.

Although human rotaviruses can now be grown in cell cultures, this is not a practical method for detection from clinical specimens. Numerous assays have been developed for the detection of rotaviruses, as outlined previously in Table 1. The most widely applied methods aim at detection directly from stool specimens. Electron microscopy is highly specific, because rotaviruses have such a distinct morphological appearance; it is limited, however, by the requirement for an electron microscope as well as a capable operator. It provides the most rapid diagnosis when dealing with only a limited number of specimens.[62] In addition, it has the advantage of recognizing the non-group-A rotaviruses as well as other viral agents of gastroenteritis in a single specimen.

Other efficient but more practical assays for large numbers of specimens include ELISA, RIA, latex agglutination, and counterimmunoelectro-osmophoresis.[38,62] ELISA is the most practical diagnostic method for large-scale studies and is limited only by the availability of suitable reagents; however, many kits are now available commercially. However, some of these are prone to false-positive reactions. Thus, we recommend that a confirmatory ELISA in which a pre- or postrotavirus immunization serum be used as the solid-phase precoat.[62,304,638,648] In this way, the difference in a specimen's reactivity with the pre- or postserum is determined. Some specimens react equally with both sera, thus preventing a diagnosis. However, without the control serum, such a specimen would be considered positive for rotavirus. A dot hybridization assay as well as the application of PCR technology have further increased the sensitivity of virus detection.[164,631] Thus, there are several efficient and practical methods for detecting rotaviruses; the method of choice will vary according to the resources and experience of individual laboratories. An enzyme immunoassay and a PCR assay have also been developed for detection of infection with group B or group C rotavirus.[146,175,198,409,561]

Rotaviruses can be serotyped (or genotyped) by various methods such as neutralization in cell culture, SPIEM, ELISA, hybridization, sequencing, and PCR.[26,28,37,104,162,166, 168,185,188,200,201,203,204,206,238,387,501,517,541,544,546,552,576,577,579] The availability of monoclonal antibodies for each of the four clinically important serotypes has made the ELISA the most practical serotyping method.

Serological evidence of rotavirus infection may be detected by a variety of techniques (see Table 2). Complement fixation is efficient and practical when testing sera from pediatric patients about 6–24 months of age.[304,646] However, it is not as efficient as other techniques when testing sera from patients less than 6 months of age and from adults.[646] Serological evidence of rotavirus infection may be detected in these age groups by ELISA or IF. ELISA has also been employed to measure specific immunoglobulin responses in rotavirus infection.[373,649] An ELISA for serum IgA antibodies (which do not cross the placenta) is particularly useful for measuring antibody responses in infants under 6 months of age because they possess passively acquired maternal IgG antibodies that may confound an IgG ELISA.[121,344,345] In addition, serum IgA antibodies may reflect the immunologic status of the intestine.[117,244,245] Neutralizing antibodies in tissue

culture systems are particularly important when serotype-specific antibody rsponses are sought.[12,283,585] A competition-solid-phase immunoassay that detects epitope-specific immune responses to individual serotypes has proven important in determining immune responses to individual epitopes.[204,207,361,504] As long as the limitations of the various methods are recognized, the method of choice will vary according to the resources and experience of individual laboratories.

9. Control and Prevention

9.1. Norwalk Group of Viruses

Methods are not available for the prevention or control of infection or illness with the Norwalk group of agents. Since this group of agents is highly contagious and transmitted by the fecal–oral (or vomitus–oral) route, it is possible that in a family or group setting where one member is ill with this form of gastroenteritis, effective handwashing and disposal or disinfection of contaminated material could decrease the likelihood of transmission. Increased vigilance concerning the purity of drinking water or of water in swimming pools as well as careful attention to hygiene by food handlers and concern regarding the source of uncooked shellfish might also limit the number of outbreaks.

Treatment of gastroenteritis caused by the Norwalk group usually consists of replacement of fluid loss by the administration of liquids orally. Parenteral intravenous fluid therapy is only rarely necessary in this form of self-limited gastroenteritis.[128–130] The impact of this group of agents in debilitated hosts has not been evaluated systematically, although a few deaths in elderly debilitated individuals have been reported.[306] In volunteers, oral administration of bismuth subsalicylate after onset of symptoms significantly reduced the severity and duration of abdominal cramps and the median duration of gastrointestinal symptoms; however, it did not significantly affect the number, weight, or water content of stools.[528]

Because of the paradoxical effect of antibody on the susceptibility or resistance to Norwalk virus infection or illness, it is premature to consider vaccination strategies. However, if the immune mechanisms are elucidated more clearly and antibodies are found to be important in preventing illness, a vaccine may be relevant in preventing epidemic gastroenteritis, especially in special groups such as military personnel and individuals living closely in an institutional setting. The need for such a vaccine in pediatric groups would need further study.

9.2. Rotaviruses

There are no methods for the prevention or control of infection or illness with rotaviruses. Although improved hygiene is of course generally desirable, it does not appear that such measures would markedly affect the transmission of rotavirus infection. As noted earlier, the prevalence of rotavirus infection is similar in both developed and developing countries, regardless of hygienic conditions. Therefore, the development of a rotavirus vaccine is of primary importance.[143]

Treatment of rotavirus gastroenteritis has as its goal the replacement of fluids and electrolytes lost by vomiting and diarrhea. Treatment must be initiated early in the course of illness because rotavirus gastroenteritis can lead to rapid dehydration. Administration of intravenous (IV) fluids and electrolytes are very effective in the treatment of rotavirus dehydration, but this form of therapy is not readily available in many parts of the world. As a result, in seeking alternate approaches, the administration of oral rehydration salts (ORS) solutions was found to be comparable in effectiveness to IV therapy, and thus has provided a major advance in treatment.[281,411,469,471,472,485] In certain instances, when ORS fails to correct the fluid and electrolyte deficit or if the patient is severely dehydrated or in shock, IV therapy must be instituted.

Passive immunization by the oral route with various rotavirus antibody-containing preparations has been effective in selective circumstances in preventing dissemination of rotavirus infections in a nursery or in prevention of nosocomial infections.[22,118,136] Various forms of antibody-containing preparations have been described.[22,118,136,639,640] Passive immunization was effective in the treatment of rotavirus illness in immunodeficient infants and young children and in a recent study in the treatment of normal infants with naturally occurring rotavirus infection.[223,491]

Bismuth subsalicylate (BSS) given orally for 5 days as an adjunct to rehydration therapy in infants and young children was associated with a more rapid recovery from illness.[518,519] The BSS group had shorter times until the last loose stool (57.5 hr vs. 104.5 hr) and the last unformed stool (113 hr vs. 167.7 hr) when compared to the placebo group. Because of the reported correlation between the use of salicylates and Reye's syndrome, the possibility of such an association with BSS or other nonacetylsalicylic acid salicylates was considered and none was found.[518,519]

As noted earlier, rotaviruses represent a major cause of severe diarrhea of infants and young children in both developed and developing countries. Thus, it is clear that a rotavirus vaccine is needed.[283,284,294,300] Although diarrheal illnesses are not a major cause of mortality in the

developed countries, they are the leading cause of death in infants and young children in many developing countries, being associated with five to ten million deaths.[593] It has been estimated that in developing countries, rotaviruses are responsible for over 870,000 deaths in children under 5 years of age.[266] It is likely that a rotavirus vaccine would make an important impact on reducing the morbidity and mortality from rotavirus diarrhea in infants and young children.[125]

It has been suggested, however, that enterotoxigenic *E. coli* may be a more important cause of death from diarrhea in developing countries than rotaviruses.[140,334] For example, in the United States in New York City in the early 1900s, there was a staggering infant mortality rate with a large proportion of deaths attributable to outbreaks of summer diarrhea in slum tenements.[334] The infant death rate declined markedly in the next decades, and it has been suggested that this decline was not because of better medical management of summer diarrhea, but rather was the result of development of improved sanitary conditions such as iceboxes, flush toilets, and water supply, which limited bacterial contamination.[334] Indeed, despite the advanced sanitary conditions and high standards of living in the United States today, almost all persons still undergo rotavirus infection by the end of the third year of life, though mortality from diarrheal illnesses is infrequent in the United States. Decline in mortality from diarrheal diseases in developed countries has resulted in part from the availabilty of fluid replacement therapy and possibly better nutrition, but undoubtedly other factors have played a major role, such as the decline in incidence of bacterial diarrheas as sanitation improved.[334] However, from longitudinal and cross-sectional studies in developing countries, it appears that rotaviruses and enterotoxigenic *E. coli* are major causes of clinically significant diarrhea of infants and young children, with rotaviruses being responsible for a disproportionately high percentage of severe illnesses in relation to their overall contribution to diarrhea of any severity.[50,51]

Animal studies cited earlier clearly indicate that antibody in the intestinal lumen plays a major role in resistance to rotavirus disease. In experimentally infected animals, serum rotavirus antibody in the absence of intestinal antibody was not effective in preventing rotavirus illness. Thus, one approach in the control of rotavirus illness may be the encouragement of breast-feeding as a means of providing local antibody to the young infant. Colostrum and milk contain IgA rotavirus antibody, and it may be that such antibody would exert some protective role against rotavirus illness in the infant and young child.[650] If a successful rotavirus vaccine were developed, it might be beneficial to immunize the mother to raise the level of antibodies in her breast milk for transfer to the intestine of the infant. One discouraging aspect of this approach is the high frequency of diarrheal diseases in general, including those associated with rotavirus, in countries where infants and young children are breast-fed for extended periods.[192] However, the nutritional status of the nursing mother may be a critical factor in this observation. This approach to vaccination is not considered to be practical because it would likely entail the parenteral rather than oral administration of a rotavirus vaccine because of the relatively high levels of preexisting maternal antibodies that would neutralize an orally administered vaccine.

The aim of a successful vaccine is to prevent serious illness during the first 2 years of life, when the outcome of such infection may be especially serious or fatal.[87,284,294,300] Thus, the vaccine would be administered orally to induce local IgA antibody within the first 6 months of life or perhaps even neonatally, since it has been estimated that 10 to 40% of infants born in developing countries are in contact with a health care provider only at the time of birth.[232]

Approaches to the development of a rotavirus vaccine range from conventional cell culture cultivation of human or animal rotavirus strains to molecular biological techniques. The most extensively evaluated method involves the "Jennerian" approach in which an antigenically related rotavirus from a nonhuman host such as a calf or monkey is used as the immunizing agent.[283,290,291,294,366,584,588,612] The feasibility of this approach was tested in calves. Calves were inoculated *in utero* with calf rotavirus (serotype 6) or with placebo (or nothing).[260,627,629] Shortly after birth, the calves were challenged with a human rotavirus serotype 1 strain, and it was found that *in utero* infection with calf rotavirus induced resistance to disease caused by challenge with this human rotavirus strain. In contrast, animals that had received placebo (or nothing) developed illness on challenge with the human rotavirus serotype 1 strain soon after birth.[260,627,629] Thus, cross-protection between the calf and human rotavirus was demonstrated, indicating that the bovine virus was sufficiently related antigenically to the human rotavirus strain to induce protection.[629]

Extensive clinical evaluation of a bovine NCDV rotavirus strain has been carried out.[585,587] Efficacy trials with this strain in Finnish children 1 year of age or less demonstrated a protection rate of over 80% against clinically significant diarrhea[586] However, such trials in developing countries yielded less-encouraging results, and therefore this vaccine was withdrawn from further studies.[585] Similarly, a related bovine rotavirus strain (WC3) also

demonstrated variable efficacy and was also withdrawn from further study in its current formulation.[585,587]

A rhesus-monkey-derived rotavirus strain has also undergone extensive clinical evaluation as a vaccine candidate.[283,284] This vaccine also demonstrated variable efficacy that ranged from 85% to nil against moderately severe to severe diarrhea. This was attributed to the failure of the vaccine to protect against heterotypic strains in individuals undergoing primary infection.

Because the "Jennerian" concept was not successful in protecting against each of the four epidemiologically important rotavirus serotypes, another approach involving the use of rotavirus reassortants as vaccines was undertaken. Such reassortants were constructed by coinfection of cell cultures with two different rotavirus strains under selective pressure of antibody against one of them.[211,388,389] In this way, the rhesus rotavirus strain was the donor of attenuating genes and a human rotavirus of a specific serotype was the donor of a single gene that codes for the major neutralization protein (VP7). Such single-gene-substitution reassortants have been constructed for human rotavirus serotypes 1, 2, 3, and 4, with the rhesus (MMU18006) and/or bovine (UK) rotavirus strains as the donor of the other ten genes.[211,388,389] Efficacy trials with monovalent or quadrivalent formulations have been completed or are underway with generally encouraging results.[95,124,284,329,330,347,470,589]

In yet another approach, rotavirus strains isolated from neonates with asymptomatic infections may be important for immunoprophylaxis because they appear to be naturally attenuated, and strains belonging to each of the human rotavirus serotypes are now available. A VP7 serotype 1 neonatal strain M37 has been evaluated for efficacy in Finland; it failed to induce protection against VP7 serotype 1 illness in a preliminary study.[167,196,258,259,433,590] Other approaches such as cold-adapted human rotavirus strains or substitution of "virulence" genes with "avirulence" counterparts are under consideration as viable vaccine candidates.[254–256,367] Finally, molecular biological approaches may also yield potential vaccine candidates.[166,169,467]

Thus, it is hoped that an effective immunogen will be developed for rotavirus. However, it should be stressed that since a human rotavirus vaccine has not yet been licensed for general use, effective treatment for rotavirus diarrhea is available in the form of fluid and electrolyte replacement therapy by the oral or parenteral route of administration.[411,471,472,486] Thus, one means of controlling the severe morbidity and mortality from rotavirus diarrhea would be to make available fluids and electrolytes necessary for rehydration. In addition, since this agent is transmitted by the fecal–oral route, careful attention to handwashing, disinfection, and disposal of contaminated material would appear to be one way of limiting the spread of this highly contagious agent, especially in nurseries and hospitals, where nosocomial infections are common.

10. Unresolved Problems and Other Agents or Putative Agents of Viral Gastroenteritis

10.1. Norwalk Group and Other Enteric Viruses

Efforts must be made to determine the number of serotypes responsible for epidemic viral gastroenteritis. Such studies entail careful IEM studies to determine antigenic relationships. The development of radio- and enzyme immunoassays for Norwalk virus has permitted the study of the epidemiologic importance of this agent. However, the cloning, sequencing, and expression of the Norwalk virus and the Southampton virus have opened up a new era of research possibilities that should enable the elucidation of the natural history and the genetic interrelationships of these agents.

A major impediment to the study of the viruses is the inability to propagate them in any cell culture. This is an important problem that must be resolved.

Studies of immunity to Norwalk agent have raised rather perplexing questions because of the paradoxical effect of preexisting antibodies on the susceptibility to illness. It has been suggested that this may be due to a genetic factor, such as a receptor for Norwalk virus, that is lacking in one cohort of individuals and present in the other.[426] The role of local IgA antibody should also be explored further. Resolution of this question is essential before considering vaccine strategies.

Finally, a major unresolved area in the etiology of epidemic viral gastroenteritis is the role of other small round viruses such as astroviruses, the morphologically classical caliciviruses, minireoviruses, the Otofuke-like viruses, and the small round viruses.[286] Some of these agents, such as the astroviruses, have been studied rather intensively.

Astroviruses, which are now classified in the new family *Astroviridae*, are 28 nm in diameter and derive their name from the five- or six-pointed star-shaped configuration observed by negative staining in about 10% of particles.[326] They have a buoyant density of 1.35–1.40 g/cm^3 in cesium chloride[326] and a positive-sense single-stranded RNA genome.[326] Seven distinct serotypes are recognized and each has been grown in cell culture.[326,332a]

These viruses have been linked to epidemic or endemic mild gastroenteritic illness in infants and young children.[242,326,336] They have been associated with outbreaks of gastroenteritis in newborn nurseries and pediatric wards, in community settings, and in nursing homes.[326] Studies of the prevalence of astrovirus antibody have demonstrated a rather rapid acquisition of antibody, so that over 70% of 5-year-old children have acquired antibody.[326] They have only rarely been associated with diarrheal illnesses requiring hospitalization. They were associated with 8.6% of gastroenteritis illnesses in an outpatient pediatric study in Thailand (2% in controls) and in Guatemala in 7.3% of diarrhea episodes (2.4% in diarrhea-free periods) with the highest frequency in children less than 1 year of age in both studies.[242] In day-care center studies in the United States, astroviruses were detected in 4% of the children with diarrhea (less than 1% of the controls).[336]

Astroviruses are transmitted by the fecal–oral route. In volunteer studies, they are of low pathogenicity in adult volunteers, with only 2 of 36 developing vomiting and diarrhea after oral challenge, although over one half developed serological evidence of infection.[327a,388a]

Diagnosis is limited to research laboratories with EM, EIA, and IF used to detect infection.[326] Astroviruses have also been detected in stools of various animals with and without diarrhea.[326]

Caliciviruslike particles, which are about 32–40 nm in diameter and have characteristic cuplike configurations on their surface, have also been studied rather intensively.[106] Gastroenteritis in infants and young children in Japan, England, and Canada has been associated with such particles.[106] In addition, such particles were found in the small intestine of a 22-month-old infant who died of acute gastroenteritis.[161a] As noted earlier, the Norwalk virus is now definitely classified as a calicivirus. Recent molecular biological advances should clarify the relationship of the Norwalk virus with the so-called "classical" (in morphology) caliciviruses.

Another particle, the Otofuke agent, 34–38 nm in diameter with a density of 1.35–1.37 g/cm^3, has been associated with an outbreak of gastroenteritis in a work-training facility for mentally deficient persons 15 years of age or older.[573] The antigenically related Sapporo agent was detected in a gastroenteritis outbreak in infants and young children in an orphanage.[313] The 30–32 nm "minireoviruses" have been found in stools of pediatric patients with nosocomial gastroenteritis and with gastroenteritis requiring hospitalization.[386,523] They have recently been found to belong to the calicivirus family by cloning and sequencing techniques.[337] Their role in diarrheal illness needs to be elucidated. Small round viruses (20–32 nm) have also been found in stools of pediatric patients with diarrhea.[40] Their role in disease is unknown.

Two other groups that have no morphological similarity to the Norwalk virus are the adenoviruses and the coronaviruses. Fastidious adenoviruses, which did not grow in conventional cell cultures known to support the growth of other adenoviruses, have been observed in stools of infants and young children hospitalized with diarrhea.[592] They are referred to as fastidious or enteric adenoviruses. The latter term is somewhat confusing since conventional adenoviruses, which are indistinguishable morphologically by EM, may also be detected in the enteric tract. The fastidious enteric adenoviruses are detected in stools by EM or by an immunoassay.[592]

The fastidious adenoviruses that have been associated with diarrheal illnesses of infants and young children measure 70–80 nm in diameter and are DNA viruses with a buoyant density of 1.34 g/cm^3 in cesium chloride.[592] They belong to serotypes 40 and 41 by neutralization and to the F subgroup based on the pattern of DNA fragments produced by the digestion of their nucleic acid by restriction endonucleases as observed on agarose gels by electrophoresis.[592] They can be propagated in adenovirus-5-transformed Graham 293 HEK cells and certain other cells also.[592] They have a worldwide prevalence. About 50% of children acquire antibodies by 4 to 5 years of age.[592] It appears that the fastidious adenoviruses rank second to rotaviruses as viral etiologic agents of diarrhea of infants and young children that requires hospitalization.[571,592] In a Washington, DC, study, 4.4% of children hospitalized with diarrhea and 1.8% of controls shed an adenovirus in stools by EM.[60] In Sweden, fastidious adenoviruses were associated with 7.9% of the acute diarrheal episodes in children who were hospitalized or seen as outpatients.[572] In comparison, rotaviruses were detected in 45% of children with acute diarrheal illnesses. In Korea, 9% of the children hospitalized with diarrhea (mean age 11 months) shed adenoviruses (2% controls), whereas in Italy, 8.3% of hospitalized infants and young children shed adenovirus presumptively.[85,311] Adenoviruses have also been associated with infection and illness in bone marrow transplant patients. They were detected in 12 of 31 bone marrow transplant patients with gastroenteritis, six of whom died.[635]

Adenoviruses have also been detected in stools of 15% of patients hospitalized with gastroenteritis in a Canadian study.[450] In addition, adenoviruses were associated with the deaths of three children with severe gastroenteritis; adenovirus antigen was detected in the jejunal cells of two of the children by IF and in one by thin-section EM.[450,602] Adenoviruses have also been associated with a gastroenteritis outbreak in a long-stay children's

ward.[159] They have also been found in small-intestinal fluid of pediatric patients with gastroenteritis[372]; in such patients, D-xylose absorption was impaired. Adenovirus infection has also been associated with intussusception.[96,179]

Coronaviruses are established as etiologic agents of diarrheal disease in many animals, but they have not yet been implicated conclusively as important etiologic agents of infantile gastroenteritis in humans.[78,430] They are difficult to detect conclusively in stools by EM because of the plethora of fringed objects reminiscent of coronaviruslike particles.[78,83,430] There are reports of the detection of coronaviruses in stools by EM in three outbreaks of gastroenteritis in adults and one in neonates, with the particles from one of the adult outbreaks being propagated in organ and cell cultures.[82,84,582] Fringed coronaviruslike particles were also associated with an outbreak of severe hemorrhagic enterocolitis in premature and full-term newborn infants in France, with two deaths.[88] In this same study, similar particles were detected in outbreaks of diarrhea in 3- to 24-month-old children.[88,89] However, these particles have not been described further, and the issue of their nature and origin is still unresolved.[331,428] Human serum has been shown to contain neutralizing antibody to calf coronavirus; however, since the human respiratory coronavirus OC43 and the calf coronavirus share some antigenic relationship, it is not certain whether this antibody is related to OC43 or to another human coronavirus.[307,503] Antibody to human enteric coronaviruslike particles has also been detected in the serum of Australian aborigines by IEM.[494]

In recent studies in Italy and the United States, coronaviruses have been associated with acute infantile gastroenteritis and necrotizing enterocolitis, respectively. In addition, representative particles were characterized and appeared to be true coronaviruses[25,186,449] distinct antigenically from the Breda–Berne group of fringed viruses, which were recently implicated in diarrheal illnesses of humans and calves.[25,609]

Other agents detected in stools of infants and young children include the pleomorphic fringed Breda or Berne-like viruses (toroviruses) with a diameter of 100–140 nm, the 35m picobirnaviruses, and a pestivirus antigen.[181,431,432,609,636] Their contribution to the etiology of viral gastroenteritis needs further study.

10.2. Rotaviruses

Important advances have been made in the development and clinical evaluation of rotavirus vaccines. However, with this progress comes the need to address fundamental questions on the nature of the immune response to rotavirus infections. What is the role of serum or local intestinal antibodies in preventing or modifying rotavirus illness? Must a rotavirus vaccine be administered by the oral route to be effective, or will parenteral immunization also be important? Will the modified "Jennerian" approach, which aims at inducing antibody responses to each of the four epidemiologically relevant serotypes, be effective? Can a rotavirus vaccine be formulated that protects against all episodes of rotavirus diarrhea and not only against all episodes of severe rotavirus diarrhea (with the latter being the current expectation)? Can neonatal rotavirus strains be used as vaccines because they are usually associated with subclinical infections? Can neonates be immunized successfully with rotavirus? Can an oral rotavirus vaccine be given simultaneously with oral poliovirus vaccine without interference?[190] How stable will an attenuated oral rotavirus vaccine strain be after being shed in stools? Will it induce secondary infections or illnesses? Must an oral rotavirus vaccine be administered with buffer because rotaviruses are acid labile at pH 3? Will breast milk interfere with the "take" of an oral rotavirus vaccine? Would vaccinating expectant mothers to boost their breast milk and serum antibody titers to rotavirus have any impact on rotavirus morbidity and mortality in infants? What will be the impact of a rotavirus vaccine on the overall occurrence of severe diarrheal disease and on mortality from diarrheal diseases in a developing country? Why are rotavirus infections predominantly subclinical in neonates? Are there reservoirs for rotaviruses? Practically every animal studied has been found to have a virulent indigenous rotavirus. Although there is evidence of transmission of animal rotaviruses or of human–animal rotavirus reassortants to humans, is this of epidemiologic relevance now or in the future? Will serotypes other than 1, 2, 3, and 4 assume epidemiologic importance? Another area to be resolved concerns the role of rotavirus infection in malnutrition and the effect of malnutrition on rotavirus infection. The possible role of breast milk in prevention or attenuation of rotavirus diarrhea must also be clarified further. Although there is evidence that breast milk can exert an effect on rotavirus shedding, its role in the prevention of rotavirus diarrhea remains to be established. The synergism, if any, between rotaviruses and bacteria needs study. In animals, certain bacteria act synergistically with rotavirus to cause more severe illness than if either were present alone.[568] The worldwide importance of rotaviruses has increased the demand for reagents for study. Suitable reagents for ELISA for detecting human rotaviruses and for typing them are necessary. Finally, the natural history, importance, and distribution of the non-group-A rotaviruses need to be determined. Methods to propagate these strains

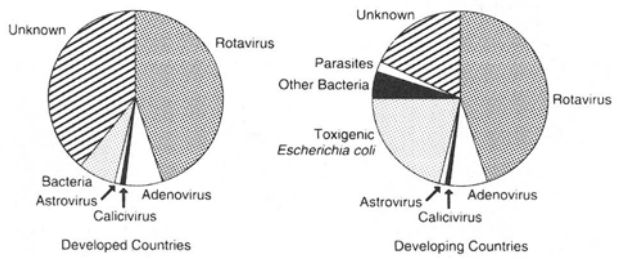


Figure 10. An estimate of the role of various etiologic agents in severe diarrheal illnesses requiring hospitalization of infants and young children in developed and developing countries. From Kapikian.[280]

efficiently in cell culture are needed as well as simple assays to detect and identify them.

11. Summary

Although numerous microbial agents infect the intestinal tract,[412] it is clear that the single most important etiologic agents of severe diarrhea of infants and young children in both developed and developing countries are the group A rotaviruses.[286] The relative role of viral, bacterial, and parasitic agents as a cause of severe diarrhea in infants and young children worldwide is estimated schematically in Fig. 10.[280] Hopefully, the unknown portions of these "pies" will be elucidated in the not-too-distant future.

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