Viruses and diarrhoea

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Acute gastro-enteritis is one of the leading causes of morbidity in infancy and childhood throughout the world and probably the leading cause of death in infancy in developing countries. Rhode & Northrup (1976) estimated that, in 1975, approximately 500 million episodes of diarrhoea occurred among children in Asia, Africa and Latin America, and this resulted in between five and 18 million deaths.

Known bacterial pathogens cannot be implicated in up to 75% of outbreaks of acute diarrhoeal disease (Cramblett & Siewers, 1965). Nevertheless, until recently gastro-enteritis provided a poor hunting ground for virologists for, although such viruses as adenoviruses, echoviruses and Coxsackie viruses could be isolated from the stools of patients with acute gastro-enteritis, they could often be recovered with almost equal frequency from those without diarrhoeal disease, particularly in developing countries. However, the examination of negatively stained faecal preparations from patients with gastro-enteritis by electron microscopy has resulted in the discovery of many viruses, some of which, for example, rotaviruses and such parvovirus-like viruses as the Norwalk agent, are undoubtedly the cause of acute gastro-enteritis. The role of such other viruses as astroviruses, caliciviruses, adenoviruses and coronaviruses remains as yet to be clearly established. In general, in vitro cultivation of viruses causing gastro-enteritis in humans is either not possible or extremely difficult and of little value for routine diagnostic purposes but, since they are often excreted in very high titres, they may be detected without difficulty by electron microscopic examination of faecal extracts.

Viruses known to cause gastro-enteritis

1. Parvovirus-like viruses

Small virus particles, approximately 27 nm in diameter, with the morphology and biophysical characteristics of parvoviruses have been detected in bacteria-free filtrates of stools from patients with the syndrome of winter vomiting disease (endemic nausea and vomiting). Family and community-wide outbreaks which occur during winter months have been described, including outbreaks in schools (Kapikian et al., 1972), in which up to 50% of the staff and children were affected. Secondary attack rate of up to 30% occurred among family contacts, all age-groups being affected. Nausea, vomiting—sometimes projectile—and vertigo are the symptoms most commonly encountered; some patients have diarrhoea and abdominal pain. The disease has an incubation period of 24 to 36 hours and runs a short course, the duration of which is usually from 24 to 72 hours. Parvovirus-like particles may be detected in the stools by electron microscopy during the acute phase of the infection, peak excretion occurring about 36 hours after onset. An immune response may be detected by immune electron-microscopy. The first outbreak in which the virus was characterized occurred in a primary school in Norwalk, Ohio (Kapikian et al., 1972). Since then, parvovirus-like viruses have been detected in further outbreaks in the U.S.A. (Wyatt et al., 1974) as well as in the U.K. (Appleton et al., 1977). Cross challenge studies in volunteers, together with studies by immune electron microscopy, indicate that these strains exhibit antigenic diversity (Wyatt et al., 1974). Thus, although the Norwalk agent is closely related to the Montgomery County agent which was detected in a family outbreak in Maryland, U.S.A., both these viruses are unrelated to that which caused an outbreak of winter vomiting disease in Hawaii (Thornhill et al., 1977). W and Ditchling agents are antigenically related and caused outbreaks in schools in England (Clarke et al., 1972; Appleton et al., 1977). However, the virus detected in faecal extracts of patients with gastro-enteritis following ingestion of cockles in 1976 (Appleton & Pereira, 1977) appears to be unrelated to W or Ditchling agents. U.K. and U.S. strains are apparently unrelated (Appleton & Pereira, 1977). Antigenic comparison of strains detected in an extensive epidemic in a primary school in Australia, in which 200 of 381 children and nine of 18 teachers were involved, has not as yet been reported (Christopher et al., 1978). Recently, a sensitive radio-immuno-assay test has been developed for detecting antibodies to Norwalk agent (Greenberg et al., 1978). Studies on sera collected in Washington, D.C., showed that antibody is acquired gradually with age, about 50% of adults having immunity. The role of parvovirus-like viruses in acute gastro-enteritis in developing countries remains to be determined but preliminary studies conducted on sera collected in Guatemala and rural Bangladesh suggest that the incidence of infection does not differ markedly from that in developed countries, since the pattern of acquisition of antibody is similar (Greenberg & Kapikian, 1978).

2. Rotaviruses

Rotaviruses form a distinct genus in the family of reoviridae. Virus particles contain double-stranded RNA and are approximately 65 to 70 nm in diameter and have a characteristic double capsid. Radiating
spoke-like capsomeres are a characteristic feature of the inner capsid, this being surrounded by the outer capsid which provides the particle with a sharply defined circular outline (Fig. 1). These viruses have been provisionally designated “rotavirus” because of the similarity of the intact particle to the appearance of a wheel (rota = Latin for wheel) (FLEWETT et al., 1974). Rotaviruses have been detected, not only in children but in the young of many animal species, including mice, calves, piglets, monkeys, lambs, voles, foals, antelopes and rabbits; virus-specific antibodies have been detected in brown bears, guinea-pigs and dogs (DERBYSHIRE & WOODE, 1978). It seems probable that the young of all mammalian species may have their own rotaviruses. Group-specific antigens associated with the inner capsid layer are probably common to rotaviruses of all animal species; antigens associated with the outer capsid are species-specific (WOODE et al., 1976).

Rotaviruses were first detected in children by electron-microscopy in ultra-thin sections of the duodenal mucosa of six of nine babies during the acute phase of gastro-enteritis but viruses were not detected in babies who did not have diarrhoeal disease (BISHOP et al., 1973). Within a short period, similar virus particles were detected by negative staining techniques in faecal extracts of children with acute gastro-enteritis in many parts of the world, including the tropics (LANCET, 1975). The accumulated data from studies in temperate climates showed that infection occurred most frequently in children between six and 24 months of age (by school age most children have antibody) (ELIAS, 1977) and that infection occurred most commonly during winter months. Thus, during the course of any one year, rotaviruses may be detected in the stools of up to 40 to 50% of children with acute gastro-enteritis but rarely among controls; during winter months the incidence might rise as high as 75 to 80% (DAVIDSON et al., 1975). The incubation period is usually of the order of one to three days and rotaviruses are generally excreted in very large quantities (up to $10^{10}$ particles per gram of faeces), virus excretion usually persisting for about a week, being maximum from three to four days after the onset of symptoms. A study among children admitted to hospital in Toronto showed that, in approximately a third of cases, rotavirus infection, often severe, was the result of hospital-acquired infection (MIDDLETON et al., 1977). Rotavirus infection may also be endemic in newborn nurseries but, in contrast with findings in older children, the newborn generally experiences mild or asymptomatic infection (CHRISTIE et al., 1978). The reason for this is unknown. Rotavirus has been reported less frequently among adults. However, approximately 25 to 35% of adult family contacts of children with rotavirus diarrhoea may show a rise in rotavirus antibody titre and some have mild diarrhoea (KAPIKIAN et al., 1976; TUFVESSON et al., 1977). However, despite the fact that most adults have antibodies to rotavirus, rotaviruses have been detected in the stools of adults with moderately severe gastro-enteritis who had not had a known close contact with children (VON BONSDORFF et al., 1976). Furthermore, outbreaks of rotavirus infection have even been reported in homes for old people.

In developing countries, such factors as malnutrition and debilitating diseases may influence the outcome of any acute diarrhoeal episode. However, there have been comparatively few studies conducted in the tropics in which specimens have been collected over a sufficiently extended period to show whether rotavirus infection is caused by climatic factors. Those that have show no consistent pattern (Table). Thus, studies in southern India (Vellore) and central Australia (Alice Springs) suggest that rotavirus infection occurs most commonly during the cooler months. However, in parts of those countries with high ambient temperatures throughout the year (Calicut and Darwin), there was an increased prevalence of rotavirus infection during or just after the rainy season (MATHAN et al., 1977; SHNAGL et al., 1977; Walker & Marshall, 1978, personal communication). However, in Central America (Guatemala and Costa Rica), there was no apparent association between ambient temperature and rotavirus infection (WYATT et al., 1978), although studies in Costa Rica suggested that rotavirus infection tended to occur during periods of low relative humidity (MATA, 1978). Rotavirus could only be detected in 14% of episodes of acute diarrhoeal disease in Guatemala. However, infections were generally more severe than those caused by other agents, since they caused vomiting and dehydration significantly more frequently (WYATT et al., 1978). In Bangladesh, rotaviruses were associated with
of episodes of acute diarrhoeal disease and, as in temperate climates, infection occurred most commonly in children between six and 24 months of age (Yolken, 1978, personal communication). It is encouraging that studies carried out in southern India suggest that oral rehydration may be used effectively in the treatment of infants with rotavirus-induced diarrhoea (MATHAN et al., 1977; Mathan, 1978, personal communication).

Currently, evidence is accumulating that there are multiple serotypes of human rotaviruses (FLEWETT et al., 1978; FONTENYE et al., 1978; ZISSIS & LAMBERT, 1978) and studies on children who have been reinfected have shown that this usually results from infection by different serotypes. Large-scale prospective studies to assess the role of rotaviruses as a cause of severe gastro-enteritis in developing countries were, until recently, hindered by the fact that such sophisticated techniques as electron microscopy were required for diagnosis. However, the ELISA test is of comparable sensitivity to electron microscopy and can be readily carried out in developing countries with little cost, provided standardized reagents are available (YOLKEN et al., 1977). The ELISA test may be used, not only to detect rotaviruses and compare their antigenicity, but also to detect immune responses to them. It is hoped that much invaluable epidemiological data relating to the role of rotavirus infection in different communities in developing countries may result from the widespread use of this technique.

3. Other viruses

The role of such other viruses as astroviruses, caliciviruses, coronaviruses and even adenoviruses as a cause of acute diarrhoeal disease in infancy and childhood must for the present remain more speculative, for these viruses may often be detected among asymptomatic controls. Other viruses may also be present in faecal extracts at the same time, and virus excretion may not always be associated with the development of immune responses.

Astroviruses are approximately 25 to 28 nm in diameter, have an entire outer edge and a star-shaped surface configuration which may be five- or six-pointed (MADELEY & COSGROVE, 1975) (Fig. 2). Evidence is beginning to accumulate to suggest that these viruses may well be human pathogens in young babies. Thus, studies in Glasgow have shown that about 80% of babies excreting virus have gastro-intestinal symptoms and that, when viruses are also present in faecal extracts, astroviruses are usually the first to appear (Madeley, 1978, personal communication). An outbreak of astrovirus-associated diarrhoea in a children's ward affecting both children and staff has recently been described, and some of those affected were shown to develop an immune response (KURTZ et al., 1977). Astroviruses have also been shown to cause mild gastro-intestinal symptoms when fed to adult volunteers (REID et al., 1978).

Caliciviruses are of approximately 30 nm diameter and appear to have characteristic stain-filled, cup-like depressions on the surface of the particle (MADELEY & COSGROVE, 1976) (Fig. 3). Their association with acute gastro-enteritis is
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Fig. 2. Electronmicrograph of astroviruses (× 200,000).

rather more tenuous than that of astroviruses as yet, although there has been a recent report of calicivirus-like virus particles being detected in the stools of children and staff in an outbreak of winter vomiting disease in a primary school (McSWIGGAN et al., 1978).

Since coronaviruses are important causes of severe diarrhoeal disease in pigs (transmissible gastro-enteritis) and calves (TAJIMA, 1970; STAIR et al., 1972), many workers have hoped to identify their counterpart in man. However, unlike rota-viruses, evidence that coronaviruses cause human gastro-intestinal infection is currently somewhat tenuous. Corona-like virus particles have been detected in patients with both acute and chronic diarrhoeal disease, as well as in asymptomatic persons. Although some workers have expressed doubt as to whether the corona-like virus particles detected were indeed viruses rather than bacterial substructures or fragments of gut epithelium, it is encouraging that coronavirus-like particles have now been propagated in both cell and organ cultures (CAUL & EGGLESTONE, 1977). Fringed, pleomorphic particles, resembling "coronaviruses, have been detected in 90% of faecal extracts obtained in parts of southern India where tropical sprue is endemic. These particles were present in all age groups except for newborn infants, but they were detected with almost equal frequency among patients and asymptomatic persons (MATHAN et al., 1975; MATHAN & MATHAN, 1978). The role of these virus-like structures in tropical sprue is therefore far from clear. However, it is important that further studies, in which attempts are made to cultivate these virus-like particles, be carried out.

Although adenoviruses may be present in the stools of children with and without diarrhoeal disease, explosive outbreaks in hospitals of acute gastro-enteritis in infancy and childhood may sometimes be caused by adenoviruses (FLEWETT et al., 1975) and, on occasion, infection may be fatal (WHITEHAW et al., 1977). It is of interest that, despite the presence of very large quantities of virus, enteric adenoviruses can rarely be propagated in cell culture (MADELEY et al., 1977).

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