CHAPTER 11

Viral Gastroenteritis

Albert Z. Kapikian, Harry B. Greenberg, Richard G. Wyatt, Anthony R. Kalica, Hyun Wha Kim, Carl D. Brandt, William J. Rodriguez, Robert H. Parrott, and Robert M. Chanock

1. Introduction

Nonbacterial gastroenteritis is a syndrome that 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. In the Cleveland Family Study, which included some 25,000 illnesses over an approximate 10-year period, infectious gastroenteritis was the second most common disease experience and accounted for 16% of all illnesses.[58] In addition, a winter survey of a sample of United States physicians engaged in pediatric practice revealed that "GI disturbance" was the second most common disease for which children were brought to the physicians' offices, accounting for 9.5% of all visits.[3] On the global scale, the impact of diarrheal diseases is staggering; World Health Organization (WHO) statistics have revealed that diarrheal diseases account for a large proportion of the total reported deaths in many countries.[280] An estimate of the total number of diarrheal episodes in 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.[217] 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.[271]

Despite the great importance of this problem,

Albert Z. Kapikian, Harry B. Greenberg, Richard G. Wyatt, Anthony R. Kalica, Hyun Wha Kim, Carl D. Brandt, William J. Rodriguez, Robert H. Parrott, and Robert M. Chanock · Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; Children's Hospital National Medical Center of Washington, D.C.; and George Washington University School of Medicine and Health Sciences, Department of Child Health and Development, Washington, D.C.

A. S. Evans (ed.), *Viral Infections of Humans*

studies failed to reveal an etiological agent for the majority of diarrheal illnesses.[51] However, discoveries made since 1972 of two new groups of viruses—the "parvoviruslike" group, of which the 27-nm Norwalk particle is the prototype, and the 70-nm rotavirus group—have brought forth an abundance of new information about viral gastroenteritis.[21,135,136] The Norwalk 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.[102,136] The 70-nm rotaviruses have been associated with 39–63% of the acute diarrheal diseases of infants and young children requiring hospitalization in several developed countries in different parts of the world and appear to be important ethiological agents of severe acute infantile gastroenteritis in developing countries also. This chapter will deal primarily with these two new groups of viruses; in addition, other viral agents that do play a role or may also play a role in this syndrome will be discussed at the end of the chapter.

2. Historical Background

Diarrhea in humans has been documented since pre-Hippocratic times. Discoveries made in the past century in the fields of bacteriology and parasitology resulted in the elucidation of the etiology of a portion of the diarrheal syndromes. 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 etiological agent. By exclusion, it was assumed that many of these infectious gastroenteritides were due to viruses. In 1945, Reimann, Price and Hodges[206] 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.*,[96] 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.*[140] induced gastroenteric illness in volunteers following oral administration of bacteria-free fecal filtrates derived from diarrhea cases in the Niigata Prefecture and other districts; serial passage was achieved, and short-term immunity was demonstrated on challenge with a single strain. Yamamoto *et al.*,[292] 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.*[92] 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 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.[117] Cross-challenge studies in volunteers revealed that the Marcy and FS strains were not antigenically related; in addition, the incubation period of and clinical illness induced by the two strains were somewhat different.[117]

Studies on the etiology of severe infantile gastroenteritis also failed to reveal an etiological agent in the majority of instances. However, in 1943, Light and Hodes[151] were able to induce diarrhea in calves with a filterable agent derived from diarrheal stools obtained from infants who developed 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 recently examined by electron microscopy (EM) and found to contain rotavirus.[112] Whether this represented a true calf rotavirus or the human strain passaged in calves could not be determined conclusively; in recent studies, the agent was not infectious when administered to a gnotobiotic calf.[112,281]

In 1972, application of the technique of immune electron microscopy (IEM) led to the discovery of 27-nm particles in stool material derived from a gastroenteritis outbreak in Norwalk, Ohio[135] (Fig. 1A).

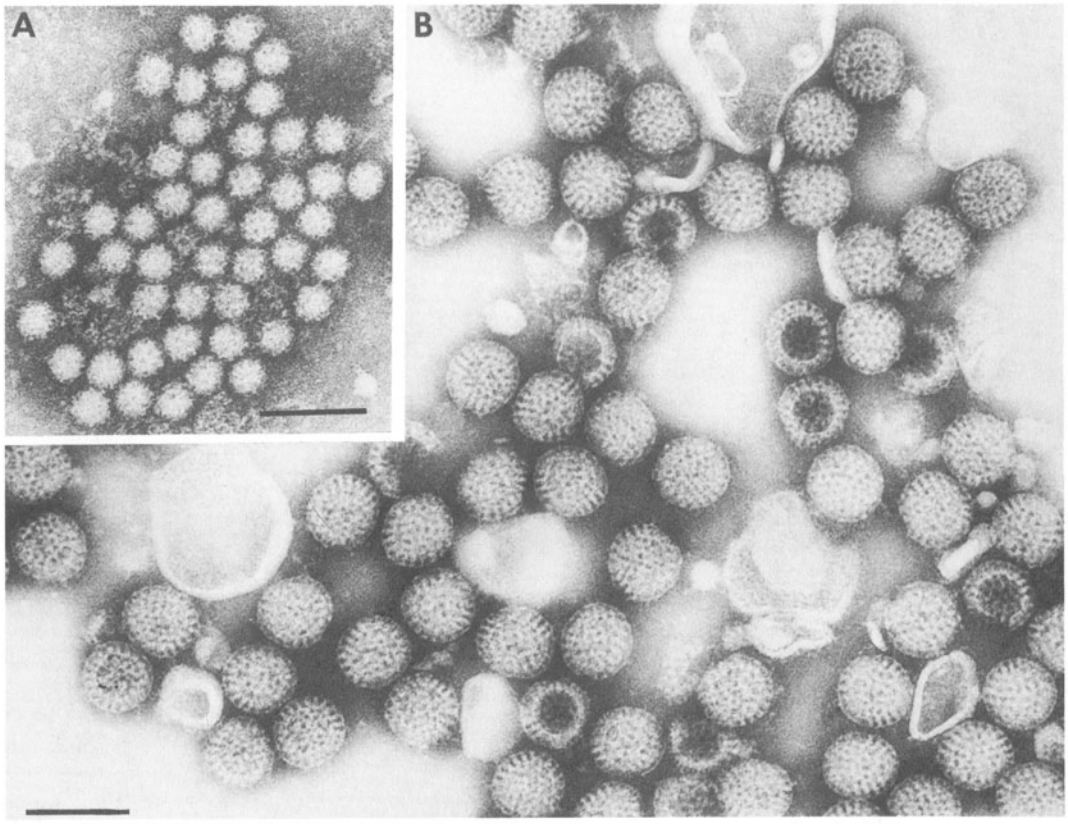


Fig. 1. (A) A group of Norwalk virus particles observed after incubation of 0.8 ml of Norwalk stool filtrate (prepared from a stool of a volunteer administered the Norwalk agent) with 0.2 ml of a 1:5 dilution of a volunteer's prechallenge serum and further preparation for EM. The quantity of antibody on these particles was rated as 1+. Scale bar: 100 nm. From Kapikian *et al.*[135] (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.*[134]

This technique, which had actually been described in 1939 but not used to its fullest potential until recently, might be considered as the direct observation of antigen–antibody interaction by EM.[7,8,12,129] The 27-nm particles were visualized in a known infectious stool filtrate derived from a volunteer who had developed illness following administration of the Norwalk agent[135]; the particle-positive specimen had also induced illness in other volunteers on serial passage.[60] The particles were recognized following reaction of the known infectious stool filtrate with a volunteer's convalescent serum prior to preparation for examination by IEM.[135] 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 concluded that the 27-nm particle was the etiological agent of the Norwalk outbreak.[135] Particles morphologically similar to Norwalk virus—such as the Hawaii, Montgomery County, Ditchling, "W," cockle, Parramatta, and Marin County agents—were later detected from patients in outbreaks of gastroenteritis by IEM or conventional EM.[9,11,48,193,256]

It soon became apparent from other studies that a 70-nm particle—formerly known by various names such as orbivirus, orbiviruslike, reoviruslike agent, duovirus, and infantile gastroenteritis virus but now officially designated rotavirus—was indeed the major etiological agent of infantile diarrhea.[21,54,66,86,104,107a,133,165,201] The human rotavirus was discovered in 1973 by Bishop *et al.*[20,21] by examination by thin-section EM of duodenal biopsies obtained from infants and young children hospitalized with acute gastroenteritis in Australia. Subsequently, it was found to be readily detectable in stool preparations by EM.[19,83] 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 viral etiological 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.*[174] 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.[86,126,127,134] It is of interest that both the Norwalk and rotavirus groups could have been discovered much sooner than they were if the concept of "direct virology" using EM had been applied to appropriate specimens.[136]

3. Methodology Involved in Epidemiological 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 of the Office of Health Research Statistics and Technology, Public Health Service, Hyattsville, Maryland. One of the causes of death enumerated in this report is "enteritis and other diarrheal diseases." Another is "bacillary dysentery and amebiasis." 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 estimate the role of specific viruses in overall mortality from diarrhea. On a worldwide scale, mortality data for 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. However, with the emergence of rotaviruses as a major cause of infantile diarrhea, it is generally assumed that this group of agents is of importance as a cause of mortality from diarrheal diseases in the developing countries. However, its relative importance in this regard in comparison to bacterial agents has not yet been ascertained.

3.2. Sources of Morbidity Data

Since the clinical manifestations of viral gastroenteritis are indistinguishable in individual cases 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. Recently, the National Institute of Allergy and Infectious Diseases initiated an enteric-diseases program that has as one of its aims the collection of gastroenteritis morbidity data from an epidemiological as well as an etiological view point. This program now includes studies in families, day-care centers, health-plan members, and other groups, and already some information has emerged on gastroenteritis morbidity associated with the new agents. With rare exceptions, such as the longitudinal study of infection with gastroenteric viruses in a Guatemalan village,[291] most of the data on morbidity associated with gastroenteritis viruses comes from cross-sectional hospital-based studies of infants and young children admitted for diarrheal illness and from studies of 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.

3.3. Serological Surveys

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.[100,127,131,286,302] Rotavirus serology has relied heavily on the complement-fixation (CF) and enzyme-linked immunosorbent assay (ELISA) techniques.[126,127,302] Until very recently, large-scale serological surveys could not be carried out with Norwalk virus, since the only assay available was immune electron microscopy (IEM); this technique was not practical for such studies, since 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) and a radioimmunoassay (RIA) has now made it possible to perform serological surveys with Norwalk virus.[104,131] Such surveys have not been carried out with other members of the Norwalk group, since the only available serological assay for them is still IEM.

3.4. Laboratory Methods

3.4.1. Norwalk Group of Viruses

a. 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. IEM entails the reaction of antibody (such as that present in the patient's convalescent serum or in pooled immune serum globulin) with virus in the patient's stool preparation.[128,136] 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 aggregating may occur. The presence of antibodies on the particle with or 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 below 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 subject in the outbreak, or a subject 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.[128,136]

An RIA for detection of the prototype strain of the Norwalk group of agents has recently been developed[99,104] that is even more efficient than IEM. The test is essentially a research tool, since suitable reagents are not generally available. An IAHA has also been developed for the Nowalk agent,[131] but is not efficient for its detection in clinical specimens. RIA and IAHA techniques are not available for the other members of the Norwalk group; thus, EM and IEM remain the only methods for their detection and IEM the only method for their identification.

The Norwalk group of agents has not been shown to produce illness in any experimental animal.[25,60,61,136,284,287] However, the Norwalk virus has been found to infect chimpanzees by the alimentary route as indicated by shedding of antigen and a serological response.[104,284]

b. 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 postillness serum specimen, and the amount of antibody coating the particle is scored on a 0–4+ scale.[128,129,135,136] 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. 2. The difference in the amount of antibody coating the Norwalk virus following its incubation with the volunteer's prechallenge serum and his 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-blocking test for measurement of Norwalk virus antibody has greatly facilitated epidemiological study of this agent.[100,101,102] This assay is as efficient as IEM for detecting a seroresponse but is much more practical, since it is much less time-consuming and also requires much less antigen and antibody. In addition, an IAHA for detection of Norwalk virus antibody has been developed.[131] It is not quite as efficient as either IEM or RIA, but is, of course, more practical than IEM. Both the RIA-blocking and IAHA techniques remain essentially research tools because of the paucity of appropriate Norwalk antigen. Neither an RIA-blocking nor an IAHA technique is available for other members of the Norwalk group of agents.

3.4.2. Rotavirus

a. Antigen Detection. Since the human rotavirus in clinical specimens does not grow readily in tissue culture or in small laboratory animals, it cannot be detected by conventional cultivation techniques.[286,288] Thus, in the early studies, EM was the

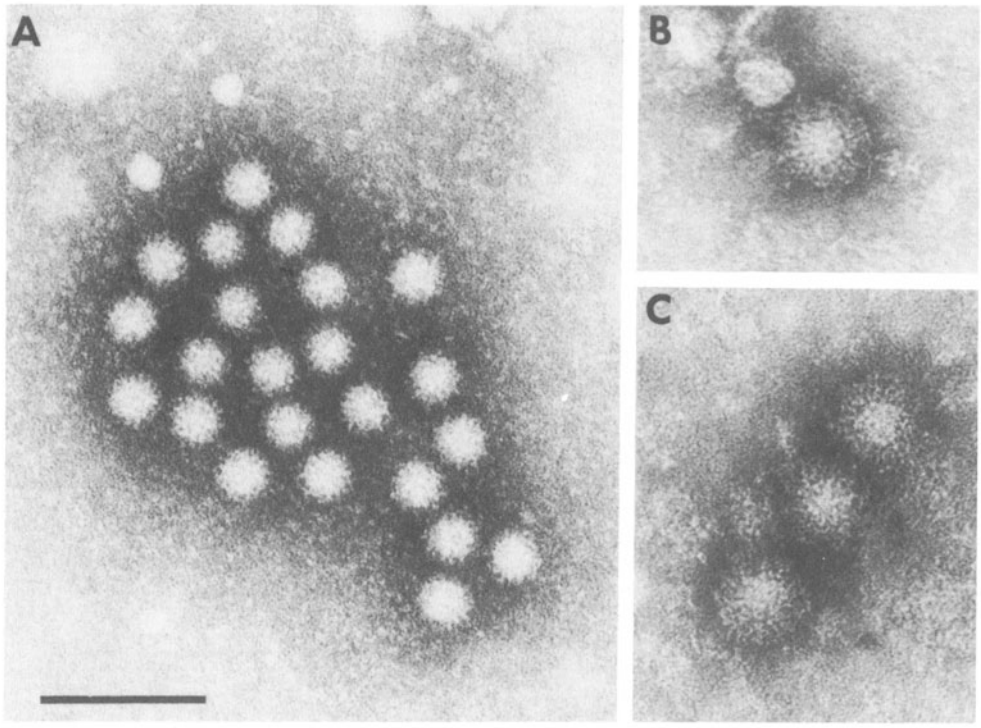


Fig. 2. (A) An aggregate observed after incubation of 0.8 ml of Norwalk (8FIIa) stool filtrate with 0.2 ml of a 1:5 dilution of a volunteer's prechallenge serum and further preparation for electron microscopy. This volunteer developed gastroenteritis following challenge with a second passage Norwalk filtrate which had been heated for 30 minutes at 60°C.[60] 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. The difference in the quantity of antibody coating the particles in the prechallenge and post challenge sera of this volunteer is clearly evident. The bar = 100 nm and applies to A, B, and C. From: Kapikian *et al.*[129]

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

Method	Efficiency[b]	Practicality for large-scale epidemiological studies (assuming 4+ efficiency)
Electron microscopy (EM)[19,83,134,182,202]	4+	1+
Immune electron microscopy (IEM)[133,134]	4+	1+
Complement-fixation (CF) (conventional)[95,141,182,228,240,265]	1+	4+
Human fetal intestinal organ culture [with immunofluorescence (IF)][288]	1+	0
Counterimmunoelectroosmophoresis[10,97,180,240,241,264]	3–4+	4+
Fluorescent virus precipitin test[91,199,299]	4+	1+
Cell culture (cytopathic effect)[186,283]	1+	2–3+
Cell culture (with IF)[6,186,204,283]	1+	1+
Cell culture (with EM)[6,204,283]	1+	1+
Centrifugation onto cell culture (with IF)[15,38,259,263]	3–4+	1+
Gel diffusion[278]	1+	4+
Smears (with IF)[274]	1+	4+
Radioimmunoassay (RIA)[24,53,119,179]	4+	3–4+
Enzyme-linked immunosorbent assay (ELISA)[294,296,300]	4+	4+
Immune adherence hemagglutination assay (IAHA)[164]	3+	2–3+
RNA electrophoresis patterns in gels[78]	3+	1+
Modified CF[304]	3–4+	2–3+
Enzyme-linked fluorescence assay (ELFA)[297]	4+	3+
Ultrasensitive enzymatic radioimmunoassay (USERIA)[110]	4+	3+
Solid-phase aggregation of coupled erythrocytes (SPACE)[31]	3–4+	3–4+

[a] From Kapikian *et al.*[136] with additions.
[b] On a scale of 1–4+, where 1+ indicates a low degree of efficiency or practicality and 4+ indicates a high degree of efficiency or practicality.

mainstay for detection of rotavirus in stool 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.[134] However, various simpler and more readily available methods for rotavirus detection have been developed as practical alternatives to EM for diagnosis and for research epidemiological 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 efficent, they are not practical, but nevertheless 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 ELISA, since it is practical and efficient and does not require sophisticated equipment. However, with this assay, all "positive" specimens detected by the conventional ELISA must be confirmed by an appropriate blocking test, or alternatively and more practically appropriate positive and negative sera should be employed in the initial detection system so that a confirmatory test is done at the time of virus detection.[136,296] Recently, a modification of this test called the enzyme-linked fluorescence assay (ELFA) was described as being even more sensitive than the conventional ELISA: a special substrate is employed that is examined for the degree of fluorescence (rather than for the degree of color change as in the conventional ELISA).[297] An even more sensitive assay designated as ultrasensitive enzymatic radioimmunoassay (USERIA) has also been described recently.[110] Another assay that has been described as being efficient for rotavirus detection—solid-phase aggregation of coupled erythrocytes (SPACE)—has recently been introduced.[31] The selection of technique will depend on the investigator's capabilities and experience and

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

Method	Efficiency[b]	Practicality for large-scale epidemiological studies (assuming 4+ efficiency)
Immune electron microscopy (IEM)[30,84,133,134,141]	4+	<1+
Complement-fixation (CF)[30,66,74,106,107,126,127,133,134]	3–4+	4+
Immunofluorescence (IF)[56,74,86,127,139,186,194,285,288]	4+	1+
Gel diffusion[278]	Not known	2+
Counterimmunoelectroosmophoresis[52,180]	Variable	2+
Neutralization of calf rotavirus in cell culture[86,108,124,127,262,278]	2+	1+
Radioimmunoassay (RIA)[24]	Not known	3+
Enzyme-linked immunosorbent assay (ELISA)[234,295,298,301,302]	4+	3–4+
Inhibition (neutralization) of fluorescent foci[38,74,257,259,285]	Not known	1+
Immune adherence hemagglutination assay (IAHA)[131,162,164]	3–4+	4+
Hemagglutination-inhibition (HI)[80,233,242]	2–3+	4+

[a] After Kapikian *et al.*[136] with certain reference changes.
[b] On a scale of 1–4+, where 1+ indicates a low degree of efficiency or practicality and 4+ indicates a high degree of efficiency or practicality.

the availability of appropriate reagents. Regardless of the methods employed, it is striking that almost all 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 recently.[136]

b. Serological Studies. Numerous assays have been described for detection of rotavirus antibody. Since the agent could not be grown in cell cultures, initial studies of serological responses relied on IEM, utilizing human rotavirus-positive stools as antigen.[134] 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.[126,127,134] 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 that animal strains could be used as substitute CF antigens for detection of infection with human rotavirus.[126,127] 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[158,169]; thus the quantity of CF antigen was virtually unlimited. Moreover, the recent efficient propagation of human rotavirus strain Wa (see Section 4.2) should make sufficient human rotavirus antigen available for this and other assays.[285] 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 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.[298,301] 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.[298] Thus, 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.[295,301] It is noteworthy that in most of the serological assays shown in Table 2 in which human rotavirus was used as antigen, the approach of "direct virology," which obviates *in vitro* cultivation of an agent, was quite successful.[136] Of course, a source of antigen such as particle-rich stools was essential for these serological assays.

4. Biological Characteristics

4.1. Norwalk Group of Viruses

This group of agents is comprised of several viruses that (1) were detected in the stool of patients with gastroenteritis, (2) have not been propagated *in vitro*, (3) share a morphological appearance similar to that of picorna- or parvoviruses and in certain preparations to the caliciviruses also, and (4) have a buoyant density in $CsCl_2$ of 1.37–1.41 g/cm^3.[9,11,130,135,136,256,287] The Norwalk virus represents the prototype strain of this group of agents.[135]

There are several other members of the group the origins and epidemic features of which are shown in Table 3 and described later in this section. The Norwalk and Hawaii viruses were shown to be distinct by cross-challenge and immune-electron-microscopic (IEM) studies, the Norwalk and Montgomery County to be related, and the relationship of Hawaii and Montgomery County to be inconclusive.[135,282] The Ditchling and W agents appear to be related but distinct from Norwalk and Hawaii by IEM.[9,129] The cockle agent is distinct from Norwalk virus, but its relationship to other agents is inconclusive.[11]

Some of the biochemical or biophysical characteristics of two of these viruses 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) and relatively heat-stable, and both the Norwalk and "W" viruses were ether-stable (20% ether at 4°C for 24 hr).[50,60]

This group has not yet been classified into any family of viruses. However, it has been suggested that the Norwalk virus was "parvoviruslike" since it shared certain characteristics with this group such as morphology by negative-stain EM, density in CsCl, and ether, acid, and relative heat stability.[60,135] This suggestion was quite tentative, since the nucleic acid content of the Norwalk virus as well as of the other agents in this group was not known. Recent studies of the polypeptides of Norwalk virus suggest that it may be caliciviruslike because a single primary virion-associated protein with molecular weight of 59,000 was detected; in addition, a single soluble protein with a molecular weight of 30,000 was also found.[102] Caliciviruses also have a single structural protein with a molecular weight of about 65,000[35,223b,224]; in addition, a small virion-associated protein with a molecular weight of about 15,000, as well as a nonstructural virus-associated protein with a molecular weight of 29,000 have been described.[35,223b]

As shown in Table 3, other potential members of this group include the Parramatta,[48] the Colorado (Snow Mountain)[185,193] and Marin County agents.[98,193] The Parramatta and Marin County agents were readily visualized by EM and IEM, respectively; only a few 27-nm particles were visualized in a filtrate of the Colorado agent. The density of each of these particles has not been described.

Evidence for the etiological 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[282]; serological evidence of infection has been demonstrated in most of a sample of volunteers who developed illness as well as in certain subjects in the original outbreak.[104,131,135] Further evidence of an etiological association was the demonstration that volunteers who developed illness after Norwalk challenge had a close temporal relationship between virus shedding and illness, with maximal shedding occurring at the onset of experimental illness.[255] Only short-term immunity characteristically occurs in volunteers who develop illness after initial challenge[60,197,282]; prechallenge serum antibody titers could not be correlated with susceptibility to illness.[197] Volunteers have also developed illness following administration of stool filtrates containing the Hawaii, Montgomery County, "W," and Colorado agents.[50,185,282] IEM seroresponses were observed in volunteers challenged with the Hawaii and Montgomery County agents.[135,256] Homologous seroresponses also occur in most individuals who develop illness associated with the Parramatta or Marin County agents.[48,193]

4.2. Rotaviruses

Human rotavirus has been detected in stools of about 30–60% of infants and young children hospitalized with acute gastroenteritis and much less often in older children and adults with this disease,[33,54,102,133,136,141] as well as in stools of numerous animals. Rotaviruses have been identified with a diarrheal illness in the calf,[169,174,276] infant mouse,[187] piglet,[148,168,210,254,277] foal,[85] lamb,[238] young rabbit,[39,203] monkey,[247] newborn deer,[268] newborn antelope,[205] young chimpanzee,[13] young go-

Table 3. Characteristics of Norwalk, Norwalklike, and Possibly Related Agents Associated with Acute Epidemic Nonbacterial Gastroenteritis in Humans

Agent	Size (nm)	Buoyant density in cesium chloride (g/cm^3)	Growth in cell culture	Administration[a] of agent induces illness in:		Particle detected by	Serological studies by	Antigenic relationships
				Humans	Animal(s)			
Norwalk[60,61,104,130,131,135,255,284]	27 × 32[b]	1.38–1.41	No	Yes	No	IEM[c]	IEM; RIA, AHA	Distinct
Hawaii[62,135,256,282]	26 × 29[b]	1.37–1.39	No	Yes	No	IEM	IEM	Distinct
Montgomery County[135,256,282]	27 × 32[b]	1.37–1.41	No	Yes	No	IEM	IEM	Related to Norwalk agent by IEM and cross-challenge studies.
Ditchling[9]	25–26	1.38–1.40	No	N.T.[d]	No	EM	IEM	Ditchling and "W" agents related to each other, but appear to be distinct from Norwalk and Hawaii agents by IEM.
"W"[9,50,129]	25–26	1.38–1.40	No	Yes	N.T.	EM	IEM	
Cockle[11]	25–26	1.40	No	N.T.	N.T.	EM	IEM	Appears to be distinct from Norwalk agent by IEM.
Parramatta[48]	23–26	N.T.	No	N.T.	N.T.	EM	IEM	Distinct from Norwalk agent by IEM.
Colorado (Snow Mountain)[98,185,193]	c	N.T.	No	Yes	N.T.	IEM	IEM	Distinct from Norwalk, Hawaii, and Marin County[e] agents by IEM or RIA or both.
Marin County[99a,193]	27	N.T.	No	Yes	No	IEM	IEM	Distinct from Norwalk, Hawaii, and Colorado[f] agents by IEM or RIA.

[a] By alimentary route. [b] Shortest × longest diameter. [c] Immune electron microscopy. [d] Not tested. [e] IEM and RIA tests with paired sera from a Colorado-agent-infected volunteer. [f] IEM test with paired sera from a Colorado-agent-infected volunteer.

rilla,[13] young turkey,[16,167] chicken,[116] young goat,[231] and young kitten[235]; pneumoenteric illness has been found in the newborn impala,[79] newborn addax,[74] and newborn gazelle.[79] The offal ("O") agent was derived from mixed intestinal washings from abattoir waste,[158] and a rotavirus has been derived from dogs with no known illness.[218] Of all these rotaviruses, human rotavirus Wa (discussed later in this Section), the calf, piglet, and monkey isolates, and the "O" agent from sheep and calf intestines have been successfully carried through two or more passages in cell cultures. Further descriptions of these animal rotaviruses have been recently reviewed.[286] With the exception of the SA-11 strain of simian rotavirus, canine rotavirus, and the "O" agent that was derived from mixed intestinal washings from an abattoir waste, 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, whereas the core has a diameter of about 37 nm.[75,88,114,159,196,200,231] 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 spokes radiating from a wide hub.[82,86] Rotaviruses resemble orbiviruses and reoviruses morphologically, but differ characteristically in their fine structure.[192,286]

The rotavirus genome is comprised of 11 segments of double-stranded RNA,[118,120,121,192,211,225,260] which distinguishes them from reoviruses and orbiviruses, both of which possess only 10 RNA segments.[89,286] The migration patterns of the RNA segments of rotaviruses as determined by polyacrylamide-gel electrophoresis are of importance not only in the biophysical characterization of these agents but also as epidemiological probes; they have served as one of the methods of differentiating human and animal rotaviruses as well as various human rotavirus strains.[118,120] The distinctive RNA migration pattern of rotaviruses has also been used for detection and identification of rotavirus strains from clinical specimens.[78] Rotaviruses contain eight to ten polypeptides, with five or six associated with the inner shell and three or possibly four with the outer shell of the double-shelled capsid.[191,192,211,212,260] Complete particles have a density of 1.36 g/cm^3 in $CsCl_2$ and a sedimentation coefficient of 520–530 S.[127,132,200,201,211,212,253] Particles that lack the double capsid (core particles) have a density of approximately 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.[75,211,253]

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.[165] This family now contains six genera: reovirus, orbivirus, rotavirus, phytoreovirus, Fijivirus, and an as yet unnamed group comprising the cytoplasmic polyhedrosis viruses.[165] Certain members of the first three infect humans, whereas the phytoreoviruses and Fijiviruses infect plants and the cytoplasmic polyhedrosis viruses infect insects.[165]

Antigenically, rotaviruses are distinct from reoviruses by complement-fixation (CF) and IEM and from those orbiviruses tested by CF.[56,88,119,126,127,134] By neutralization assay, there are at least 3 human rotavirus serotypes.[15a,88a,98,98a,281,285] Rotaviruses can also be separated into at least 2 distinct subgroups by IEM, specific CF, ELISA, and IAHA.[33,70a,90,118a,125a,215,302,303] It has recently been shown that the neutralization and the subgroup specifities are coded for by different genes.[118a] As noted earlier, the various animal and human rotaviruses are morphologically similar and possess common CF and fluorescent-antibody antigens[5,86,126,127,278]; however, by enzyme-linked immunosorbent assay (ELISA) blocking and by neutralization of immunofluorescent (IF) focus formation in cell culture, human rotavirus may be differentiated from various animal rotaviruses, and various animal rotaviruses may be distinguished from one another.[259,278,293]

The human rotaviruses are rather fastidious agents, and until very recently, not a single strain had been propagated efficiently in any cell- or organ-culture system. However, a few strains had been cultivated to a limited extent with only a small percentage of cells exhibiting evidence of infection.[6,79,283,288] Recently, human rotavirus Wa, a subgroup 2 virus was adapted to grow efficiently in primary African green monkey kidney (AGMK) cells following 11 serial

passages of a strain in gnotobiotic piglets.[285] Pretreatment of porcine-grown virus with trypsin was required for optimal growth of this strain in AGMK cells; low-speed centrifugation of the virus inoculum onto cell cultures was also employed. The availability of this cell-culture-adapted strain has implications for vaccine development, for the production of specific antigens for use in various immunological assays and for assessment of the relationships among rotaviruses by neutralization in tissue culture. In addition, recently 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.[98a] The cultivatable reassortants had mixed genotypes, but also had the neutralization specificity of human rotavirus. Also, efficient propagation of human rotaviruses in cell cultures has recently been reported.[65,223a]

Experimentally, human rotavirus induces a diarrheal illness in various newborn animals including gnotobiotic calves, gnotobiotic and conventional piglets, rhesus monkeys, and gnotobiotic lambs.[118,147,148,173,181,184,237,261,262,290]; subclinical infections also occur in newborn puppy dogs.[267] Particle-positive stools from calves have been an important source of human rotavirus for biophysical and serological studies.

Firm evidence exists for the association of rotavirus with gastrointestinal illness. The virus has been detected significantly more often in stools from patients 6–24 months old with gastroenteritis than in those without gastroenteritis[33,54] 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."[133] The virus is detectable predominantly during the acute phase of illness.[54] In addition, illness has been induced in volunteers with a stool filtrate containing human rotavirus D strain.[124] Serum antibody appeared to be associated with resistance.

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 general population are not available. Estimates of the importance of this group of agents are suggested from various sources. Infectious gastroenteritis was the second most common disease experience in the Cleveland Family Study over an approximate 10-year period.[58] and the Norwalk group was probably associated with at least some of these illnesses, especially in the adults. About one third of all outbreaks of nonbacterial gastroenteritis studied are associated with Norwalk virus infection.[101,102] It is probable that other members of the group are also responsible for a portion of epidemic gastroenteritis, but appropriate tests are not available to confirm this. 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 less than 2% of infants and children hospitalized with diarrhea at the Children's Hospital, Washington, D.C., a value not significantly different from that observed in controls,[33] nor was serological evidence of Norwalk virus infection detected in selected diarrhea patients from this study.[131]

Prevalence data are available for the Norwalk virus, since the development of both an immune adherence hemagglutination assay (IAHA) and a radioimmunoassay (RIA) has permitted the study of serum specimens from different age groups and from different locations. By IAHA, the acquisition of antibody to Norwalk virus and rotavirus was compared in infants and young children in the metropolitan Washington, D.C., area, young adults at the University of Maryland, and adults in the metropolitan Washington, D.C., area.[131] As shown in Fig. 3, 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 5th decade of life, 50% of the subjects 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.[107,250,251] 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 an-

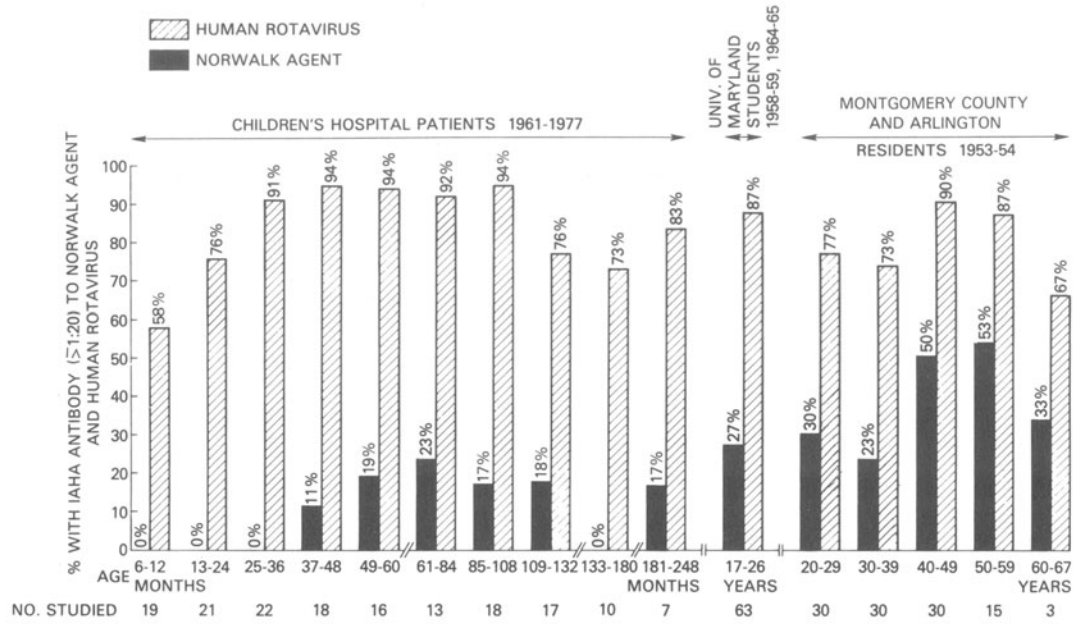


Fig. 3. Prevalence of antibody to Norwalk agent and rotavirus by IAHA in three groups. From Kapikian *et al.*[131]

tibody in a welfare institution for homeless but otherwise normal children yielded a pattern similar to that just described.[131] In a very limited 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.[131]

The prevalence of Norwalk antibody was studied in subjects from various parts of the world with the RIA-blocking assay.[100] As shown in Fig. 4, 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 subjects 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 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 adult blood donors in the United States studied by RIA or from adults studied by IAHA as described above.[100]

Children from the United States and to a lesser extent Yugoslavia acquired antibody more slowly than did children from less well developed countries such as Ecuador and Bangladesh (Fig. 5).[100] The high antibody prevalence in the pediatric age group in Bangladesh and Ecuador was unexpected and indicates that the Norwalk or an antigenically related virus infects early in life in at least these parts of these less developed countries; its importance as an etiological agent of clinical gastroenteritis in this age group remains to be determined. Prevalence data are not available for the other members of the Norwalk group, since suitable serological assays have yet to be developed.

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. Although the term "winter vomiting disease" has been applied to certain outbreaks of epidemic viral gastroenteritis, a clear-cut

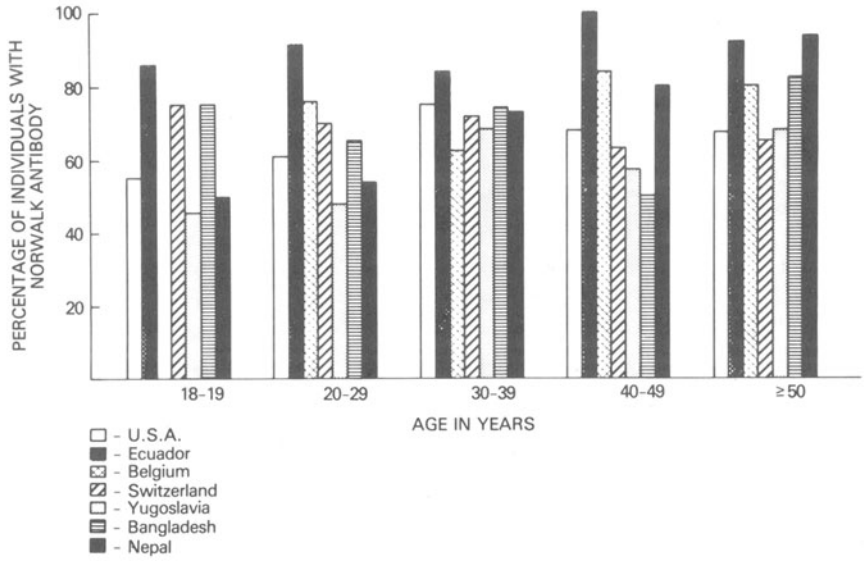


Fig. 4. Prevalence by age of serum antibody to Norwalk virus in healthy adults from various parts of the world. Note that no specimens were tested in the 18- to 19-year age group from Belgium. From Greenberg *et al.*[100]

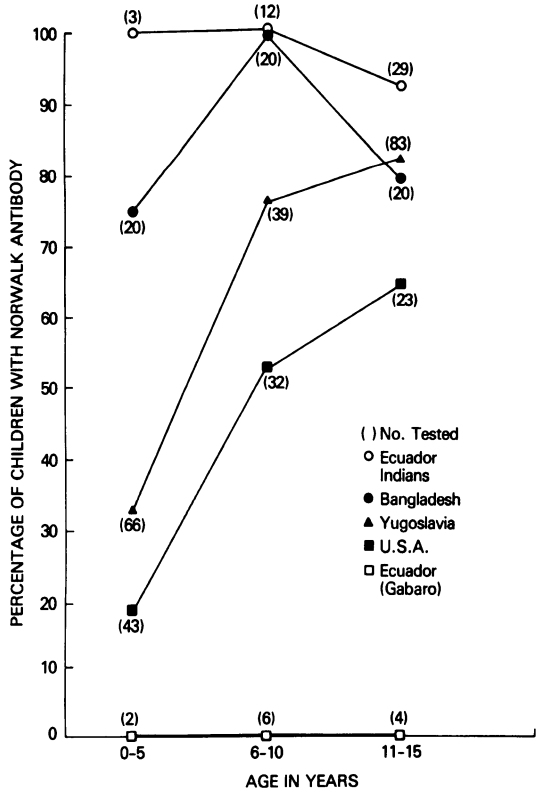


Fig. 5. Age-related prevalence of serum antibody to Norwalk virus in children from various countries. From Greenberg *et al.*[100]

seasonality does not appear to occur, at least for Norwalk virus-associated outbreaks.[2,101,102,302a]

A summary of several outbreaks of gastroenteritis associated with the Norwalk group of viruses (Table 4) indicates primary attack rates of 24–55% and secondary household attack rates of 11–32%. Some outbreaks have been brief, others extended over several months. The incubation period, where measured, was short, with an average of 48 hr in secondary cases in the Norwalk, Ohio, outbreak. The Hawaii and Montgomery County agents were obtained in family outbreaks.[256,282] A "cockle" agent derives its name from two outbreaks of gastroenteritis in England that occurred 24–30 hr after the ingestion of cockles.[11] In Australia, a large outbreak, in which a portion of the cases was associated with Norwalk agent, occurred in the winter months of June–July 1978 in over 2000 persons after ingestion of oysters.[102,105,189]

In a systematic study of selected paired sera from 23 outbreaks of nonbacterial gastroenteritis over a 12-year period employing the RIA-blocking assay, 6 outbreaks appeared related to Norwalk virus.[102] The outbreaks involved two cruise ships, two groups of college students (one affecting over 1000 students), a family, and a primary school in Japan in which over 100 students were ill with an attack rate of 40%. If the Norwalk, Hawaii, and Montgomery County family outbreaks studied by immune electron microscopy[135,256] are added to the 23 studied by RIA, then 8 of 26, or 31%, were Norwalk-virus-associated. Recent serological studies by RIA, including the 23 cited above, incriminated Norwalk agent in 24 of 70 outbreaks (34%).[102] Of the 24 outbreaks, 4 occurred in each of four settings: recreational camps, cruise ships, contaminated drinking or swimming water, and a community or family; 3 involved elementary or college students; 2, nursing homes; 1, shellfish; and 2 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; however, it is thus possible that a majority might have a recognizable etiology when satisfactory serological as-

Table 4. Summary of Outbreaks of Norwalk Group Gastroenteritis

Agent	Group involved	Number at risk	Attack rate (%)	Dates of outbreak	Other information
Norwalk[2]	Students and teachers in elementary school, Norwalk, Ohio	232	50	Oct. 30–31, 1968	Incubation period 12–24 hr Secondary attack rate in families 32%; with an average incubation period 48 hr
"W"[50]	Students in boys' boarding school, England	830	24	Mar. 14–22, 1963	Only 1 teacher and kitchen staff ill. Attack rate: higher in younger boys than in older boys
Ditchling[9]	Students in primary school, Ditchling, England	138	24	Oct. 3–7, 1975	Related to "W" agent
Parramatta[48]	Students and teachers in primary school in Sydney, Australia	381	54	July 18–Aug. 18, 1977	9 of 18 teachers ill
Marin County[193]	Elderly residents and employees of convalescent home in Marin, California	187	51	Mar.–May, 1978	7% of employees also ill
Colorado (Snow Mountain)[185]	Persons at a resort camp in Colorado	760	55	Dec. 1976	Contaminated water supply suspected; secondary attack rate in households 11%

says are available for the other members or possible members of the Norwalk group of agents.

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, and Australia.[100–102, 104,105,131] The only population studied that lacked detectable antibody was the very isolated Gabaro Ecuadorian Indians, who also lacked antibody to hepatis B virus (anti-HBc negative).[100] In contrast, they were found to have serum antibody to rotavirus, respiratory syncytial virus, and hepatitis A virus.[100]

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 have revealed that 9 of 24 Norwalk outbreaks occurred during the warmer months of the year.[102] The temporal distribution in tropical countries is not known.

5.1.5. Age. In developed countries, the Norwalk agents appear to induce illness in all age groups. During outbreaks, the peak incidence is observed in school-aged children and adults who are in close contact in various group settings.[102] However, close contact is not always essential, since outbreaks have occurred after ingestion of contaminated water or seafood.[11,102,105,185,189] In the United States, antibody-prevalence data indicate that the Norwalk virus is not an important cause of gastroenteritis in infants and young children,[131] nor has it played an important role in diarrheal illness of early life serious enough to require hospitalization.[33,131,133] Its overall importance in the developing countries is not known, but in parts of Bangladesh and Ecuador, Norwalk virus infection is common in infants and young children, suggesting that it may cause gastroenteritis in this age group.[100] Further studies are needed to answer this question.

5.1.6. Sex, Race, and 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 epidemiological 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.

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 Bangladesh and in certain groups in Ecuador in comparison to that in developed countries may reflect a role of crowding or other socioeconomic factors in facilitating the spread of this agent.[100]

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.[197] These 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 etiological 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.[68–70,73] The illness rate among family contacts of patients with rotavirus gastroenteritis is low, although subclinical infections in contacts occur frequently.[133,139,252,266] In the metropolitan Washington, D.C. area, the pattern of acquisition of rotavirus antibody contrasted sharply to that of the Norwalk agent.[131] By the end of the 3rd 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.[131,138a,198] 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.[74,106,115,127,302] In the metropolitan Washington, D.C., area study, acquisition of antibody to both rotavirus serotypes followed a

similar pattern, so that by the 3rd year of life, over 90% of infants and young children tested had antibody to type 1 and type 2 rotaviruses.[302]

In a cross-sectional study of patients admitted with a diarrheal illness to Children's Hospital National Medical Center in Washington, D.C., from January 1974 to June 1978, 39% of 604 patients shed rotavirus in a stool or rectal swab specimen (Fig. 7).[33] The major role of rotaviruses in diarrheal illnesses requiring hospitalization has also been observed in many other countries of the world, including Australia, Canada, England, and Japan.[37,54,142,182,183] For example, in an Australian study that embraced a period of 1 year, 52% of 378 patients admitted with gastroenteritis shed rotavirus.[54] In a Japanese study that covered three peaks of rotavirus prevalance, December 1974 through March 1977, rotavirus was detected in stools of 320 (63%) of 506 infants and young children.[142] 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. In the Children's Hospital, Washington, D.C., study, each of the 313 rotavirus strains was subgrouped by ELISA: 238 (76%) were subgroup 2, and 75 (24%) were subgroup 1.[33,302] The greater frequency of subgroup 2 infections in hospitalized patients may indicate that serotypes in this subgroup have a greater pathogenic potential than those of subgroup 1. Further evidence to support this view was observed in a longitudinal study of Guatemalan infants and young children studied during the first 3 years of life. Each of 16 infections with a subgroup 2 virus was associated with diarrheal illness, whereas only 6 of 11 subgroup 1 infections were associated with such disease.[291] Sequential infections with human rotavirus have been documented in 9 infants and young children: 8 of the 9 shed a different subgroup virus during each of the sequential infections, and all but 1 of the 8 developed a diarrheal illness during the second infection, suggesting that infection with one subgroup does not protect against illness associated with the other subgroup.[302] Neutralization assays with specific serotypes within each subgroup are needed to clarify the implications of such sequential infections.

The relative role of rotaviruses and bacterial agents in the etiology of gastroenteritis was recently summarized for 6352 patients for a 1-year period, February 1978–January 1979, at the Matlab Treatment Center in Bangladesh.[22,102] This study showed

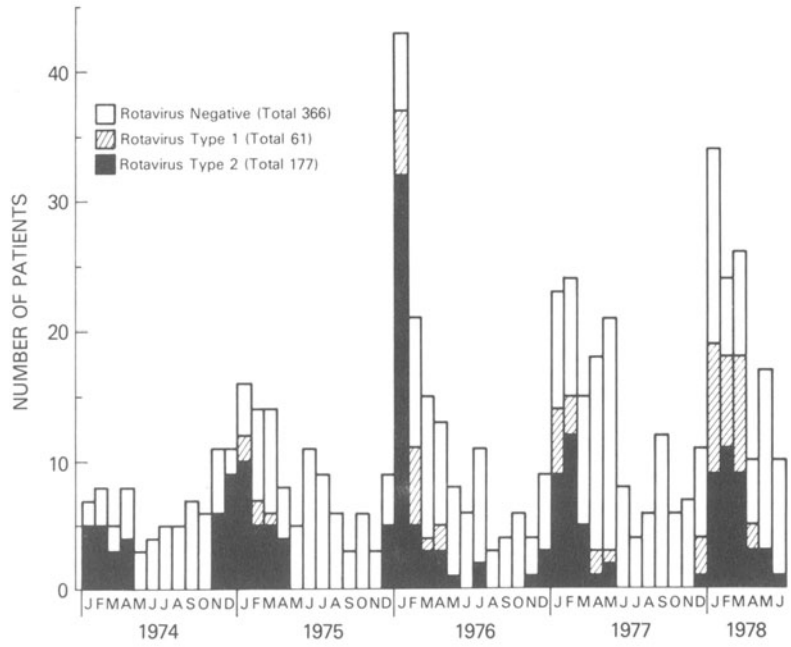


Fig. 6. Temporal distribution of rotavirus subgroup 1 and subgroup 2 infections in 604 infants and young children hospitalized with gastroenteritis at Children's Hospital National Medical Center, Washington, D.C., Jan. 1974(partial)–June 1978. From Brandt *et al.*[33]

that 46% of the patients under 2 years of age shed rotavirus and that 28% shed toxigenic *Escherichia coli*. In the 2-year-and-over age group, bacterial agents were detected more frequently than rotaviruses.

Although rotaviruses have been implicated during cross-sectional studies as a major cause of gastroenteritis necessitating hospitalization of infants and young children, rates of hospitalization were not available, since it was not possible to define accurately the population base from which the hospitalized patients had come. Recently, however, the incidence of hospitalization was estimated in the Washington, D.C., area.[214] Medical care for the population under study was provided by Group Health Association, Inc., and 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. 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 year or part of a year. Of the 38 children hospitalized, 31 were under 2 years old. Of 30 studied microbiologically, 19 (63%) shed rotavirus, and 1 who did not, developed serological evidence of infection. From this analysis, it was calculated that 1 in 272 (3.7/1000) subjects less than 1 year of age and 1 in 451 (2.2/1000) 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 $2\frac{1}{4}$-year period. 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, 1 of every 7.8 infants. The incidence declined sharply in the 25-month-to-15-year age group.[214] Longitudinal studies that are in progress should provide additional data, including the incidence of subclinical rotavirus infections that do not require a visit to a clinic.

An incidence of about 1.1 rotavirus diarrheal episodes per child during the first 3 years of life was estimated in a group of 24 infants and young children residing in the Guatemalan highland village of Santa Maria Cauque[291] on the basis of selected ciated with bacterial and parasitic pathogens (about 42%) and those from whom stools were not available for study (about 5%). The true incidence may thus actually be greater than this estimate. It should be noted that specimens were not examined for enterotoxigenic *E. coli*.

5.2.2. Epidemic Behavior. Unlike the situation with the Norwalk group, true outbreaks of rotavirus gastroenteritis are rare. In the temperate climates, rotavirus infections have characteristically demonstrated a consistent temporal pattern similar to that observed in studies at Children's Hospital, Washington, D.C., during 1974–1978 (Fig. 6),[33,37,54,133,142,182] which was characterized by a large number of hospitalizations in infants and young children for gastroenteritis during the cooler months of each year.[33] Rotaviruses were 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. Community outbreaks of rotavirus illness occur rarely if at all, 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.[133,139,252,266] In one study, 22 (55%) of 40 adult contacts of patients hospitalized with gastroenteritis which 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.[139] Only 3 of the 26 adult contacts with rotavirus infection gave a history of an associated gastrointestinal 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.[71,139,252,266]

Although community outbreaks of rotavirus gastroenteritis are rare, one unusual outbreak involving not only children and mothers in a playgroup but also fathers and grandparents was recently described[216]: all 9 children 15 months through 5 years of age, 3 of 5 mothers who shared playgroup activities, 4 of 5 fathers, and each of 2 grandparents developed gastroenteritis. The incubation period was

24–48 hr. The index cases were most likely nonplaygroup siblings who had been cared for (about 48 hr prior to the playgroup meeting) by the mother at whose home the playgroup met. The suspected index cases had gastroenteritis, the mother who took care of the index cases developed diarrhea 24 hr before the playgroup met, and her daughter had onset of diarrhea just before the playgroup met, and vomited during the playgroup 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 for both. This unusual outbreak further attests to the contagiousness of rotavirus infection.

5.2.3. Geographic Distribution. Rotavirus infection has been detected in virtually all parts of the world. In developed countries where the etiology of gastroenteritis of infants and young children has been studied, rotaviruses have emerged as the major etiological agents of diarrheal illnesses severe enough to warrant hospitalization. In each locality, the pattern was similar to that described for Washington, D.C.[68–70,73] In less developed countries, such as Bangladesh and Guatemala, rotaviruses have been shown to be important etiological agents of severe diarrheal illness in infants and young children[111,157,220,291]; however, it appears that toxigenic *E. coli* also play an important role in underdeveloped countries such as Bangladesh where intensive etiological studies have been pursued.[22,67,102,149] Further studies must be carried out in the developing countries to determine the relative importance of the rotavirus and toxigenic *E. coli*. In practically every area of the world studied, rotaviruses have exhibited an important role in acute gastrointestinal disease of the young.

5.2.4. 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. The pattern for Washington, D.C., is shown in Fig. 6[5,33,37,54,133,141,182]: during five Januarys, from 1974 (partial) through 1978, 87 (71%) of 123 patients admitted for diarrheal illness were found to be shedding rotavirus;[33] similarly, during three Januarys, from 1976 to 1978, 21 (62%) of 34 gastroenteritis outpatients were rotavirus-positive.[33] In Japanese studies, rotavirus was detected in stools of 288 (79%) of 365 hospitalized gastroenteritis patients during the cooler months (December to March) in a 28-month study.[142] The reason for this striking seasonal pattern of infection is not known.

The pattern of infection with rotavirus strains belonging to subgroups 1 or 2 from 1974 to 1978 in the Children's Hospital, Washington, D.C., study is also shown in Fig. 6.[33] Viruses in subgroup 2 were first observed in January 1974 and during each succeeding yearly winter peak; 75% of the rotavirus strains detected over the 4½-year period belonged to subgroup 2. Strains belonging to subgroup 1 rotavirus were first observed in January 1975 and then detected yearly thereafter in December or January. Although this subgroup represented only about 25% of the 238 strains detected over the 4½-year period, a marked fluctuation in the temporal distribution of rotavirus subgroups was observed. For example, whereas subgroup 1 strains accounted for 14% of the total from October 1974 to September 1976, in the last epidemic year reported, subgroup 1 strains were identified in 31 (46%) of the 68 rotavirus-positive patients studied. Also, clustering of subgroups was observed in shorter periods within some of the seasons. The distribution of subgroups in gastroenteritis outpatients was similar to that observed for inpatients. The overall distribution of subgroups in populations in Guatemala, Costa Rica, Belgium, England, Australia, Asia, and Africa was also similar to that in the Washington, D.C., study in that rotavirus subgroup 2 strains predominated over subgroup 1 strains, ranging from a low of 62% (16 of 26) of the strains in Guatemala to a high of 100% in Costa Rica (10 of 10) and England (25 of 25).[302] It will be important to ascertain the pattern of infection of specific serotypes (as determined by neutralization) within each subgroup. Such studies have not been feasible until very recently because of the fastidious nature of rotaviruses.

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, and in all seasons in a newborn nursery in Australia.[14,66,111,157,160,188,227,228,246,263,270] In both the 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 have led to variable results.[111,160,270]

5.2.5. Age. In studies from various parts of the world, infants and young children, characteristically from about 6 months to 2 years of age, experience the highest frequency of rotavirus gastroenteritis that requires hospitalization[33,37,54,142]; infants under 6 months of age have the next highest frequency. An unexplained paradox in the epidemiology of rotavirus infection is the low rate of clinical illness in neonates who shed rotavirus.[14,188,263] In one study, breast-fed infants shed rotavirus significantly less often than those who were not breast fed; however, the effect of breast feeding on illness could not be determined, since most of the rotavirus infections in both the breast-fed and bottle-fed neonates were subclinical.[263]

Rotavirus strains in subgroups 1 and 2 were found in the Children's Hospital (D.C.) study to have quite different patterns of infection in relation to age: the number of subgroup 1 infections declined with increasing age during the first year of life and was quite low thereafter, whereas the number of subgroup 2 infections peaked at 10–12 months and gradually declined thereafter.[33] The percentage of gastroenteritis patients who had infections with subgroup 1 viruses showed relatively little variation with increasing age, whereas the percentage with infections with subgroup 2 viruses increased steadily, with a peak at 13–15 months of age. The largest number of rotavirus infections with either subgroup 1 or subgroup 2 viruses was observed in the 10- to 12-month age group, and the group with the greatest percentage of rotavirus infections was 13–15 months of age. 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.[28–30,108,109,133,139,176,195,266,305]

5.2.6. Sex. A somewhat larger number of males (335) than females (269) (M/F = 1.2) was hospitalized for gastroenteritis, irrespective of age, in the Children's Hospital of Washington, D.C., study.[33] This was also reflected in the number of males or females positive for each of the rotavirus serotypes, but this difference was not striking.[33] A higher frequency of hospitalization of males for acute gastroenteritis was also observed in a Canadian study.[183]

5.2.7. 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.[33] This difference was reflected in hospitalizations for gastroenteritis associated with either subgroup 1 or subgroup 2 rotavirus, since black patients were about 6 months younger than nonblacks with respect to each subgroup. The median age of black vs. nonblack patients for rotavirus subgroup 1 was 5 months vs. 11.5 months; for subgroup 2 rotavirus, it was 8 months vs. 14 months. Also, D.C. residents and Medicaid recipients who were hospitalized with rotavirus infection tended to be younger than non-D.C. residents and non-Medicaid recipients. In addition, there was a tendency for rotavirus illness to occur earlier in the course of the outbreak in blacks and in D.C. residents. Transmission of rotavirus might be facilitated by crowding and poor sanitation, and this may explain the earlier appearance of rotavirus infection in D.C. when compared to the suburbs, in blacks as compared to nonblacks, and in Medicaid recipients as compared to non-Medicaid recipients.[33]

Malnutrition is probably an important factor in increasing the susceptibility of an infant or young child to develop severe clinical manifestations following rotavirus infection. In addition, 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.[161]

5.2.8. Occurrence in Different Settings. Rotavirus gastroenteritis occurs predominantly in infants and young children; rotavirus infection has been observed by the 36th month of age in almost all children studied who were residing in a family setting.[26,74,106,131] Family contacts are also frequently infected with rotavirus, but usually subclinically.[133,139,252,266] Rotavirus infections have also been observed for extended periods in newborn nurseries as described above.[5,14,263] In addition, nosocomial rotavirus infections occur commonly.[183,219] In one study, 10 (17%) of the 60 children admitted to the hospital without diarrhea (but during a period of rotavirus prevalence) de-

veloped diarrheal illness associated with rotavirus infection while hospitalized.[219] In another hospital study, over a 1-year period, about 1 of every 5 rotavirus infections appeared to be hospital-acquired.[183] Outbreaks of rotavirus gastroenteritis have been observed in school-aged children, in a home playgroup setting, and in a military group, but characteristically, rotavirus illness occurs sporadically and not in widespread community outbreaks as does the Norwalk group of agents.[29,30,108,109,176,216] Thus, rotavirus illness is not common beyond the first few years of life.

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, "W," and Colorado agents can be transmitted via the oral route, i.e., following the ingestion of stool material containing the infectious agent.[60,61,150,185,282] It is unlikely that this group of agents is transmitted by the respiratory route. In one study, nasopharyngeal washings from a volunteer acutely ill with experimentally induced Norwalk illness failed to induce illness in three volunteers.[60] Recently, Norwalk virus has been detected in vomitus from certain infected volunteers.[103]

The explosive nature of some of these outbreaks in which large numbers of people 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 the Colorado outbreak, 61% of the 418 cases had onset of illness on a single day.[185] Epidemiological 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 thus 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 during a 2-day period.[2] Although a common-source exposure was sought, none could 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 cockles (cockle agent) and oysters (Norwalk agent) has also been described as a mode of transmission of this group of agents.[11,72,105,189] In addition, outbreaks of Norwalk gastroenteritis have now been associated with ingestion of contaminated drinking water and with swimming in a contaminated lake.[43,44,102]

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.[124] The rapid acquisition of rotavirus antibody in the first few years of life in all populations studied has led to the suggestion that rotaviruses might also be transmitted by the respiratory route.[26,74,106,115,127,131] No experimental evidence for this exists. Throat gargles obtained from volunteers with an experimentally induced rotavirus diarrheal illness failed to yield rotavirus.[124] Although the possibility of common-source exposure to rotavirus, such as a contaminated water supply, has been suggested, it is unlikely that such exposure plays an important role in its transmission.

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.[133,139,252,266] 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 due in part 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.[84] It is likely that human rotavirus is also quite stable and may remain viable in the environ-

ment unless destroyed by careful disinfection. The persistence of rotavirus infections in certain newborn nurseries and the high frequency of nosocomial rotavirus infection in hospitals provide additional evidence for this possibility. Effective disinfection of contaminated material and care in hand-washing may be important measures in containing rotavirus infection, especially in a hospital setting.[139,219]

The role, if any, of animals in transmitting rotaviruses to humans is not known. Although rotaviruses are established causes of diarrhea in newborn animals of many species, there is no evidence of transmission of an animal rotavirus to humans. On the other hand, human rotavirus has been shown to induce a diarrheal illness in various newborn animals under experimental conditions.[118,147,148,173,181,184,237,261,262,290] It appears unlikely, however, that even if animal-to-human transmission of rotavirus could be documented, such transmission would account for an appreciable number of infections.

7. Pathogenesis and Immunity

7.1. Norwalk Group of Viruses

7.1.1. Incubation Period. From studies of outbreaks associated with this group of agents, the incubation period is estimated to be 24–48 hr.[2,9,11,185] The incubation period in volunteer studies with the Norwalk agent ranged from 10 to 51 hr, and the illness usually lasted less than 48 hr.[25,60,61,282] Norwalk virus shedding as determined by immune electron microscopy coincided with the onset of illness and usually could not be detected after 72 hr following onset.[255]

7.1.2. Pathogenesis. The pathogenesis of Norwalk- and Hawaii-induced illness studied in volunteers by light microscopy of biopsies of the proximal small intestine has characteristically revealed broadening and blunting of villi, with mucosa itself being intact histologically; mononuclear cell infiltration and cytoplasmic vacuolization were also observed.[4,62,229,230] Transmission electron microscopy of the proximal small intestine showed intact epithelial cells with shortening of microvilli.[4,62,229,230] 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 of normal volunteers challenged with the Norwalk agent.[273] Brush-border small-intestinal enzyme levels (including alkaline phosphatase, sucrase, and trehalase) were decreased during illness; adenylate cyclase activity was not elevated.[4,81,150,222] Recently, it was found that volunteers with Norwalk-induced gastroenteritis or the characteristic small-intestinal pathological changes, or both, experienced marked delays in gastric emptying.[175]

7.1.3. Immunity. Volunteer studies with the Norwalk agent have raised rather perplexing questions about the mechanism of immunity to the Norwalk agent. It appears that two forms of clinical immunity to Norwalk-virus-induced illness exist—one with a short-term and the other with a long-term immunity.[60,197,282] 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 later resistant to challenge with Norwalk virus.[60,282]

The situation with regard to long-term immunity is different, as indicated when 12 volunteers were challenged with the Norwalk agent on two separate occasions 27–42 months apart[197] and 4 were rechallenged again 4–8 weeks after the second challenge. Of these 12 volunteers, 6 developed illness following challenge and again after rechallenge 27–42 months later. In contrast, 6 of the other volunteers failed to become ill after initial challenge or after rechallenge 31–34 months later. Of the 6 volunteers who developed illness after each of the two sequential challenges, 4 were inoculated a third time with the same inoculum, but only 1 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, it seems that serum antibody is not a critical factor in immunity to Norwalk gastroenteritis. It is also 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 subjects,

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.[197]

Further evidence for the possible role of nonimmunological factors in resistance to Norwalk illness was observed when the prechallenge serum and local jejunal antibody levels in 23 volunteers were studied by the radioimmunoassay (RIA)-blocking technique.[102] 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 titer of serum tended to be greater in volunteers who became ill after challenge than in those who did not become ill.[102] 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 measured also by RIA.[23]

7.2. Rotaviruses

7.2.1. Incubation Period. From clinical studies, rotavirus diarrheal illness has been estimated as having an incubation period of less than 48 hr.[54] 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 2 to 4 days. Virus shedding began the 2nd, 3rd, or 4th day after inoculation and lasted a total of at least 6 days.[124]

7.2.2. Pathogenesis. Limited studies of biopsies from the proximal small intestine of a few infants and children hospitalized with rotavirus infections have shown shortening of the villi, mononuclear-cell infiltration in the lamina propria, distended cisternae of the endoplasmic reticulum, mitochondrial swelling, and sparse, irregular microvilli.[114,248] Impaired D-xylose absorption was also observed.[166] In addition, some patients had depressed disaccharidase levels (maltase, sucrase, and lactase).[21]

The pathogenesis of human rotavirus D strain, a subgroup 2 virus, infection was studied experimentally in newborn gnotobiotic colostrum-deprived calves that developed illness following intraduodenal administration of this virus.[172] Morphological changes in the small intestine proceeded in a cephalocaudad direction: within $\frac{1}{2}$ 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 immunofluorescence [fluorescent-antibody (FA) test]; at this time, the lower small intestine was intact, but abundant rotaviral antigens were observed by FA test in swollen epithelial cells. 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 could not be detected by FA test. 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. In contrast, adenylate cyclase and cyclic AMP were not stimulated.[55,93]

7.2.3. Immunity. Epidemiological observations, as well as experimental studies in animals and humans, have helped in understanding certain mechanisms involved in rotavirus immunity.[34,124,148,171,237,239,279] The observation was made that newborn calves frequently develop rotavirus diarrhea despite a high level of circulating rotavirus antibody acquired from ingestion of colostrum.[279] 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.[34,279]

A similar study in gnotobiotic lambs also examined the relative role of local and systemic rotavirus antibody by evaluating the clinical response of two groups of lambs to challenge with lamb rotavirus.[237,238,239] From these and other studies in animals, it appears that antibody in the lumen of the intestine was of prime importance in resistance to rotavirus illness in animals.[148,179]

The mechanism of immunity was also studied in 18 volunteers who were administered a human ro-

tavirus by the oral route.[124] Of these 18, 5 shed rotavirus, and 4 of these 5 developed a diarrheal illness. Examination of the relationship of prechallenge rotavirus antibody measured by immunofluorescence (IF), neutralization (to bovine rotavirus), complement-fixation, and to the development of diarrheal illness revealed that the absence of IF antibody was significantly associated with the development of illness, whereas a similar trend was observed with antibodies measured by other assays. The role of local intestinal rotavirus antibody 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.

Reinfections with rotavirus occur commonly in adult contacts of patients with rotavirus illness; however, most of these reinfections are subclinical.[133,139,252,266] Whether those that are manifested clinically are the result of a low level or absence of local intestinal rotavirus IgA antibody is not known. Sequential rotavirus illnesses have been observed in infants and young children.[90,215,291,302] However, such sequential illnesses have thus far been associated with strains belonging to different subgroups, suggesting that immunity does develop. The duration of such immunity, however, is not known. However, neutralization assays with specific serotypes within each subgroup are needed to clarify immune mechanisms in rotavirus infections.

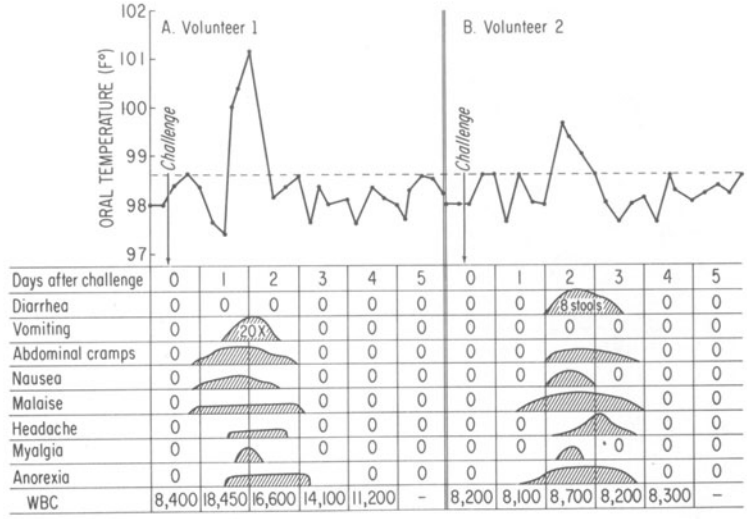
One of the perplexing areas in the study of rotavirus epidemiology has been the unexplained relative sparing of neonates from rotavirus illness despite frequent infection in this age group.[5,14,263] In one study of newborn babies, rotavirus infections occurred significantly less often in breast-fed infants than in bottle-fed infants. 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.[14,263] 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.[33,133]

8. Patterns of Host Response

8.1. Norwalk Group of Viruses

8.1.1. Clinical Features. Clinical features observed in the original Norwalk outbreak from which the Norwalk particle was derived are characteristic of those observed with this group of agents. Of the 604 subjects tabulated as 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 clinical manifestations was 12–24 hr; none of the affected subjects was hospitalized. These clinical findings are similar but not identical to those observed in a report describing 31 of 52 volunteers who developed definite or probable illness following administration of the Norwalk agent.[282] Of the 31 volunteers, 45% had fever ($\geq$ 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.[59] Of 16 volunteers who became ill following Norwalk- or Hawaii-agent challenge, 14 developed a transient lymphopenia.[63] 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.[25,60,61,282] 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. 7.[61] 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 immune electron microscopy (IEM) was maximal around the onset of illness and was rarely detected after 3 days following onset.[255] A valid estimate of the ratio of subclinical to clinical Norwalk infections has not been made. However, serologically proven infection without definite gastroenteric illness has been observed.[123,245]

8.1.2. Diagnosis. A specific diagnosis of infection with the Norwalk group is not possible on the basis of a patient's clinical manifestations. Thus, since this group of agents does not grow in cell culture or produce disease in an experimental ani-



Days after challenge	0	1	2	3	4	5	0	1	2	3	4	5
Diarrhea	0	0	0	0	0	0	0	0	8 stools		0	0
Vomiting	0	20 X		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	–

Fig. 7. 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 inoculum. From Dolin *et al.*[61]

mal, diagnosis of infection remains essentially a research procedure. For virus detection and identification, IEM remains the mainstay procedure for the group as a whole[128,129,135,136]; however, for the Norwalk virus, a recently developed radioimmunoassay (RIA) has been shown to be even more sensitive than IEM for detection of Norwalk antigen in clinical specimens.[99,104] Direct EM examination of negatively stained stool material with or without prior concentration may also be attempted.[32] Although EM examination of stools is a simple and relatively rapid procedure, caution must be used in interpreting the significance of "particles" visualized, since stools contain a myriad of small objects that have no relationship to the illness being studied.[128] 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 observed. Ideally, a patient's paired sera should show an IEM antibody increase with the particle that has been visualized being used as the antigen (see Fig. 2); in addition, if paired sera are not available, careful IEM studies with γ-globulin, paired sera from other subjects in the same outbreak or from other 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, demonstrate aggregation without the addition of serum. These nonspecific aggregates may appear to be coated with a small or moderate amount of antibody. Thus, it is essential to determine the amount of antibody coating the particles even if they are aggregated. If there is any question about the significance of aggregation, the concentration of antigen or antibody may 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.[128,136] Awareness of specificity of aggregation is essential, since certain stools contain groups of 22-nm particles that characteristically appear in "aggregate" form with little or no "antibody" on them and that have had no known relationship to the illness being studied.[128,136] These aggregates generally appear similar with paired acute or convalescent sera. IEM remains the only method for detecting serological evidence of infection for other members of the group not antigenically related to the Norwalk virus. Serological diagnosis of Norwalk infection can of course be made by IEM, but recently a practical RIA-blocking test has been developed for this agent.[23,104] The development of an RIA-blocking test has permitted the study of a large number of serum specimens from outbreaks in different localities and thus paved the way to an understanding

of the epidemiology of this virus.[102,104] An immune adherence hemagglutination assay has also proved useful for detecting serological evidence of Norwalk infection, but it is not quite as sensitive as RIA and also uses larger amounts of antigen.[131] Although practical, these assays are limited to use in research laboratories, since reagents are not generally available.

8.2. Rotaviruses

8.2.1. Clinical Features. Clinical characterization of rotavirus illness in infants and young children has been heavily weighted toward disease severe enough to warrant hospitalization. The three major clinical manifestations observed in rotavirus gastroenteritis in such studies are vomiting, diarrhea, and dehydration. A comparison was made of signs and symptoms observed in 72 patients hospitalized with a diarrheal illness associated with rotavirus and 78 patients hospitalized with a diarrheal illness that could not be associated with rotavirus[213] (Table 5). The rotavirus group experienced both vomiting and dehydration significantly more often then the nonrotavirus group. The dehydration was isotonic in 95% of the patients in the rotavirus 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 than in the nonrotavirus group (2.6 vs. 0.9 days). Diarrhea began later but lasted longer than vomiting in the rotavirus group (mean duration 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 (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.[213] 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.

Table 5. 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] From Rodriguez *et al.*[213] with minor changes. [b] $p < 0.01$. [c] $p < 0.01$.

Deaths have been reported in infants and young children with rotavirus infection.[40,54,177,178,182,183] In a Canadian study, 21 deaths were reported in infants and young children with rotavirus infection from May 1972 to March 1977. Of these 21 children, 10 were dead on arrival at the hospital, while 10 were moribund and could not be successfully resuscitated on arrival.[40] One child was already in the hospital when he acquired the disease; this patient had congestive cardiomyopathy that contributed to his death. All except this patient and one other had been healthy previously. The patients with fatal disease ranged in age from 4 to 30 months, with a mean of 11 months. The deaths occured 1–3 days after onset of symptoms. The major factor causing death was believed to be dehydration and electrolyte imbalance in 16 cases, aspiration of vomitus in 3 cases, and in the remaining 2, seizures were a contributing factor. It is striking that the parents of 16 of the 20 children brought to the hospital moribund or already dead had had some contact with a physician during the course of the illness.[40]

Rotavirus infection has also been observed in pediatric patients with intussusception and with self-limited gastrointestinal bleeding.[57,143] One of the patients in the latter group had clinical findings compatible with Henoch–Schoenlein purpura. One patient with rotavirus gastroenteritis was observed to develop a fatal Reye's syndrome with severe CNS manifestations.[223] Another patient with rotavirus gastroenteritis developed encephalitis with severe CNS manifestations and was making a slow recovery.[223] Hemolytic uremic syndrome or disseminated intravascular coagulation have been reported in several children with rotavirus infection[207]; one child with rotavirus gastroenteritis developed severe neurological sequelae after marked dehydration and intravascular coagulation.[183] Elevated serum transaminases have also been reported in patients with rotavirus-associated gastroenteritis.[64,252] The relationship or frequency, or both, of rotavirus infection with these unusual clinical manifestations should be clarified in future studies. 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.[125,272]

In the volunteer studies in which the D strain, a subgroup 2 human rotavirus was administered orally to volunteers, four developed a diarrheal illness that began 2–4 days after inoculation.[124] 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). Average duration of diarrhea was 2.5 days, with a range of 1–4 days. The number of diarrheal stools per illness ranged from 1 to 24 stools, with one volunteer having a maximum of 11 in one day. Thus, under experimental as well as natural conditions, adults can develop a rotaviral diarrheal illness. However, subclinical rotavirus infection in adults appears to be much more common, as demonstrated in one study in which 22 of 50 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[139]; however, only 3 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, specific diagnosis of infection with human rotavirus is not possible on the basis of clinical manifestations. Even though rotavirus infection follows a predictable seasonal pattern of recurrent high prevalence during the "winter" or cooler months in temperate climates, a laboratory diagnosis is essential, since other agents may also cause gastroenteritis even during peak rotavirus periods.

The human rotaviruses do not grow readily in tissue culture or in conventional laboratory animal models.[286] However, numerous assays have been developed for the detection of rotaviruses, as outlined previously in Table 1. The most widely applied methods are able to detect rotavirus in stool specimens by direct visualization or by immunological assay ("direct virology").[136] EM has the distinct advantage of being highly specific, since the rotavirus has such a characteristic morphological appearance; it is limited, however, by the requirement for an electron microscope as well as a capable operator. EM is the most rapid method of diagnosis when dealing with only a few specimens. Other efficient but more practical assays for large numbers of specimens include counterimmunoelectroosmophoresis, RIA, and enzyme-linked immunosorbent assay (ELISA). The ELISA is probably the most practical diagnostic method for large-scale studies and is limited only by the availability of suitable re-

agents. False-positive reactions may occur, and the laboratory should be able to confirm all positive samples by appropriate methods. 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.

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.[136,298] However, it is not as efficient as certain other techniques when testing sera from patients less than 6 months of age and from adults.[298] Serological evidence of rotavirus infection may be detected in these age groups by ELISA or immunofluorescence. ELISA has also been employed to measure specific immunoglobulin responses in rotavirus infection.[301] 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

There are no methods 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 hand-washing 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 might also limit the number of outbreaks due to these agents.

Treatment of gastroenteritis caused by the Norwalk group characteristically consists of replacement of fluid loss by the administration of liquids orally. Parenteral intravenous fluid therapy is only rarely necessary in this form of generally self-limited gastroenteritis.[59–61] The impact of this group of agents in debilitated hosts has not been evaluated extensively. In addition, it should be noted that recent volunteer studies with the Norwalk agent revealed that 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. This treatment did not significantly affect the number, weight, or water content of stools.[245]

Neither the need for the development of a vaccine for this group nor the techniques required for such a vaccine (such as propagation of these agents in cell culture) have been established. For example, the number of serotypes in this group and their overall importance in epidemic gastroenteritis must be understood before immunoprophylaxis can be considered. Furthermore, the unusual aspects of Norwalk virus immunity require more precise definition. This represents a clear priority in view of the apparent nonimmunological basis for long-term immunity. Thus, it is premature to consider immunoprophylaxis for the Norwalk group of agents.

9.2. Rotaviruses

In both the developed and developing countries, rotaviruses represent a major cause of severe diarrhea of infants and young children. Thus, it is clear that a rotavirus vaccine is needed. Although diarrheal illnesses are not a major cause of mortality in the developed countries, such illnesses are the leading cause of death in infants and young children in many developing countries. The role of rotaviruses in the estimated 5–10 million fatal diarrheal illnesses that occur in developing countries each year[271] has not been established, although their major importance in the etiology of severe gastroenteritis in these populations is now well documented. Moreover, rotaviruses are known to cause a severe dehydrating diarrheal illness, and such illness if untreated can be fatal. Indeed, in a Canadian study, 21 deaths associated with rotavirus gastroenteritis were observed.[40] All but one of the fatalities occurred in infants and young children who died on the way to the hospital or in the emergency clinic at the time of hospitalization. Although rotavirus infections can be fatal, it is not known what the impact of an effective rotavirus vaccine would be on the staggering death toll from diarrheal disease in the developing countries. Recently, it has been suggested that enterotoxigenic *E. coli* may be a more important cause of death from diarrhea in developing countries than rotavirus.[67,149] For example, in the United States in New York City in the early 1900s, these authors note that there was a staggering

infant mortality rate with a large proportion of deaths attributable to outbreaks of summer diarrhea in slum tenements.[149] 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.[149] 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 3rd year of life, though mortality from diarrheal illnesses is infrequent in the United States. Decline in mortality from diarrheal diseases in developed countries is due in part to the availability of fluid replacement therapy and possibly better nutrition, but undoubtedly other factors have played a major role, such as the decline of incidence of bacterial diarrheas as sanitation improved.[149] The relative role of bacteria, the rotaviruses, and other agents in infant mortality from diarrheal illness in developing countries needs intensive investigation.

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.[295] 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 frequency of diarrheal diseases, including those associated with rotavirus, in countries where infants and young children are breast fed almost exclusively for extended periods. However, the nutritional status of the nursing mother may be a critical factor.

It is likely that a rotavirus vaccine would be most effective if administered orally to stimulate local IgA antibody, since this antibody may be a major determinant of resistance to rotavirus illness.

The aim of a successful vaccine would be to prevent serious illness during the first 2 years of life, when the outcome of such infection may be especially serious or fatal. Thus, the vaccine would be administered within the first 6 months of life with a possible need for boosters at appropriate intervals.[45] With the recent successful cultivation of the Wa strain rotavirus, the development of a vaccine against at least one serotype appears possible.[285] It will be essential to propagate other rotavirus serotypes, since a vaccine should probably contain prevalent serotypes. Approaches to the development of a vaccine center about the development of mutants that have lost their ability to induce illness but are capable of inducing immunity. Such a vaccine would be tested initially in experimental animals for safety, antigenicity, and protective effect. If the vaccine were shown to be safe, immunogenic, and protective, studies would be carried out in adult volunteers initially. If safe, immunogenic, and protective in adult volunteers, the vaccine would be tested for safety and immunogenicity in a stepwise progression in younger age groups with the ultimate aim of a small field trial for efficacy.

Another approach to vaccine development takes advantage of the antigenic relatedness of human and animal rotavirus strains.[126,127,163,278] Thus, if an animal rotavirus, such as a bovine rotavirus, can be shown capable of infecting humans and inducing immunity without causing illness, such a strain might be an ideal vaccine candidate, especially if it protected against prevalent serotypes. The feasibility of this approach has been tested in calves. Calves were inoculated *in utero* with calf rotavirus or with placebo (or nothing).[289] Shortly after birth, the calves were challenged with the strain D of human rotavirus 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 human rotavirus D strain soon after birth. Thus, cross-protection between the calf and human rotavirus was demonstrated, indicating that the bovine virus was sufficiently related antigenically to human rotavirus D strain, a subgroup 2 virus, to induce protection, a finding that warrants further evaluation. Of course, prior to human studies, extensive safety tests would have to be performed.

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 developed, effective treatment for rotavirus diarrhea is available in the form of fluid and electrolyte replacement therapy by the oral or parenteral route of administration.[190,221] 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 hand-washing, 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

10.1. Norwalk Group and Miscellaneous Enteric Agents

Numerous unresolved problems remain for the Norwalk group. Intensive efforts are needed to determine the number of serotypes of agents responsible for epidemic viral gastroenteritis. Such studies entail careful electron-microscopic (EM) or immune-EM (IEM) studies for detection of viral particles. IEM must then be used to determine the significance of any particles observed and their antigenic relatedness to previously recognized viruses of the group. The development of a radioimmunoassay for Norwalk virus has permitted the study of the epidemiological importance of this agent. Such a practical assay is needed for the other known agents of this syndrome, such as the Hawaii, Ditchling, cockle, Parramatta, Marin County, and Colorado agents. It is conceivable that the etiology of most of epidemic viral gastroenteritis could be accounted for by the known members of the Norwalk group.

Efforts are also needed to find a suitable cell-culture system to propagate these agents. Such a system would facilitate epidemiological studies and assist in further characterization of these agents. For example, it is not yet known whether the Norwalk agent is an RNA or a DNA virus.

Studies of immunity to Norwalk agent have raised rather perplexing questions, since there appears to be one cohort of individuals who demonstrates immunity to Norwalk infection and illness, whereas there is another who characteristically demonstrates short-term but not long-term immunity. One explanation for this phenomenon postulates a genetic factor, such as a receptor for Norwalk virus, that is lacking in one cohort and present in the other.[197] The role of local IgA antibody should also be explored further.

Finally, a major unresolved area in the etiology of epidemic viral gastroenteritis is the role of other agents such as astroviruses, caliciviruses, minireoviruses, and other small, round viruses.[10,36,37,54,66,77,83,87,144–146,152–156,166,177,184,226,236] Some of these agents, such as the astroviruses, have been studied rather intensively: for example, astroviruses, which are 28 nm in diameter and derive their name from the five- or six-pointed star-shaped configuration observed by negative staining in certain particles,[152] have been administered to volunteers and found to induce a diarrheal illness in 1 of 17 volunteers but to infect a substantial number[145]; studies of the prevalence of astrovirus antibody have demonstrated a rather rapid acquisition of antibody, so that by the 10th year, 75% of persons have antibody.[144] Astroviruses have also been detected in stools of lambs with diarrhea and in calves without diarrhea.[236,275] The lamb astrovirus has been shown to induce illness in lambs under experimental conditions, whereas the calf astrovirus did not induce illness under such conditions. Caliciviruslike particles, which are about 32–40 nm in diameter and have characteristic cuplike configurations on their surface,[152] have also been studied rather intensively. For example, gastroenteritis in infants and young children in Japan, England, and Canada has been associated with such particles.[47,52a,243a,249] As noted earlier, recent evidence suggests that the Norwalk agent may be a calicivirus.[102] Another particle 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. These particles were believed to be different from caliciviruses and astroviruses morphologically.[269] The classification of all these particles needs further study.

Two other groups that have no morphological similarity to Norwalk agent are the adenoviruses and the coronaviruses. Fastidious adenoviruses that

do not grow readily in cell culture have been observed in stools of infants and young children hospitalized with diarrhea.[18,37,54,66,133,265] In the Washington, D.C., Children's Hospital study, adenoviruses were detected by EM significantly more often in stools or rectal-swab specimens of patients hospitalized with gastroenteritis than in those hospitalized for other than diarrheal illness (5.1 vs. 1.9%).[33] A large proportion of these adenoviruses could not be cultivated in cell culture. In addition, gastroenteritis outpatients were also found to shed adenoviruses significantly more often than nongastroenteritis outpatients (2.5 vs. 0.3%).[33] Adenoviruses have also been detected in stools of 15% of patients hospitalized with gastroenteritis in a Canadian study.[208] In addition, adenoviruses were associated with the deaths of two infants who had dehydration from severe gastroenteritis; adenovirus antigen was detected in their jejunal cells by immunofluorescence.[208] Adenoviruses have also been associated with a gastroenteritis outbreak in a long-stay children's ward.[84] Adenoviruses have also been found in small-intestinal fluid of pediatric patients with gastroenteritis[166]; in such patients, D-xylose absorption appears to have been impaired. Overall, the contribution of adenoviruses to etiology of pediatric gastroenteritis appears to be small. Adenovirus infection has also been associated with intussusception.[49,94]

Coronaviruses are established as etiological agents of diarrheal disease in many animals, but they have not yet been implicated conclusively in published reports as etiological agents of infantile gastroenteritis.[17,27,76,122,138,170,209,244] Coronaviruses have been reported to have been detected by EM in stools obtained from three outbreaks of gastroenteritis in adults, and the particles in a stool from one of the outbreaks were propagated in organ and cell cultures.[41,42] In February, 1980, enteric coronaviruses were described as associated with an outbreak of severe hemorrhagic enterocolitis in newborn infants in France, with two deaths.[46] In addition, the occurrence of enteric coronavirus in epidemics of diarrhea in 4- to 30-month-old patients was described.[46] Human serum has been shown to contain neutralizing antibody to calf coronavirus; however, since the human respiratory coronavirus organ culture (OC)43 and the calf coronavirus share some antigenic relationship, it is not certain whether this antibody is related to OC43 or to another human coronavirus.[137,232] Thus, a major area of future research involves delineation of the role of these miscellaneous enteric agents in viral gastroenteritis.

10.2. Rotaviruses

There are numerous unresolved problems relating to the epidemiology of rotaviruses. The impact of rotavirus diarrhea on the staggering mortality rate from diarrheal diseases in the developing countries must be elucidated. Although rotaviruses are an important cause of severe diarrheal illness, the role of rotaviruses in infant mortality needs to be elucidated.

The incidence of rotavirus diarrhea in the general population in the United States and worldwide is not yet known. It is anticipated that in the United States, this information will be gathered from ongoing longitudinal studies, whereas worldwide information will be obtained from a recently launched WHO diarrheal diseases control program.

The paradox of rotavirus infections that are predominantly subclinical in neonates in certain nurseries has not been explained. The mechanism of this overall decreased susceptibility should be elucidated. In addition, the effect of such neonatal infection on future response to rotavirus should be determined.

Another area of interest is an understanding of the possible reservoirs of rotaviruses. Practically every animal studied has been found to have an indigenous rotavirus capable of causing diarrhea. However, there is no documented evidence of natural spread of an animal rotavirus to humans or vice versa. It is known, however, that the human rotavirus can induce diarrhea in piglets, calves, and monkeys under experimental conditions.

The inability to propagate rotavirus efficiently from clinical material remains a hindrance to the study of this agent. However, as described in Section 4.2, recently important advances have been made in this regard. Efficient cultivation of all prevalent serotypes in cell cultures is a major research goal.

The question of the number of rotavirus serotypes must also be resolved. There is agreement on at least two serotypes; however, the existence of up to five serotypes has been reported. The number of serotypes and their importance epidemiologically should be elucidated.

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 of rotavirus diarrhea must also be evaluated. 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 should be studied. In animals, it is described that the presence of certain bacteria acts synergistically with rotavirus to cause more severe illness than if either were present alone.

Finally, with the worldwide importance of rotaviruses as a cause of diarrhea established, there is an ever-increasing demand for reagents for study of these agents. Suitable reagents for ELISA for detecting human rotavirus are available from various sources. The availability of such reagents should facilitate worldwide study of rotavirus.

11. References

1. Adams, W. R., and Kraft, L. M., Epizootic diarrhea of infant mice: Identification of the etiologic agent, *Science* **141**:359–360 (1963).
2. Adler, I., and Zickl, R., Winter vomiting disease, *J. Infect. Dis.* **119**:668–673 (1969).
3. Aldrich, R. A., Introduction to pediatrics: The change from pediatrics to child health and human development, in: *Brennemann's Practice of Pediatrics* (V. C. Kelley, ed.), pp. 1–28, Harper and Row, Hagerstown, Maryland, 1972.
4. Agus, S. G., Dolin, R., Wyatt, R. G., Tousimis, A. J., and Northrup, R. S., Acute infectious nonbacterial gastroenteritis: Intestinal histopathology. Histologic and enzymatic alterations during illness produced by the Norwalk agent in man, *Ann. Intern. Med.* **79**:18–25 (1973).
5. Albrey, M. B., and Murphy, A. M., Rotavirus and acute gastroenteritis of infants and children, *Med. J. Aust.* **1**:82–85 (1976).
6. Albrey, M. B., and Murphy, A. M., Rotavirus growth in bovine monolayers, *Lancet* **1**:753 (1976).
7. Almeida, J. D., and Waterson, A. P., The morphology of virus antibody interaction, *Adv. Viral Res.* **15**:307–338 (1969).
8. Anderson, T. F., and Stanley, W. M., A study by means of the electron microscope of the reaction between tobacco mosaic virus and its antiserum, *J. Biol. Chem.* **139**:339–344 (1941).
9. Appleton, H., Buckley, M., Thom, B. T., Cotton, J. L., and Henderson, S., Virus-like particles in winter vomiting disease, *Lancet* **1**:409–411 (1977).
10. Appleton, H., and Higgins, P. G., Viruses and gastroenteritis in infants, *Lancet* **1**:409–411 (1977).
11. Appleton, H., and Pereira, M. S., A possible virus etiology in outbreaks of food-poisoning from cockles, *Lancet* **1**:780–781 (1977).
12. Ardenne, M. v., Friedrich-Freska, H., and Schramm, G., Electronenmikrokopischen Untersuchung der Präcipitinreaktion von Tabakmosaik Virus mit Kaninchenanti-serum, *Arch. Gesamte Virusforsch.* **2**:80–86 (1941).
13. Ashley, C. R., Carl, E. O., Clark, S. K. R., Corner, B. D., and Dunn, S., Rotavirus infections of apes, *Lancet* **2**:477 (1979).
14. Banatvala, J. E., Chrystie, I. L., and Totterdell, B. M., Rotaviral infections in human neonates, *J. Am. Vet. Med. Assoc.* **173**:527–530 (1978).
15. Banatvala, J. E., Totterdell, B., Chrystie, I. L., and Woode, G. N., *In vitro* detection of human rotaviruses, *Lancet* **2**:821 (1975).

15a. Beards, G. M., Pilfold, J. N., Thouless, M. E., and Flewett, T. H., Rotavirus serotypes by serum neutralization *J. Med. Virol.* **5**:231–237 (1980).

16. Berglund, M. E., McAdaragh, J. P., and Stotz, I., Proceedings of the 26th Western Poultry Disease Conference at University of California, Davis, pp. 129–130, 1966.
17. Binn, L. N., Lazar, E. C., Keenan, K. P., Huxsoll, D. L., Marchwicki, R. H., and Srano, A. J., Recovery and characterization of a coronavirus from military dogs with diarrhea, Proceedings of the 78th Annual Meeting of the U.S. Animal Health Association, pp. 359–366, 1974.
18. Birch, C. J., Lewis, F. A., Kennett, M. L., Homola, M., Pritchard, H., and Gust, I. D., A study of prevalence of rotavirus infection in children with gastroenteritis admitted to an infectious diseases hospital, *J. Med. Virol.* **1**:69–77 (1977).
19. Bishop, R. F., Davidson, G. P., Holmes, I. H., and Ruck, B. J., Detection of a new virus by electron microscopy of fecal extracts from children with acute gastroenteritis, *Lancet* **1**:149–151 (1974).
20. Bishop, R. F., Davidson, G. P., Holmes, I. H., and Ruck, B. J., Evidence for viral gastroenteritis, *N. Engl. J. Med.* **289**:1096–1097 (1973).
21. Bishop, R. F., Davidson, G. P., Holmes, I. H., and Ruck, B. J., Virus particles in epithelial cells of duodenal mucosa from children with acute gastroenteritis, *Lancet* **2**:1281–1283 (1973).
22. Black, R. E., Merson, M. H., Mizanur Rahman, A. S. M., Yanus, M. D., Alim, A. R. M. A., Huq, I., Yolken, R. H., and Curlin, G. T., A 2 year study of bacterial, viral, and parasitic agents associated

with diarrhea in rural Bangladesh, *J. Infect. Dis.* **142**:660–664 (1980).

23. Blacklow, N. R., Cukor, G., Bedigian, M. K., Echeverria, P., Greenberg, H. B., Schreiber, D. S., and Trier, J. S., Immune response and prevalence of antibody to Norwalk enteritis virus as determined by radioimmunoassay, *J. Clin. Microbiol.* **10**:903–909 (1979).
24. Blacklow, N. R., Cukor, G., Panjavani, Z., Capozza, F., and Bednarek, F., Simplified radioimmunoassay for detection of rotavirus in pediatric and adult stools, for assessment of duration of antibody to rotavirus in human breast milk (abstract), Fourth International Congress for Virology, The Hague, p. 464, 1978.
25. Blacklow, N. R., Dolin, R., Fedson, D. S., DuPont, H., Northrup, R. S. Hornick, R. B., and Chanock, R. M., Acute infectious nonbacterial gastroenteritis: Etiology and pathogenesis (A combined clinical staff conference at the Clinical Center of the National Institutes of Health), *Ann. Intern. Med.* **76**:993–1008 (1972).
26. Blacklow, N. R., Echeverria, P., and Smith, D. A., Serological studies with reovirus-like enteritis agent, *Infect. Immun.* **13**:1563–1566 (1976).
27. Bohl, E. H., Transmissible gastroenteritis, in: *Diseases of Swine*, 3rd ed. (H. W. Dunne, ed.), pp. 158–176, Iowa State University Press, Ames, 1970.
28. Bolivar, R., Conklin, R. H., Vollett, J. J., Pickering, L. K., DuPont, H. L., Walters, D. L., and Kohl, S., Rotavirus in travelers' diarrhea: Study of an adult student population in Mexico, *J. Infect. Dis.* **137**:324–327 (1978).
29. Bonsdorff, C. H. von, Hovi, T., Makela, P., Hovi, L., and Tevalusto-Aarnio, M., Rotavirus associated with acute gastroenteritis in adults, *Lancet* **2**:423 (1976).
30. Bonsdorff, C. H. von, Hovi, T., Makela, P., and Morttinen, A., Rotavirus infections in adults in association with acute gastroenteritis, *J. Med. Virol.* **2**:21–28 (1978).
31. Bradburne, A. F., Almeida, J. D., Gardner, P. S., Moosai, R. B., Nash, A. A., and Coombs, R. R. A., A solid phase system (SPACE) for the detection and quantification of rotavirus in faeces, *J. Gen. Virol.* **44**:615–623 (1979).
32. Brandt, C. D., Kim, H. W., Rodriguez, W. J., Thomas, L., Yolken, R. H., Arrobio, J. O., Kapikian, A. Z., Parrott, R. H., and Chanock, R. M., Comparison of direct electron microscopy, immune electron microscopy, and rotavirus enzyme-linked immunosorbent assay for detection of gastroenteritis viruses in children, *J. Clin. Microbiol.* **13**:976–981 (1981).
33. Brandt, C. D., Kim, H. W., Yolken, R. H., Kapikian, A. Z., Arrobio, J. O., Rodriguez, W. J., Wyatt, R. G., Chanock, R. M., and Parrott, R. H., Comparative epidemiology of two rotavirus serotypes and other viral agents associated with pediatric gastroenteritis, *Am. J. Epidemiol.* **110**:243–254 (1979).
34. Bridger, J. C., and Woode, G. N., Neonatal calf diarrhea: Identification of a reovirus-like (rotavirus) agent in feces by immunofluorescence and immune electron microscopy, *Br. Vet. J.* **131**:528–535 (1975).
35. Burroughs, J. N., and Brown, F., Presence of a conalently linked protein in calicivirus RNA, *J. Gen. Virol.* **41**:443–446 (1978).
36. Bruce-White, G. B., Ashton, C. I., Roberts, C., and Parry, H. E., "Rotavirus" in gastroenteritis, *Lancet* **2**:726 (1974).
37. Bryden, A. S., Davies, H. A., Hadley, R. E., Flewett, T. H., Morris, C. A., and Oliver, P., Rotavirus enteritis in the West Midlands during 1974, *Lancet* **2**:241–243 (1974).
38. Bryden, A. S., Davies, H. A., Thouless, M. E., and Flewett, T. H., Diagnosis of rotavirus infection by cell culture, *J. Med. Microbiol.* **10**:121–125 (1977).
39. Bryden, A. S., Thouless, M. E., and Flewett, T. H., Rotavirus in rabbits, *Vet. Rec.* **99**:232 (1976).
40. Carlson, J. A. K., Middleton, P. J., Szymanski, M., Huber, J., and Petric, M., Fatal rotavirus gastroenteritis: An analysis of 21 cases, *Am. J. Dis. Child.* 132:477–479 (1978).
41. Caul, E. O., and Egglestone, S. I., Further studies on human enteric coronaviruses, *Arch. Virol.* **54**:107–117 (1977).
42. Caul, E. O., Paver, W. K., and Clarke, S. K. R., Coronavirus particles in faeces from patients with gastroenteritis, *Lancet* **1**:1192 (1975).
43. Center for Disease Control, Viral gastroenteritis—Pennsylvania, *Morbid. Mortal. Weekly Rep.* **27**:403–404 (1978).
44. Center for Disease Control, Gastroenteritis associated with lake swimming—Michigan, *Morbid. Mortal. Weekly Rep.* **28**:413–416 (1979).
45. Chanock, R. M., Wyatt, R. G., and Kapikian, A. Z., Immunization of infants and young children against rotavirus gastroenteritis: Prospects and problems, *J. Am. Vet. Med. Assoc.* **173**:570–572 (1978).
46. Chany, C., Discussion at Perspectives in Virology, XI Meeting, *Perspect. Virol.* **11**:185–187 (1981).
47. Chiba, S., Sakuma, Y., Kogasaka, R., Akihara, M., Horino, K., Nakao, T., and Fukui, S., An outbreak of gastroenteritis associated with caliciviruses in an infant home. *J. Med. Virol.* **4**:249–254 (1979).
48. Christopher, P. J., Grohmann, G. S., Millsom, R. H., and Murphy, A. M., Parvovirus gastroenteritis—a new entity for Australia, *Med. J. Aust.* **1**:121–124 (1978).
49. Clarke, E. J., Jr., Phillips, I. A., and Alexander,

E. R., Adenovirus infection in intussusception in children in Taiwan, *J. Am. Med. Assoc.* **208**:1671–1674 (1969).

50. Clarke, S. K. R., Cook, G. T., Egglestone, S. I., Hall, T. S., Miller, D. L., Reed, S. E., Rubenstein, D., Smith, A. J., and, Tyrrell, D. A. J., A virus from epidemic vomiting disease, *Br. Med. J.* **3**:86–89 (1972).
51. Connor, J. D., and Barrett-Connor, E., Infectious diarrheas, *Pediatr. Clin. North Am.* **14**:197–221 (1967).
52. Cook, D. A., Zbitnew, A., Dempster, G., and Gerrard, J. W., Detection of antibody to rotavirus by counterimmunoelectrophoresis in human serum, colostrum and milk, *J. Pediatr.* **93**:967–970 (1978).

52a. Cubitt, W. D., McSwiggan, D. A., and Moore, W., Winter vomiting disease caused by calicivirus. *J. Clin. Pathol.* **32**:786–793 (1979).

53. Cukor, G., Berry, M. K., and Blacklow, N. R., Simplified radioimmunoassay for detection of human rotavirus in stools, *J. Infect. Dis.* **138**:906–910 (1978).
54. Davidson, G. P., bishop, R. F., Townley, R. R. W., Holmes, I. H., and Ruck, B. J., Importance of a new virus in acute sporadic enteritis in children, *Lancet* **1**:242–245 (1975).
55. Davidson, G. P., Butler, D. G., Gall, D. G., Petric, M., and Hamilton, J. R., Ion transport in enteritis caused by human rotavirus, *American Society for Microbiology Annual Meeting*, abstract A-20/1043, May 1977, American Society of Microbiology, Washington, D.C., 1977.
56. Davidson, G. P., Goller, I., Bishop, R. F., Townley, R. R. W., Holmes, I. H., and Ruck, B. J., Immunofluroesecence in duodenal mucosa of children with acute enteritis due to a new virus, *J. Clin. Pathol.* **28**:263–266 (1975).
57. Delage, G., McLaughlin, B., and Bethaume, L., A clinical study of rotavirus gastroenteritis, *J. Pediatr.* **93**:455–457 (1978).
58. Dingle, J. H., Badger, G. F., and Jordan, W. S., *Illness in the home: A Study of 25,000 Illnesses in a Group of Cleveland Families*, p. 19, The Press of Western Reserve Univerity, 1964.
59. Dolin, R., Norwalk-like agents of gastroenteritis, in: *Principles and Practices of Infectious Diseases* (G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett, eds.) pp. 1364–1370, John Wiley, New York, Chichester, Brisbane, Toronto, 1979.
60. Dolin, R., Blacklow, N. R., DuPont, H., Buscho, R. J., Wyatt, R. G., Kasel, J. A., Hornick, R., and Chanock, R. M.: Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis, *Proc. Soc. Exp. Biol. Med.* **140**:578–583 (1972).
61. Dolin, R., Blacklow, N. R., DuPont, H., Formal, S., Buscho, R. F., Kasel, J. A., Chames, R. P., Hornick, R., and Chanock, R. M., Transmission of acute infectious nonbacterial gastroenteritis to volunteers by oral administration of stool filtrates, *J. Infect. Dis.* **123**:307–312 (1971).
62. Dolin, R., Levy, A. G., Wyatt, R. G., Thornhill, T. S., and Gardner, J. D., Viral gastroenteritis induced by the Hawaii agent: Jejunal histopathology and seroresponse, *Am. J. Med.* **59**:761–767 (1975).
63. Dolin, R., Reichman, R. C., and Fauci, A. S., Lymphocyte populations in acute viral gastroenteritis, *Infect. Immun.* **14**:422–428 (1976).
64. Dominick, H. C., and Maass, G., Rotavirus Infectionen im Kindersalter, *Klin. Paediatr.* **191**:33–39 (1979).
65. Drozdov, S. G., Shekoyan, L. A., Korolev, M. B., and Andzhaparidze, A. G., Human rotavirus in cell culture: Isolation and passaging, *Vopr. Virusol.* **4**:389–392 (1979).
66. Echeverria, P., Ho, M. T., Blacklow, N. R., Quinnan, G., Portnoy, B., Olson, J. G., Conklin, R., DuPont, H. L., and Cross, J. H., Relative importance of viruses and bacteria in the etiology of pediatric diarrhea in Taiwan, *J. Infect. Dis.* **136**:383–390 (1977).
67. Edelman, R., and Levine, M. M., Acute diarrheal infections in infants. II. Bacterial and viral causes, *Hosp. Pract.* **15**:96–104 (1980).
68. Editorial, Reovirus-like agent as a cause of human diarrheal disease, *South. Med. J.* **70**:390–392 (1977).
69. Editorial, Rotavirus gastroenteritis, *Br. Med. J.* **2**:784–785 (1977).
70. Editorial, Rotaviruses of man and animals, *Lancet* **1**:257–259 (1975).

70a. Editorial, Towards a rotavirus vaccine, *Lancet* **2**:619–620 (1981).

71. Editorial: Viral cross-infections in wards, *Lancet* **1**:1391–1393 (1976).
72. Editorial, Viruses from shellfish, *Lancet* **2**:1224–1225 (1979).
73. Editorial, Viruses of infantile gastroenteritis, *Br. Med. J.* **3**:555–556 (1975).
74. Elias, M. M., Distribution and titers of rotavirus antibodies in different age groups, *J. Hyg. (Cambridge)* **79**:365–372 (1977).
75. Elias, M. M., Separation and infectivity of two particle types of human rotavirus, *J. Gen. Virol.* **37**:191–194 (1977).
76. Ellens, D. J., and DeLeeuw, P. W., ELISA for detection of bovine coronavirus in faeces, Abstract of the International Congress for Virology, No. 627, The Hague, 1978.
77. Esparza, J., Viera de Torres, B., Pinero, A., Carmona, F. O., and Mazzali De Ilia, R., Rotavirus in Venezuelan children with gastroenteritis, *Am. J. Trop. Med. Hyg.* **26**:148–151 (1977).
78. Espejo, R. T., Calderon, E., and Gonzalez, N.,

Distinct reovirus-like agents associated with acute infantile gastroenteritis, *J. Clin. Microbiol.* **6**:502–506 (1977).

79. Eugster, A. K., Strother, J., and Hartfield, D. A., Rotavirus (reovirus-like) infection of neonatal ruminants in a zoo nursery, *J. Wildl. Dis.* **14**:351–354 (1978).
80. Fauvel, M., Spence, L., Babiuk, L. A., Petro, R., and Bloch, S., Hemagglutination and hemagglutination inhibition studies with a strain of Nebraska calf diarrhea virus (bovine rotavirus), *Intervirology* **9**:95–105 (1978).
81. Field, M., Modes of action of enterotoxins from *Vibrio cholerae* and *Escherichia coli*, *Rev. Infect. Dis.* **1**:918–925 (1979).
82. Flewett, T. H., Diagnosis of enteritis virus, *Proc. R. Soc. Med.* **69**:693–696 (1976).
83. Flewett, T. H., Bryden, A. S., and Davies, H., Virus particles in gastroenteritis, *Lancet* **2**:1497 (1973).
84. Flewett, T. H., Bryden, A. S., and Davies, H., Epidemic viral enteritis in a long-stay children's ward, *Lancet* **1**:4–5 (1975).
85. Flewett, T. H., Bryden, A. S., and Davies, H., Virus diarrhea in foals and other animals, *Vet. Rec.* **97**:477 (1975).
86. Flewett, T. H., Bryden, A. S., Davies, H., Woode, G. N., Bridger, J. C., and Derrick, J. M., Relation between viruses from acute gastroenteritis of children and newborn calves, *Lancet* **2**:61–63 (1974).
87. Flewett, T. H., and Davies, H., Caliciviruses in man, *Lancet* **1**:311 (1976).
88. Flewett, T. H., Davies, H., Bryden, A. S., and Robertson, M. J., Diagnostic electron microscopy of faeces. II. Acute gastroenteritis associated with reovirus-like particles, *J. Clin. Pathol* **27**:608–614 (1974).

88a. Flewett, T. H., Thouless, M. E., Polfold, J. N., Bryden, A. S., and Candeias, J. A. N., More serotypes of human rotavirus, *Lancet* **2**:632 (1978).

89. Flewett, T. H., and Woode, G. N., The rotaviruses (brief review), *Arch. Virol.* **57**:1–23 (1978).
90. Fonteyne, J., Zissis, G., and Lambert, J. P., Recurrent rotavirus gastroenteritis, *Lancet* **1**:983 (1978).
91. Foster, L. G., Peterson, H., and Spendlove, R. S., Fluorescent virus precipitin test, *Proc. Soc. Exp. Biol. Med.* **150**:155–160 (1975).
92. Fukumi, H., Nakaya, R., Hatta, S., Noriki, H., Yunoki, H., Akagi, K., Saito, T., Uchiyama, K., Kobari, K., and Nakanishi, R., An indication as to identity between the infectious diarrhea in Japan and the afebrile infectious non-bacterial gastroenteritis by human volunteer experiments, *Jpn. M. Med. Sci. Biol.* **10**:1–17 (1957).
93. Gall, D. G., Pathophysiology of viral diarrhea, in: Proceedings of the 73rd Ross Conference on Pediatric Research: Etiology, Pathology, and Treatment of Acute Gastroenteritis, Ponte Vedra Beach, Florida, March 20–22, 1977.
94. Gardner, P. S., Knox, E. G., Court, S. D. M., and Green, C. A., Virus infection and intussusception in childhood, *Br. Med. J.* **2**:697–700 (1962).
95. Gomez-Barreto, J., Palmer, E., Nahmias, A. J., Hatch, M. N., Acute enteritis associated with reovirus-like agents, *J. Am. Med. Assoc.* **235**:1857–1860 (1976).
96. Gordon, I., Ingraham, H. S., and Korns, R. F., Transmission of epidemic gastroenteritis to human volunteers by oral administration of fecal filtrates, *J. Exp. Med.* **86**:409–422 (1947).
97. Grauballe, P. C., Genner, J., Meyling, A., and Hornsleth, A., Rapid diagnosis of rotavirus infections: Comparison of electron microscopy and immunoelectro-osmophoresis for detection of rotavirus in human infantile gastroenteritis, *J. Gen. Virol.* **35**:203–218 (1977).
98. Greenberg, H. B., *et al.*, Unpublished studies.

98a. Greenberg, H. B., Kalica, A. R., Wyatt, R. G., Jones, R. W., Kapikian, A. Z., and Chanock, R. M., Rescue of noncultivatable human rotavirus by gene reassortment during mixed infection with ts mutants of a cultivatable bovine rotavirus, *Proc. Nat. Acad. Sci. USA* **78**:420–424 (1981).

99. Greenberg, H. B., and Kapikian, A. Z., Detection of Norwalk agent antibody and antigen by solid-phase radioimmunoassay and immune adherence hemagglutination assay, *J. Am. Vet. Med. Assoc.* **173**:620–623 (1978).

99a. Greenberg, H. B., Singh, N., Oshiro, L., London, W. T., Sly, D. L., unpublished studies.

100. Greenberg, H. B., Valdesuso, J., Kapikian, A. Z., Chanock, R. M., Wyatt, R. G., Szmuness, W., Larrick, J., Kaplan, J., Gilman, R. H., and Sack, D. A., Prevalence of antibody to the Norwalk virus in various countries, *Infect. Immun.* **26**:270–272 (1979).
101. Greenberg, H. B., Valdesuso, J., Yolken, R. H., Gangarosa, E., Gary, W., Wyatt, R. G., Konno, T., Suzuki, H., Chanock, R. M., and Kapikian, A. Z., Role of Norwalk virus in outbreaks of nonbacterial gastroenteritis, *J. Infect. Dis.* **139**:564–568 (1979).
102. Greenberg, H. B., Wyatt, R. G., Kalica, A. R., Yolken, R. H., Black, R., Kapikian, A. Z., and Chanock, R. M., New insights in viral gastroenteritis, *Perspect. Virol.* **11**:163–187 (1981).
103. Greenberg, H. B., Wyatt, R. G., and Kapikian, A. Z., Norwalk virus in vomitus, *Lancet* **2**:55 (1979).
104. Greenberg, H. B., Wyatt, R. G., Valdesuso, J., Kalica, A. R., London, W. T., Chanock, R. M., and Kapikian, A. Z., Solid-phase microtiter radioimmunoassay for detection of the Norwalk strain of acute nonbacterial epidemic gastroenteritis virus and its antibodies, *J. Med. Virol.* **2**:97–108 (1978).

105. GROHMANN, G. S., GREENBERG, H. B., WELCH, B. M., AND MURPHY, A. M., Oyster-associated gastroenteritis in Australia: The detection of Norwalk virus and its antibody by immune electron microscopy and radioimmunoassay, *J. Med. Virol.* **6**:11–19 (1980).

106. GUST, I. D., PRINGLE, R. C., BARNES, G. L., DAVIDSON, G. P., AND BISHOP, R. F., Complement-fixing antibody response to rotavirus infection, *J. Clin. Microbiol.* **5**:125–130 (1977).

107. GWALTNEY, J. M., JR., Medical reviews: Rhinoviruses, *Yale J. Biol. Med.* **48**:17–45 (1975).

107a. HAMILTON, J. R., GALL, D. G., KERZNER, B., BUTLER, D. G., AND MIDDLETON, P. J., Recent developments in viral gastroenteritis, *Pediatr. Clin. N. Am.* **22**:747–755 (1975).

108. HARA, M., MUKOYAMA, J., TSURUHARA, T., ASHIWARA, Y., SAITO, Y., AND TAGAYA, I., Acute gastroenteritis among school children associated with reovirus-like agent, *Am. J. Epidemiol.* **107**:161–169 (1978).

109. HARA, M., MUKOYAMA, J., TSURUHARA, T., SAITO, Y. AND TAGAYA, I., Duovirus in school children with gastroenteritis, *Lancet* **1**:311 (1976).

110. HARRIS, C. C., YOLKEN, R. H., KROKAN, H., AND HSU, I. C., Ultrasensitive enzymatic radioimmunoassay: Application to detection of cholera toxin and rotavirus, *Proc. Nat. Acad. Sci. USA* **76**:5335–5339 (1979).

111. HIEBER, J. P., SHELTON, S., NELSON, J. D., LEON, J., AND MOHS, E., Comparison of human rotavirus disease in tropical and temperate settings, *Am. J. Dis. Child.* **132**:853–858 (1978).

112. HODES, H. L., American Pediatric Society Presidential Address, *Pediatr. Res.* **10**:201–204 (1976).

113. HOLMES, I. H., Viral gastroenteritis, *Prog. Med. Virol.* **25**:1–36 (1979).

114. HOLMES, I. H., RUCK, B. J., BISHOP, R. F., AND DAVIDSON, G. P., Infantile enteritis viruses: Morphogenesis and morphology, *J. Virol.* **16**:937–943 (1975).

115. JESUDOSS, E. S., JOHN, T. J., MATHAN, M., AND SPENCE, L., Prevalence of rotavirus antibody in infants and children, *India J. Med. Res.* **68**:383–386 (1978).

116. JONES, R. C., HUGHES, C. S., AND HENRY, R. R., Rotavirus infection in commercial laying hens, *Vet. Rec.* **104**:22 (1979).

117. JORDAN, W. S., GORDON, I., AND DORRANCE, W. R., A study of illness in a group of Cleveland families. VII. Transmission of acute nonbacterial gastroenteritis to volunteers: Evidence for two different etiologic agents, *J. Exp. Med.* **98**:461–475 (1953).

118. KALICA, A. R., GARON, C. F., WYATT, R. G., MEBUS, C. A., VANKIRK, D. H., CHANOCK, R. M., AND KAPIKIAN, A. Z., Differentiation of human and calf reovirus-like agents associated with diarrhea using polyacrylamide gel electrophoresis of RNA, *Virology* **74**:86–92 (1976).

118a. KALICA, A. R., GREENBERG, H. B., WYATT, R. G., FLORES, J., SERENO, M. M., KAPIKIAN, A. Z., AND CHANOCK, R. M., Genes of human (strain Wa) and bovine (strain UK) rotaviruses that code for neutralization and subgroup antigens, *Virology* **112**:385–390 (1981).

119. KALICA. A. R., PURCELL, R. H., SERENO, M. M., WYATT, R. G., KIM, H. W., CHANOCK, R. M., AND , KAPIKIAN, A. Z., A microtiter solid phase radioimmunoassay for detection of the human reovirus-like agent in stools, *J. Immunol.* **118**:1275–1279 (1977).

120. KALICA, A. R., SERENO, M. M., WYATT, R. G., MEBUS, C. A., CHANOCK, R. M., AND KAPIKIAN, A. Z., Comparison of human and animal rotavirus strains by gel electrophoresis of RNA, *Virology* **87**:247–255 (1978).

121. KALICA, A. R., WYATT, R. G., AND KAPIKIAN, A. Z., Detection of differences among human and animal rotaviruses using analysis of viral RNA, *J. Am. Vet. Med. Assoc.* **173**:531–537 (1978).

122. KAPIKIAN, A. Z., The coronaviruses, in: *CRC Chemoprophylaxis and Virus Infections of the Respiratory Tract*, Vol II, 9th ed. (G. S. OXFORD, ed.), pp. 95–117, CRC Press, Cleveland, 1977.

123. KAPIKIAN, A. Z., Unpublished studies.

124. KAPIKIAN, A. Z., WYATT, R. G., LEVINE, M. M., YOLKEN, R. H., VAN KIRK, D. H., DOLIN, R., GREENBERG, H. B., AND CHANOCK, R. M., Oral administration of a human rotavirus to volunteers: Induction of illness and correlates of resistance (manuscript submitted).

125. KAPIKIAN, A. Z., BARILE, M. F., WYATT, R. G., YOLKEN, R. H., TULLY, J. G., GREENBERG, H. B., KALICA, A. R., AND CHANOCK, R. M., Mycoplasma contamination in cell culture of Crohn's disease material, *Lancet* **2**:466–467 (1979).

125a. KAPIKIAN, A. Z., CLINE, W. L., GREENBERG, H. B., WYATT, R. G., KALICA, A. R., BANKS, C. E., JAMES, H. D., JR., FLORES, J., AND CHANOCK, R. M., Antigenic characterization of human and animal rotaviruses by immune adherence hemagglutination assay (IAHA): Evidence for distinctness of IAHA and neutralization antigens, *Infect. Immun.* **33**:415–425 (1981).

126. KAPIKIAN, A. Z., CLINE, W. L., KIM, H. W., KALICA, A. R., WYATT, R. G., VANKIRK, D. H., CHANOCK, R. M., JAMES, H. D., JR., AND VAUGHN, A. L., Antigenic relationships among five reovirus-like (RVL) agents by complement-fixation (CF) and development of a new substitute CF antigen for the human RVL agent of infantile gastroenteritis, *Proc. Soc. Exp. Biol. Med.* **152**:535–539 (1976).

127. KAPIKIAN, A. Z., CLINE, W. L., MEBUS, C. A., WYATT, R. G., KALICA, A. R., JAMES, H. D., JR., VAN KIRK, D., CHANOCK, R. M., AND KIM, H. W., New complement-fixation test for the human reovirus-like agent of infantile gastroenteritis: Nebraska calf diarrhea virus used as antigen, *Lancet* **1**:1056–1069 (1975).

128. KAPIKIAN, A. Z., DIENSTAG, J. L., AND PURCELL, R

H., Immune electron microscopy as a method for the detection, identification, and characterization of agent not cultivable in an *in vitro* system, in: *Manual of Clinical Immunology*, 2nd. ed. (N. R. ROSE AND H. FRIEDMAN, eds.), pp. 70–83, American Society for Microbiology, Washington, D.C., 1980.

129. KAPIKIAN, A. Z., FEINSTONE, S. M., PURCELL, R. H., WYATT, R. G., THORNHILL, T. S., KALICA, A. R., AND CHANOCK, R. M., Detection and identification by immune electron microscopy of fastidious agents associated with respiratory illness, acute nonbacterial gastroenteritis, and hepatitis A, *Perspect. Virol.* **9**:9–47 (1975).
130. KAPIKIAN, A. Z., GERIN, J. L., WYATT, R. G., THORNHILL, T. S., AND CHANOCK, R. M., Density in cesium chloride of the 27-nm "8FIIa" particle associated with acute infectious nonbacterial gastroenteritis: Determination by ultracentrifugation and immune electron microscopy, *Proc. Soc. Exp. Biol. Med.* **142**:874–877 (1974).
131. KAPIKIAN, A. Z., GREENBERG, H. B., CLINE, W. L., KALICA, A. R., WYATT, R. G., JAMES, H. D., JR., LLOYD, N. L., CHANOCK, R. M., RYDER, R. W., AND KIM, H. W., Prevalence of antibody to Norwalk agent by a newly developed immune adherence hemagglutination assay, *J. Med. Virol.* **2**:281–294 (1978).
132. KAPIKIAN, A. Z., KALICA, A. R., SHIH, J. W., CLINE, W. L., THORNHILL, T. S., WYATT, R. G., CHANOCK, R. M., KIM, H. W., AND GERIN, J. L., buoyant density in cesium chloride of the human reovirus-like agent of infantile gastroenteritis by ultracentrifugation, electron microscopy, and complement-fixation, *Virology* **70**:564–569 (1976).
133. KAPIKIAN, A. Z., KIM, H. W., WYATT, R. G., CLINE, W. L., ARROBIO, J. O., BRANDT, C. D., RODRIGUEZ, W. J., SACK, D. A., CHANOCK, R. M., AND PARROTT, R. H., Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children, *N. Engl. J. Med.* **294**:965–972 (1976).
134. KAPIKIAN, A. Z., KIM, H. W., WYATT, R. G., RODRIGUEZ, W. J., ROSS, S., CLINE, W. L., PARROTT, R. H., AND CHANOCK, R. M., Reovirus-like agent in stools: Association with infantile diarrhea and development of serologic tests, *Science* **185**:1049–1053 (1974).
135. KAPIKIAN, A. Z., WYATT, R. G., DOLIN, R., THORNHILL, T. S., KALICA, A. R., AND CHANOCK, R. M., Visualization by immune electron microscopy of a 27 nm particle associated with acute infectious nonbacterial gastroenteritis, *J. Virol.* **10**:1075–1081 (1972).
136. KAPIKIAN, A. Z., YOLKEN, R. H., GREENBERG, H. B., WYATT, R. G., KALICA, A. R., CHANOCK, R. M., AND KIM, H. W., Gastroenteritis viruses, in: *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, 5th ed. (E. H. LENNETTE AND N. J. SCHMIDT, eds.), pp. 927–995, American Public Health Association, Washington, D.C., 1979.
137. KAYE, H. S., YARBROUGH, W. B., AND REED, C. J., Calf diarrhea coronavirus, *Lancet* **2**:509 (1975).
138. KEENAN, K. P., JERVIS, H. R., MARCHWICKI, R. H., AND BINN, L. N., Intestinal infection of neonatal dogs and canine coronavirus 1-71: Studies by virologic, histologic, histochemical and immunofluorescent techniques, *Am. J. Vet. Res.* **27**:247–256 (1976).

138a. KIM, H. W., ARROBIO, J. O., BRANDT, C. D., JEFFRIES, B. C., PYLES, G., REID, J. L., CHANOCK, R. M., AND PARROTT, R. H., Epidemiology of respiratory syncytial virus infection in Washington, D.C. I. Importance of the virus in different respiratory tract disease syndromes and temporal distribution of infection, *Am. J. Epidemiol.* **98**:216–225 (1973).

139. KIM, H. W., BRANDT, C. D., KAPIKIAN, A. Z., WYATT, R. G., ARROBIO, J. O., RODRIGUEZ, W. J., CHANOCK, R. M., AND, PARROTT, R. H., Human reovirus-like agent (HRVLA) infection: Occurrence in adult contacts of pediatric patients with gastroenteritis, *J. Am. Med. Assoc.* **237**:404–407 (1977).
140. KOJIMA, S., FUKUMI, H., KUSAMA, H., YAMAMOTO, S., SUZUKI, S., UCHIDA, T., ISHIMARAU, T., OKA, T., KURETANI, K., OHMURA, K., NISHIKAWA, F., FUJIMOTO, J., FUJITA, K., NAKANO, A., AND SUNAKAWA, S., Studies on the causative agent of the infectious diarrhea: Records of the experiments on human volunteers, *Jpn. Med. J.* **1**:467–476 (1948).
141. KONNO, T., SUZUKI, H., IMAI, A., AND ISHIDA, N., Reovirus-like agent in acute epidemic gastroenteritis in Japanese infants: Fecal shedding and serologic response, *J. Infect. Dis.* **135**:259–266 (1977).
142. KONNO, T., SUZUKI, H., IMAI, A., KUTSUZAWA, T., ISHIDA, N., KATSUSHIMA, N., SAKAMOTO, M., KITAOKA, S., TSUBOI, R., AND ADACHI, M., A long term survey of rotavirus infection in Japanese children with acute gastroenteritis, *J. Infect. Dis.* **138**:569–576 (1978).
143. KONNO, T., SUZUKI, H., KUTSUZAWA, T., IMAI, A., KATSUSHIMA, M., SAKAMOTO, M., AND KITAOKA, S., Human rotavirus and intussuception, *N. Engl. J. Med.* **297**:945 (1977).
144. KURTZ, J. B., AND LEE, T. W., Astrovirus gastroenteritis age distribution of antibody, *Med. Microbiol. Immun.* **166**:227–230 (1978).
145. KURTZ, J. B., LEE, T. W., CRAIG, J. W., AND REED, S. E., Astrovirus infection in volunteers, *J. Med. Virol.* **3**:221–230 (1979).
146. KURTZ, J. B., LEE, T. W., AND PICKERING D., Astrovirus associated gastroenteritis in a children's ward, *J. Clin. Pathol.* **30**:948–952, (1977).
147. LAMBETH, L., AND MITCHELL, J. D., Transmission of human gastroenteritis virus to a monkey (abstract), *Aust. Paediatr. J.* **11**:127–128 (1975).

147a. LAPORTE, J., AND BOBULESCO, P., Growth of human

and canine enteric coronaviruses in a highly susceptible cell line: HRT 18, *Perspect. Virol.* **11**:189–193 (1981).

148. Lecce, J. G., King, M. W., and Mock, R., Reovirus-like agent associated with fatal diarrhea in neonatal pigs, *Infect. Immun.* **14**:816–825 (1976).
149. Levine, M. M., and Edelman, R., Acute diarrheal infections in infants. I. Epidemiology, treatment and prospects for immunoprophylaxis, *Hosp. Pract.* **14**:89–100 (1979).
150. Levy, A. G., Widerlite, L., Schwartz, C. J., Dolin, R., Blacklow, N. R., Gardner, J., Kimberg, D. V., and Trier, J. S., Jejunal adenylate cyclase activity in human subjects during viral gastroenteritis, *Gastroenterology* **70**:321–325 (1976).
151. Light, J. S., and Hodes, H. L., Studies on epidemic diarrhea of the newborn: Isolation of a filtrable agent causing diarrhea in calves, *Am. J. Public Health* **33**:1451–1454 (1943).
152. Madeley, C. R., Comparison of the features of astroviruses and caliciviruses, *J. Infect. Dis.* **139**:519–523 (1979).
153. Madeley, C. R., and Cosgrove, B. P., Caliciviruses in man, *Lancet* **1**:199–200 (1976).
154. Madeley, C. R., and Cosgrove, B. P., 28 nm particles in faeces in infantile gastroenteritis, *Lancet* **2**:451–452 (1975).
155. Madeley, C. R., and Cosgrove, B. P., Viruses in infantile gastroenteritis, *Lancet* **2**:124 (1975).
156. Madeley, C. R., Cosgrove, B. P., Bell, E. J., and Fallon, R. J., Stool viruses in babies in Glasgow. I. Hospital admissions with diarrhea, *J. Hyg. (Cambridge)*. **78**:261–273 (1977).
157. Maiya, P. P., Pereira, S. M., Mathan, M., Bhat, P., Albert, M. J., and Baker, J. J., Aetiology of acute gastroenteritis in infancy and early childhood in southern India, *Arch. Dis. Child.* **52**:482–485 (1977).
158. Malherbe, H. H., and Strickland-Cholmley, M., Simian viruse SA-11 and the related "O" agent, *Arch. Gesamte Virusforsch.* **22**:235–245 (1967).
159. Martin, M. L., Palmer, E. L., and Middleton, P. J., Ulstrastructure of infantile gastroenteritis virus, *Virology* **68**:146–153 (1975).
160. Mata, L., Rotavirus diarrhea in Costa Rica (abstract), 4th International Congress for Virology, The Hague, p. 469, 1978.
161. Mata, L. J., Urrutia, J. J., and Gordon, J. E., Diseases and disabilities, in: *The Children of Santa Maria Cauque: A Prospective Field Study of Health and Growth*, by L. J. Mata, pp. 254–292, The MIT Press, Cambridge, Massachusetts, and London, 1978.
162. Matsuno, S., Inouye, S., and Kono, R., Antigenic relationship between human and bovine rotaviruses as determined by neutralization, immune adherence hemagglutination and complement-fixation tests, *Infect. Immun.* **17**:661–662 (1977).
163. Matsuno, S., Inouye, S., and Kono, R., Plaque assay of neonatal calf diarrhea virus and the neutralizing antibody in human sera, *J. Clin. Microbiol.* **5**:1–4 (1977).
164. Matsuno, S., and Nagayoshi, S., Quantitative estimation of infantile gastroenteritis virus antigens in stools by immune adherence hemagglutination test, *J. Clin. Microbiol.* **7**:310–311 (1978).
165. Matthews, R. E. F., The classification and nomenclature of viruses: Summary of results of meetings of the international committee on taxonomy of viruses in the Hague, September 1978, *Intervirology* **11**:133–135 (1979).
166. Mavromichalis, J., Evans, N., McNeish, A. S., Bryden, A. S., Davies, H. A., and Flewett, T. H., Intestinal damage in rotavirus and adenovirus gastroenteritis assessed by D-xylose malabsorption, *Arch. Dis. Child.* **52**:589–591 (1977).
167. McNulty, M. S., Allan, G. M., and Stuart, J. C., Rotavirus infection in avian species, *Vet. Rec.* **103**:319–320 (1978).
168. McNulty, M. S., Pearson, G. R., McFerran, J. B., Collins, D. S., and Allan, G. M., A reovirus like agent (rotavirus) associated with diarrhea in neonatal pigs, *Vet. Microbiol.* **1**:55–63 (1976).

168a. McSwiggan, D. A., Cubitt, D., and Moore, W., Calicivirus associated with winter disease, *Lancet* **2**:1215 (1978).

169. Mebus, C. A., Kono, M., Underdahl, N. R., and Twiehaus, M. J., Cell culture propagation of neonatal calf diarrhea (scours) virus, *Can. Vet. J.* **12**:69–72 (1971).
170. Mebus, C. A., Stair, E. L., Rhodes, M. B., and Twiehaus, M. J., Pathology of neonatal calf diarrhea induced by a coronavirus-like agent, *Vet. Pathol.* **10**:45 (1973).
171. Mebus, C. A., Torres-Medina, A., Twiehaus, M. J., and Bass, E. P., Immune response to orally administered calf reovirus-like agent and coronavirus vaccine, *Dev. Biol. Stand.* **33**:346–403 (1976).
172. Mebus, C. A., Wyatt, R. G., and Kapikian, A. Z., Intestinal lesions induced in gnotobiotic calves by the virus of human infantile gastroenteritis, *Vet. Pathol.* **14**:273–282 (1977).
173. Mebus, C. A., Wyatt, R. G., Sharpee, R. L., Sereno, M. M., Kalica, A. R., Kapikian, A. Z., and Twiehaus, M. J., Diarrhea in gnotobiotic calves caused by the reovirus-line agent of human infantile gastroenteritis, *Infect. Immun.* **14**:471–474 (1976).
174. Mebus, C. A., Underdahl, N. R., Rhodes, M. B., and Twiehaus, M. J., Calf diarrhea (scours): Reproduced with a virus from a field outbreak, *Univ. Nebraska Res. Bull.* **233**:1–16 (1969).

175. Meeroff, J. C., Schreiber, D. S., Trier, J. S., and Blacklow, N. R., Abnormal gastric motor function in viral gastroenteritis, *Ann. Intern. Med.* **92**:370–373 (1980).
176. Meurman, O. H., and Laine, M. J., Rotavirus epidemic in adults, *N. Engl. J. Med.* **296**:1298–1299 (1977).
177. Middleton, P. J., Analysis of the pattern of viral infection, in: Report of the 74th Ross Conference on Pediatric Research: Etiology, Pathology, and Treatment of Acute Gastroenteritis, Ponte Vedra Beach, Florida, March 20–22, 1977.
178. Middleton, P. J., Pathogenesis of rotaviral infection, *J. Am. Vet. Med. Assoc.* **173**:544–546 (1978).
179. Middleton, P. J., Holdaway, M. D., Petric, M., Szymanski, M. T., and Tam, J. S., Solid-phase radioimmunoassay for the detection of rotavirus, *Infect. Immun.* **16**:439–444 (1977).
180. Middleton, P. J., Petric, M., Hewitt, C. M., Szymanski, M. T., and Tam, J. S., Counter-immunoelectro-osmophoresis for the detection of infantile gastroenteritis virus (orbi-group) and antibody, *J. Clin. Pathol.* **29**:191–197 (1976).
181. Middleton, P. J., Petric, M., and Szymanski, M. T., Propagation of infantile gastroenteritis virus (orbi-group) in conventional and germfree piglets, *Infect. Immun.* **12**:1276–1280 (1975).
182. Middleton, P. J., Szymanski, M. T., Abbott, G. D., Bortolussi, R., and Hamilton, J. R., Orbivirus acute gastroenteritis of infancy, *Lancet* **1**:1241–1244 (1974).
183. Middleton, P. J., Szymanski, M. T., and Petric, M., Viruses associated with acute gastroenteritis in young children, *Am. J. Dis. Child.* **131**:733–737 (1977).
184. Mitchell, J. D., Lambeth, L. A., Sosula, L., Murphy, A., and Albrey, M., Transmission of rotavirus gastroenteritis from children to a monkey, *Gut* **18**:156–160 (1977).
185. Morens, D. M., Zweighaft, R. M., Vernon, T. M., Gary, G. W., Eslien, J. J., Wood, B. T., Holman, R. C., and Dolin, R., A waterbourne outbreak of gastroenteritis with secondary person-to-person spread association with a viral agent, *Lancet* **1**:964–966 (1979).
186. Morishima, T., Nagayoshi, S., Ozaki, T., Isomura, S., and Suzuki, S., Immunofluorescence of human reovirus-like agent of infantile diarrhoea, *Lancet* **2**:695–696 (1976).
187. Much, D., and Zajac, I., Purification and characterization of epizootic diarrhea of infant mice virus, *Infect. Immun.* **6**:1019–1024 (1972).
188. Murphy, A. M., Albrey, M. B., and Crewe, E. B., Rotavirus infections of neonates, *Lancet* **2**:1149–1150 (1977).
189. Murphy, A. M., Grohmann, G. S., Christopher, P. J., Lopez, W. A., and Millsom, R. H., Oyster food poisoning, *Med. J. Aust.* **2**:439 (1978).
190. Nalin, D. R., Levine, M. M., Mata, L., DeCespedes, C., Vargas, W., Lizano, C., Loria, A. R., Simhon, A., and Mohs, E., Comparison of sucrose with glucose in oral therapy of infant diarrhea, *Lancet* **2**:277–279 (1978).
191. Newman, J. F. E., Brown, F., Bridger, J. D., and Woode, G. N., Characterization of a rotavirus, *Nature (London)* **258**:631–633 (1975).
192. Obijeski, J. F., Palmer, F. L., and Martin, M. L., Biochemical characterization of infantile gastroenteritis virus (IGV), *J. Gen. Virol.* **34**:485–497 (1977).
193. Oshiro, L. S., Haley, C. E., Roberto, R. R., Riggs, J. L., Croughan, M., Greenberg, H., and Kapikian, A., A 27 nm virus isolated during an outbreak of acute infectious nonbacterial gastroenteritis in a convalescent hospital: A possible new serotype *J. Infect. Dis.* **143**:791–795, 1981.
194. Orstavik, I., Figenschau, K. J., Haug, K. W., and Ulstrup, J. C., A reovirus-like agent (rotavirus) in gastroenteritis of children, *Scand. J. Infect. Dis.* **8**:1–5 (1976).
195. Ostravik, I., Haug, K. W., and Sovde, A., Rotavirus-associated gastroenteritis in two adults probably caused by virus reinfection, *Scand. J. Infect. Dis.* **8**:277–278 (1976).
196. Palmer, E. L., Martin, M. L., and Murphy, F. A., Morphology and stability of infantile gastroenteritis virus: Comparison with reovirus and bluetongue virus, *J. Gen. Virol.* **35**:403–414 (1977).
197. Parrino, T. A., Schreiber, D. S., Trier, J. S., Kapikian, A. Z., and Blacklow, N. R., Clinical immunity in acute gastroenteritis caused by the Norwalk agent, *N. Engl. J. Med.* **297**:86–89 (1977).
198. Parrott, R. H., Vargosko, A. J., Kim, H. W., Bellanti, J. A., and Chanock, R. M., Myxovirus parainfluenza, *Am. J. Public Health* **52**:907–917 (1962).
199. Peterson, M. W., Spendlove, R. S., and Smart, R. A., Detection of neonatal calf diarrhea virus, infant reovirus-like diarrhea virus and a coronavirus using the fluorescent virus precipitin test, *J. Clin. Microbiol.* **3**:376–377 (1976).
200. Petric, M., Szymanski, M. T., and Middleton, P. J., Purification and preliminary characterization of infantile gastroenteritis virus (orbivirus group), *Intervirology* **5**:233–238 (1975).
201. Petric, M., Tam, J. S., and Middleton, P. J., Preliminary characterization of the nucleic acid of infantile gastroenteritis virus (orbivirus group), *Intervirology* **7**:176–180 (1976).
202. Portnoy, B. L., Conklin, R. H., Menn, M., Olarte, J., and DuPont, H. L., Reliable identification of reovirus-like agent in diarrheal stools, *J. Lab. Clin. Med.* **89**:560–563 (1977).

203. Public Health Service Laboratory (England), Rotavirus in rabbits, Communicable Disease Report 32 (1976).
204. Purdham, D. R., Purdham, P. A., Evans, N., and McNeish, A. S., Isolation of human rotavirus using human embryonic gut monolayers, *Lancet* **2**:977 (1975).
205. Reed, D. E., Daley, C. A., and Shave, H. J., Reovirus-like agent associated with neonatal diarrhea in pronghorn antelope, *J. Wildl. Dis.* **12**:488–491 (1976).
206. Reimann, H. A., Price, A. H., and Hodges, J. H., The cause of epidemic diarrhea, nausea and vomiting (viral dysentery?), *Proc. Soc. Exp. Biol. Med.* **59**:8–9 (1945).
207. Report of a WHO Scientific Working Group, Rotavirus and other viral diarrhoeas, *Bull. W.H.O.* **58**(2):183–198 (1980).
208. Retter, M., Middleton, P. J., Tam, J. S., and Petric, M., Enteric adenoviruses: Detection, implication and significance, *J. Clin. Microbiol.* **10**:574–578 (1979).
209. Ritchie, A. E., Deshmukh, D. R., Larsen, C. T., and Pomeroy, B. S., Electron microscopy of coronavirus-like particles characteristic of turkey bluecomb disease, *Avian Dis.* **17**:546–558 (1973).
210. Rodger, S. M., Craven, J. A., and William, I., Demonstration of reovirus-like particles in intestinal contents of piglets with diarrhea, *Aust. Vet. J.* **51**:536 (1975).
211. Rodger, S. M., Schnagl, R. D., and Holmes, I. H., Biochemical and biophysical characterization of diarrhea viruses of human and calf origin, *J. Virol.* **16**:1229–1235 (1975).
212. Rodger, S. M., Schnagl, R., and Holmes, I. H., Further biochemical characterization, including the detection of surface glucoproteins, of human, calf, and simian rotaviruses, *J. Virol.* **24**:91–98 (1977).
213. Rodriguez, W. J., Kim, H. W., Arrobio, J. O., Brandt, C. D., Chanock, R. M., Kapikian, A. Z., Wyatt, R. G., and Parrott, R. H., Clinical features of acute gastroenteritis associated with human reovirus-like agent in infants and young children, *J. Pediatr.* **91**:188–193 (1977).
214. Rodriguez, W. J., Kim, H. W., Brandt, C. D., Bise, B., Kapikian, A. Z., Chanock, R. M., Curlin, G., and Parrott, R. H., Incidence of pediatric rotavirus gastroenteritis resulting in admission to hospital in Washington, D.C. area, *Am. J. Dis. Child.* **134**:777–779 (1980).
215. Rodriguez, W. J., Kim, H. W., Brandt, C. D., Yolken, R. H., Arrobio, J. O., Kapikian, A. Z., and Chanock, R. M., Sequential enteric illnesses associated with different rotavirus serotypes, *Lancet* **2**:37 (1978).
216. Rodriguez, W. J., Kim, H. W., Brandt, C. D., Yolken, R. H., Richard, M., Arrobio, J. O., Schwartz, R. H., Kapikian, A. Z., Chanock, R. M., and Parrott, R. H., Common exposure outbreak of type 2 rotavirus gastroenteritis with high secondary attack rate within families, *J. Infect. Dis.* **140**:353–357 (1979).
217. Rohde, J. E., and Northrup, R. S., Taking science where the diarrhea is, in: *Acute Diarrhea in Childhood: Ciba Found. Symp. (new Ser.)* **42**:339–366 (1976).
218. Roseto, A., Lema, F., Sitbon, M., Cavalieri, F., Dianoux, L., and Peries, J., Detection of rotavirus in dogs, *Social Occupat. Med.* **7**:478 (1979).
219. Ryder, R. W., McGowan, J. E., Hatch, M. H., and Palmer, E. L., Reovirus-like agent as a cause of nosocomial diarrhea in infants, *J. Pediatr.* **90**:698–702 (1977).
220. Ryder, R. W., Sack, D. A., Kapikian, A. Z., McLaughlin, J. C., Chakraborty, J., Willis, J. G., Mizanur Rahman, A. S. M., and Merson, M. H., Entertotoxigenic *Escherichia coli* and reovirus-like agent in rural Bangladesh, *Lancet* **1**:659–663 (1976).
221. Sack, D. A., Chowdhury, A. M. A. K., Eusof, A., Ali, Md. A., Merson, M. H., Islam, S., Black, R. E., and Brown, K. H., A double blind comparison of sucrose with glucose electrolyte solution, *Lancet* **2**:280–283, (1978).
222. Sack, R. G., Human diarrheal disease caused by enterotoxigenic *Escherichia coli*, *Annu. Rev. Microbiol.* **29**:333–353 (1975).
223. Salmi, T. T., Arstila, P., and Koivikko, A., Central nervous system involvement in patients with rotavirus gastroenteritis, *Scand. J. Infect. Dis.* **10**:29–31 (1978).
223a. Sato, K., Inaba, Y., Shinozaki, T., Fujii, R., and Matumoto, M., Isolation of human rotavirus in cell cultures, *Arch. Virol.* **69**:155–160 (1981).
223b. Schaffer, F. L., Caliciviruses, in: *Comprehensive Virology, Vol. 14*, Plenum Press, New York and London, pp. 249–284, 1979.
224. Schaffer, F. L., and Soergel, M. E., Single major polypeptide of a calicivirus and characterization by polyacrylamide gel electrophoresis and stabilization of virions by cross-linking with dimethyl suberimidate, *J. Virol.* **19**:925–931 (1976).
225. Schnagl, R. D., and Holmes, I. H., Characteristics of the genome of human infantile enteritis (rotavirus) *J. Virol.* **19**:267–270 (1976).
226. Schnagl, R., Holmes, I. H., Moore, B., Lee, P., Dickinson-Jones, F., and Gust, I. D., An extensive rotavirus outbreak in aboriginal infants in Central Australia, *Med. J. Aust.* **1**:259–260 (1977).
227. Schoub, B. D., Koornof, J. H., Lecatsas, G., Prozesky, O. W., Freiman, I., Hartman, E., and Kassel, H., Viruses in acute summer gastroenteritis in black infants, *Lancet* **1**:1093–1094 (1975).
228. Schoub, B. D., Nel, J. D., Lecatsas, G., Greef, A.

S., PROZESKY, O. W., HAY, I. T., AND PRINSLOO, J. G., Rotavirus as a cause of gastroenteritis in black South African infants, *South Afr. Med. J.* **50**:1124 (1976).
229. SCHREIBER, D. S., BLACKLOW, N. R., AND TRIER, J. S., The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis, *N. Engl. J. Med.* **288**:1318–1323 (1973).
230. SCHREIBER, D. S., BLACKLOW, N. R., AND TRIER, J. S., The small intestinal lesion induced by Hawaii agent acute infectious nonbacterial gastroenteritis, *J. Infect. Dis.* **129**:705–708 (1974).
231. SCOTT, A. C., LUDDINGTON, J., LUCAS, M., AND GILBERT, F. R., Rotavirus in goats, *Vet. Rec.* **103**:145 (1978).
231. SHARPEE, R., AND MEBUS, C. A., Rotaviruses (sic) of man and animals, *Lancet* **1**:639 (1975).
233. SHINOZAKI, T., FUJII, R., SATO, K., TAKAHASHI, E., ITO, Y., AND INABA, Y., Hemagglutinin from human reovirus-like agent, *Lancet* **1**:878 (1978).
234. SIMHON, A., AND MATA, L., Anti-rotavirus antibody in human colostrum, *Lancet* **1**:39–40 (1978).
235. SNODGRASS, D. R., ANGUS, K. W., AND GRAY, E. W., A rotavirus from kittens, *Vet. Rec.* **104**:222–223 (1979).
236. SNOPGRASS, D. R., AND GRAY, E. W., Detection and transmission of 30 nm virus particles (astroviruses) in faeces of lambs with diarrhea, *Arch. Virol.* **55**:287–291 (1977).
237. SNODGRASS, D. R., MADELEY, C. R., WELLS, P. W., AND ANGUS, K. W., Human rotavirus in lambs: Infection and passive protection, *Infect. Immun.* **16**:268–270 (1977).
238. SNODGRASS, D. R., SMITH, W., GRAY, E. W., AND HERRING, J. A., A rotavirus in lambs with diarrhea, *Res. Vet. Sci.* **20**:113–114 (1976).
239. SNODGRASS, D. R., AND WELLS, P. W., Rotavirus infection in lambs: Studies on passive protection, *Arch. Virol.* **52**:201–205 (1976).
240. SPENCE, L., FAUVEL, M., BOUCHARD, S., BABIUK, L., AND SAUNDERS, J. R., Test for reovirus-like agent, *Lancet* **2**:322 (1975).
241. SPENCE, L., FAUVEL, M., PETRO, R., AND BLOCH, S., Comparison of counterimmunoelectrophoresis and electron microscopy for laboratory diagnosis of human reovirus-like agent-associated infantile gastroenteritis, *J. Clin. Microbiol.* **5**:248–249 (1977).
242. SPENCE, L., FAUVEL, M., PETRO, R., AND BLOCH, S., Hemagglutinin from rotavirus, *Lancet* **2**:1023 (1976).
243. SPENDLOVE, R. S., Reoviridae: Rotavirus, in *Section H: Virology and Rickettsiology* (G. D. HSUING AND R. H. GREEN, sect. eds.), *Handbook Series in Clinical Laboratory Science* (D. SELIGSON, ed.-in-chief), pp. 251–265, CRC Press, West Palm Beach, Florida, 1978.
243a. SPRATT, H. C., PARK, M. I., GOMERSALL, M., GILL, P., AND PAI, C. H., Nosocomial infantile gastroenteritis associated with minirotavirus and calicivirus, *J. Pediatr.* **93**:922–926 (1978).
244. STAIR, E. L., RHODES, M. B., WHITE, R. G., AND MEBUS, C. A., Neonatal calf diarrhea: Purification and electron microscopy of a coronavirus-like agent, *Am. J. Vet. Res.* **33**:1147–1156 (1972).
245. STEINHOFF, M. C., DOUGLAS, R. G., JR., GREENBERG, H. B., AND CALLAHAN, D. R., Bismuth subsalicylate therapy of viral gastroenteritis, *Gastroenterology* (in press).
246. STINTZING, G., TUFVESSON, B., HABTE, D., BACK, E., JOHNSSON, T., AND WADSTROM, T., Aetiology of acute diarrhoeal disease in infancy and childhood during the peak season Addis Ababa 1977: A preliminary report, *Ethiop. Med. J.* **15**:141–146 (1977).
247. STUKER, G., OSHIRO, L., AND SCHMIDT, N. H., Antigenic comparisons of two new rotaviruses from rhesus monkeys, *J. Clin. Microbiol.* **11**:202–203 (1980).
248. SUZUKI, H., AND KONNO, T., Reovirus-like particles in jejunal mucosa of a Japanese infant with acute infectious non-bacterial gastroenteritis, *Tohoku J. Exp. Med.* **115**:119–211 (1975).
249. SUZUKI, H., KONNO, T., KUTSUZAWA, T., IMAI, A., TAZAWA, F.; AND ISHIDA, N., The occurrence of calciviruses in infants with acute gastroenteritis, *J. Med. Virol.* **4**:321–326 (1979).
250. SZMUNESS, W., DIENSTAG, J. L., PURCELL, R. H., HARLE, E. J., STEVENS, C. E., AND WONG, D. C., Distribution of antibody to hepatitis A antigen in urban adult populations, *N. Engl. J. Med.* **295**:755–759 (1976).
251. SZMUNESS, W., DIENSTAG, J. L., PURCELL, R. H., STEVENS, C. E., WONG, D. C., IKRAM, H., BAR-SHANY, S., BEASLEY, R. P., DESMYTER, J., AND GAON, J. A., The prevalence of antibody to hepatitis A antigen in various parts of the world: A pilot study, *Am. J. Epidemiol.* **106**:392–398 (1977).
252. TALLETT, S., MACKENZIE, C., MIDDLETON, P., KERZNER, B., AND HAMILTON, R., Clinical, laboratory, and epidemiological features of a viral gastroenteritis in infants and children, *Pediatrics* **60**:217–222 (1977).
253. TAM, J. S., SZYMANSKI, M. T., MIDDLETON, P. J., AND PETRIC, M., Studies on the particles of infantile gastroenteritis virus (orbivirus group), *Intervirology* **7**:181–191 (1976).
254. THEIL, K. W., BOHL, E. H., AND AGNES, A. G., Cell culture propagation of porcine rotavirus (reovirus-like agent), *Am. J. Vet. Res.* **28**:1765–1768 (1977).
255. THORNHILL, T. S., KALICA, A. R., WYATT, R. G., KAPIKIAN, A. Z., AND CHANOCK, R. M., Pattern of shedding of the Norwalk particle in stools during experimentally induced gastroenteritis in volunteers as determined by immune electron microscopy, *J. Infect. Dis.* **132**:28–34 (1975).
256. THORNHILL, T. S., WYATT, R. G., KALICA, A. R.,

DOLIN, R., CHANOCK, R. M., AND KAPIKIAN, A. Z., Detection by immune electron microscopy of 26–27 nm virus-like particles associated with two family outbreaks of gastroenteritis, *J. Infect. Dis.* **135**:20–27 (1977).

257. THOULESS, M. E., BRYDEN, A. S., AND FLEWETT, T. H., Rotavirus neutralization by human milk, *Br. Med. J.* **2**:1390 (1977).
258. THOULESS, M. E., BRYDEN, A. S., AND FLEWETT, T. H., Serotypes of human rotavirus, *Lancet* **1**:39 (1978).
259. THOULESS, M. E., BRYDEN, A. S., FLEWETT, T. H., WOODE, G. N., BRIDGER, J. C., SNODGRASS, G. R., AND HERRING, J. A., Serological relationships between rotaviruses from different species as studied by complement-fixation and neutralization, *Arch. Virol.* **53**:287–294 (1977).
260. TODD, D., AND MCNULTY, M. S., Biochemical studies on a reovirus-like agent (rotavirus) from lambs, *J. Virol.* **21**:1215–1218 (1977).
261. TORRES-MEDINA, A., WYATT, R. G., MEBUS, C. A., UNDERDAHL, N. R., AND KAPIKIAN, A. Z., Diarrhea in gnotobiotic piglets caused by the human reovirus-like agent of infantile gastroenteritis, *J. Infect. Dis.* **133**:22–27 (1976).
262. TORRES-MEDINA, A., WYATT, R. G., UNDERDAHL, N. R., AND KAPIKIAN, A. Z., Patterns of shedding of human reovirus-like agent in gnotobiotic newborn piglets with experimentally-induced diarrhea, *Intervirology* **7**:250–255 (1976).
263. TOTTERDELL, B. M., CHRYSTIE, I. L., AND BANATVALA, J. E., Rotavirus infections in a maternity unit, *Arch. Dis. Child.* **51**:924–928 (1976).
264. TUFVESSON, B., AND JOHNSSON, T., Immunoelectroosmophoresis for detection of reo-like virus: Methodology and comparison with electron microscopy, *Acta Pathol. Microbiol. Scand B* **84**:225–228 (1976).
265. TUFVESSON, B., AND JOHNSSON, T., Occurrence of reo-like viruses in young children with acute gastroenteritis, *Acta Pathol. Microbiol. Scand. B* **84**:22–28 (1976).
266. TUFVESSON, B., JOHNSON, T., AND PETERSON, B., Family infections by reo-like virus, *Scand. J. Infect. Dis.* **9**:257–261 (1977).
267. TZIPORI, S., Human rotavirus in young dogs, *Med. J. Austr.* **2**:922–923 (1977).
268. TZIPORI, S., CAPLE, I. W., AND BUTLER, R., Isolation of a rotavirus from deer, *Vet. Rec.* **99**:398 (1976).
269. URASAWA, S., TANIGUCHI, K., URASAWA, T., SAKURADA, N., KAWAMURA, S., AND AKATSUKA, K., Virus particle detected in an institutional outbreak of acute gastroenteritis, *Igaku no Ayumi* **109**(1):25–27 (1979).
270. VIERA DE TORRES, B., MAZZALI DE ILJA, R., AND ESPARZA, J., Epidemiological agents of rotavirus infection in hospitalized Venezuelan children with gastroenteritis, *Am. J. Trop. Med. Hyg.* **27**:567–572 (1978).
271. WALSH, J. A., AND WARREN, K. S., Selective primary health care and interim strategy for disease control in developing countries, *N. Engl. J. Med.* **301**:967–974 (1979).
272. WHORWELL, P. J., PHILLIPS, C. A., BEEKEN, W. L., LITTLE, P. K., AND ROESSNER, K. D., Isolation of reovirus-like virus agents from patients with Crohn's disease, *Lancet* **1**:1169–1171 (1977).
273. WIDERLITE, L., TRIER, J. S., BLACKLOW, N. R., AND SCHREIBER, D. S., Structure of the gastric mucosa in acute infectious nonbacterial gastroenteritis, *Gastroenterology* **68**:425–430 (1975).
274. WILLIAMS, T., BOURKE, P., AND GURWITH, M., Program abstracts, 15th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C. (Abstr 234), p. 232, 1975.
275. WOODE, G. N., AND BRIDGER, J. C., Isolation of small viruses resembling astroviruses and calciviruses from acute enteritis of calves, *J. Med. Microbiol.* **11**:441–452 (1978).
276. WOODE, G. N., BRIDGER, J. C., HALL, G., AND DENNIS, M. J., The isolation of a reovirus-like agent associated with diarrhea in colostrum-deprived calves in Great Britain, *Res. Vet. Sci.* **16**:102–105 (1974).
277. WOODE, G. N., BRIDGER, J., HALL, G. A., JONES, J. M., AND JACKSON, G., The isolation of reoviruslike agents (rotavirus) from acute gastroenteritis of piglets, *J. Med. Microbiol.* **9**:203–209 (1976).
278. WOODE, G. N., BRIDGER, J. C., JONES, J. M., FLEWETT, T. H., BRYDEN, A. S., DAVIES, H. A., AND WHITE, G. B. B., Morphological and antigenic relationships between viruses (rotaviruses) from acute gastroenteritis of children, calves, piglets, mice, and foals, *Infect. Immun.* **14**:804–810 (1976).
279. WOODE, G. N., JONES, J., AND BRIDGER, J., Levels of colostral antibodies against neonatal calf diarrhoea virus, *Vet. Rec.* **97**:148–149 (1975).
280. WORLD HEALTH ORGANIZATION, Mortality due to diarrheal diseases in the world *Weekly Epidemiol. Rec.* **48**:409–416 (1973).
281. WYATT, R. G., Unpublished studies.
282. WYATT, R. G., DOLIN, R., BLACKLOW, N. R., DUPONT, H. L., BUSCHO, R. F., THORNHILL, T. S., KAPIKIAN, A. Z., AND CHANOCK, R. M., Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers, *J. Infect. Dis.* **129**:709–714 (1974).
283. WYATT, R. G., GILL, V. W., SERENO, M. M., KALICA, A. R., VAN KIRK, D. H., CHANOCK, R. M., AND KAPIKIAN, A. Z., Probable *in vitro* cultivation of human reovirus-like agent of infantile diarrhea, *Lancet* **1**:98 (1976).
284. WYATT, R. G., GREENBERG, H. B., DALGARD, D. W., ALLEN, W. P., SLY, D. L., THORNHILL, T. S., CHANOCK, R. M., AND KAPIKIAN, A. Z., Experimental infection of chimpanzees with the Norwalk agent of

epidemic viral gastroenteritis, *J. Med. Virol.* **2**:89–96 1978.

285. Wyatt, R. G., James, W. D., Bohl, E. H., Theil, K. W., Saif, L. J., Kalica, A. R., Greenberg, H. B., Kapikian, A. Z., and Chanock, R. M., Human rotavirus type 2: Cultivation *in vitro*, *Science* **207**:189–191 (1980).
286. Wyatt, R. G., Kalica, A. R., Mebus, C. A., Kim, H. W., London, W. T., Chanock, R. M., and Kapikian, A. Z., Reovirus-like agents (rotaviruses) associated with diarrheal illness in animals and man, *Perspect. Virol.* **10**:121–145 (1978).
287. Wyatt, R. G., and Kapikian, A. Z., Viral agents associated with acute gastroenteritis in humans, *Am. J. Clin. Nutr.* **30**:1857–1870 (1977).
288. Wyatt, R. G., Kapikian, A. Z., Thornhill, T. S., Sereno, M. M., Kim, H. W., and Chanock, R. M., *In vitro* cultivation in human fetal intestinal organ culture of a reovirus-like agent associated with nonbacterial gastroenteritis in infants and children, *J. Infect. Dis.* **130**:523–528 (1974).
289. Wyatt, R. G., Mebus, C. A., Yolken, R. H., Kalica, A. R., James, H. D., Jr., Kapikian, A. Z., and Chanock, R. M., Rotaviral immunity in gnotobiotic calves: Heterologous resistance to human virus induced by bovine virus, *Science* **203**:548–550 (1979).
290. Wyatt, R. G., Sly, D. L., London, W. T., Palmer, A. E., Kalica, A. R., Van Kirk, D. H., Chanock, R. M., and Kapikian, A. Z., Induction of diarrhea in colostrum-deprived newborn rhesus monkeys with the human reovirus-like agent of infantile gastroenteritis, *Arch. Viol.* **50**:17–27 (1976).
291. Wyatt, R. G., Yolken, R. H., Urrutia, J. J., Mata, L., Greenberg, H. B., Chanock, R. M., and Kapikian, A. Z., Diarrhea associated with rotavirus in rural Guatemala: A longitudinal study of 24 infants and young children, *Am. J. Trop. Med. Hyg.* **28**:325–328 (1979).
292. Yamamoto, A., Zennygogi, H., Yanagita, K., and Kato, S., Research into the causative agent of epidemic gastroenteritis which prevailed in Japan in 1948, *Jpn. Med. J.* **1**:379–384 (1948).
293. Yolken, R. H., Barbour, B., Wyatt, R. G., Kalica, A. R., Kapikian, A. Z., and Chonick, R. M., Enzyme-linked immunosorbent assay (ELISA) for identification of rotaviruses from different animal species, *Science* **201**:259–262 (1978).
294. Yolken, R. H., Kim, H. W., Clem, T., Wyatt, R. G., Kalica, A. R., Chanock, R. M., and Kapikian, A. Z., Enzyme linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis, *Lancet* **2**:263–267 (1977).
295. Yolken, R. H., Wyatt, R. G., Mata, L., Urrutia, J. J., Garcia, B., Chanock, R. M., and Kapikian, A. Z., Secretory antibody directed against rotavirus in human milk—measurement by means of enzyme linked immunosorbent assay, *J. Pediatr.* **93**:916–921 (1978).
296. Yolken, R. H., and Stopa, P. J., Analysis of nonspecific reactions in enzyme-linked immunosorbent assay testing for human rotavirus, *J. Clin. Microbiol.* **10**:703–707 (1979).
297. Yolken, R. H., and Stopa, P. J., Enzyme-linked fluorescence assay: Ultrasensitive solid-phase assay for detection of human rotavirus, *J. Clin. Microbiol.* **10**:317–321 (1979).
298. Yolken, R. H., Wyatt, R. G., Barbour, B. A., Kim, H. W., Kapikian, A. Z., and Chanock, R. M., Measurement of anti-rotavirus antibody by an enzyme-linked immunosorbent (ELISA) blocking assay, *J. Clin. Microbiol.* **8**:283–287 (1978).
299. Yolken, R. H., Wyatt, R. G., Kalica, A. R., Kim, H. W., Brandt, C. D., Parrott, R. H., Kapikian, A. Z., and Chanock, M. R., Use of a free viral immunofluorescence assay to detect human reovirus-like agent in human stools, *Infect. Immun.* **16**:467–470 (1977).
300. Yolken, R. H., Wyatt, R. G., and Kapikian, A. Z., ELISA for rotavirus, *Lancet* **2**:818 (1977).
301. Yolken, R. H., Wyatt, R. G., Kim, H. W., Kapikian, A. Z., and Chanock, R. M., Immunological response to infection with human reovirus-like agent: Measurement of anti-human reovirus-like agent immunoglobulin G and M levels by the method of enzyme-linked immunosorbent assay, *Infect. Immun.* **19**:540–546 (1978).
302. Yolken, R. H., Wyatt, R. G., Zissis, G. P., Brandt, C. D., Rodriguez, W. J., Kim, H. W., Parrott, R. H., Urrutia, J. J., Mata, L., Greenberg, H. B., Kapikian, A. Z., and Chanock, R. M., Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay, *N. Engl. J. Med.* **299**:1156–1161 (1978).

302a. Zahorsky, J., Hyperemesis hiemis or the winter vomiting disease, *Arch. Pediatr.* **46**:391–395 (1929).

303. Zissis, G., and Lambert, J. P., Different serotypes of human rotaviruses, *Lancet* **1**:38–39 (1978).
304. Zissis, G., Lambert, J. P., and De Kegel, D., Routine diagnosis of human rotavirus in stools, *J. Clin. Pathol.* **31**:175–178 (1978).
305. Zissis, G., Lambert, J. P., Fonteyne, J., and De Kegel, D., Child–mother transmission of rotavirus, *Lancet* **1**:96 (1976).

12. Suggested Reading

Brandt, C. D., Kim, H. W., Yolken, R. H., Kapikian, A. S., Arrobio, J. O., Rodriguez, W. J., Wyatt, R. G., Chanock, R. M., and Parrott, R. H., Comparative epidemiology of two rotavirus serotypes and other

viral agents associated with pediatric gastroenteritis, *Am. J. Epidemiol.* **110**:243–254 (1979).

EDELMAN, R., AND LEVINE, M. M., Acute diarrheal infections in infants. II. Bacterial and viral causes, *Hosp. Pract.* **15**:97–104 (1980).

GREENBERG, H. B., WYATT, R. G., KALICA, A. R., YOLKEN, R. H., BLACK, R., KAPIKIAN, A. Z., AND CHANOCK, R. M., New insights in viral gastroenteritis, *Perspect. Virology* **11**:163–187 (1981).

HOLMES, I. H., Viral gastroenteritis, *Prog. Med. Virol.* **25**:1–36 (1979).

KAPIKIAN, A. Z., WYATT, R. G., GREENBERG, H. B., KALICA, A. R., KIM, H. W., BRANDT, C. D., RODRIGUEZ, W. J., PARROTT, R. H., AND CHANOCK, R. M., Approaches to immunization of infants and young children against gastroenteritis due to rotaviruses, *Rev. Infect. Dis.* **2**:459–469 (1980).

KAPIKIAN, A. S., YOLKEN, R. H., GREENBERG, H. B., WYATT, R. G., KALICA, A. R., CHANOCK, R. M., AND KIM, H. W., Gastroenteritis viruses, in: *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, 5th ed. (E. H. LENNETTE AND N. J. SCHMIDT, eds.), pp. 927–995, American Public Health Association, Washington, D.C., 1979.

SPENDLOVE, R. S., Reoviridae: Rotavirus, in: *Section H: Virology and Rickettsiology* (G. D. HSUING AND R. H. GREEN, sect. eds.), *Handbook Series in Clinical Laboratory Science* (D. SELIGSON, ed.-in-chief), pp. 251–265, CRC Press, West Palm Beach, Florida, 1978.

YOLKEN, R. H., WYATT, R. G., ZISSIS, G. P., BRANDT, C. D., RODRIGUEZ, W. J., KIM, H. W., PARROTT, R. H., URRUTIA, J. J., MATA, L., GREENBERG, H. B., KAPIKIAN, A. Z., AND CHANOCK, R. M., Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay, *N. Engl. J. Med.* **299**:1156–1161 (1978).