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New Concepts in Viral Gastroenteritis

Summary: The use of negative contrast electronmicroscopy of stool suspension in the investigation of the aetiology of childhood gastroenteritis has led to the recognition of a number of candidate viral agents. There is convincing evidence that rotavirus is the single most important cause of community acquired gastroenteritis, and is responsible for some nosocomial outbreaks. The epidemiology of rotavirus acquisition, differential clinical susceptibilities of young and older infants, pathogenesis of disease, mechanisms of immunity and breast milk protection, and the role of different viral serotypes are aspects as yet poorly understood; and attempts to propagate human rotavirus in tissue culture have met with only limited success. Moreover, the aetiologically unaccountable one third of cases of infantile diarrhoea, and the association of enteritis with up to six other virus-like particles add to the complexity of the problem. This review considers the available data from human and animal studies, and based on the experience of ourselves and others comments on the present state of knowledge and trends in continuing research.

Zusammenfassung: Neuere Vorstellungen zur Virus-Gastroenteritis. Die Untersuchung von Stuhl-Aufschwemmungen mit Negativ-Kontrast-Elektronenmikroskopie zur Erforschung der Ätiologie der Gastroenteritis im Kindesalter hat zur Entdeckung einer Reihe von in Frage kommenden viralen Erregern geführt. Es bestehen überzeugende Hinweise dafür, daß das Rotavirus die allerwichtigste Ursache für die nicht im Krankenhaus erworbene Gastroenteritis ist und daß es für einige Ausbrüche von nosokomialen Erkrankungen verantwortlich ist. Die Epidemiologie der Rotavirus-Infektion, die unterschiedliche klinische Empfänglichkeit von jungen und älteren Säuglingen, die Pathogenese der Erkrankung, Mechanismen der Immunität und Schutz durch Muttermilch sowie die Rolle verschiedener viraler Serotypen sind Aspekte, von denen wir bisher noch wenig wissen. Versuche, menschliches Rotavirus in Gewebekultur zu züchten, waren bisher nur von begrenztem Erfolg. Darüber hinaus wird die Komplexität des Problems durch das ätiologisch nicht zuordenbare Drittel von Fällen kindlicher Diarrhoe und die Assoziation der Enteritis mit bis zu sechs weiteren virus-

ähnlichen Partikeln noch vermehrt. Die vorliegende Übersicht befaßt sich mit derzeit verfügbaren Fakten von Untersuchungen bei Menschen und Tieren und diskutiert auf der Basis eigener Erfahrung und derjenigen anderer Untersucher den gegenwärtigen Wissensstand und den Trend weiterer Untersuchungen.

Introduction

Until the nineteen seventies evidence for the existence of viral gastroenteritis relied on animal and human passage experiments, and studies using tissue culture of stool. *Cramblett* et al. (1) provided a contemporary critique of much of this data, which has since been reviewed by *Dupont* et al. (2) and *Steinhoff* (3). Some studies of diarrhoea occurring in closed populations gave encouraging results, but most studies of sporadic diarrhoea yielded negative or conflicting information. Certain enteroviruses (4) and adenoviruses (5, 6) were considered potential enteric pathogens, but confirmatory evidence was lacking in other studies (7, 8, 9).

Investigations of an outbreak of gastroenteritis which occurred in Norwalk, Ohio in 1968 suggested that the epidemic had been caused by a featureless 27 nm virus (10). Experiments with this particle are continuing and the accumulated data have been reviewed by *Steinhoff* (3). Clinical symptoms could be reproduced in adult volunteers infected by ingestion of faecal filtrate containing Norwalk agent (11), and subsequently histopathological abnormalities were observed in small intestinal biopsies obtained from symptomatic and asymptomatic subjects (12). Immune electronmicroscopic studies using paired sera from volunteers suggested that some had seroconverted to Norwalk antigen (10), but this method is particularly difficult to apply using faecal material as a source of virus since spontaneous aggregation of particles is very common in stool, and the surface appearance of particles can be highly variable even within the same specimen. The use of control materials and a coded system for results adds a measure of confidence to the work quoted. Three other outbreaks of gastroenteritis have been attributed to morphologically similar particles (13, 14), one of which may be antigenically related to Norwalk agent.

Extension of the use of negative contrast electronmicroscopy of stool suspension in the investigation of the aetiology of mainly childhood gastroenteritis has resulted in recognition of a number of candidate viral agents. *Flewett* (15) and *Middleton* et al. (16) have proposed a morphological classification which recognises seven virus-like particles:

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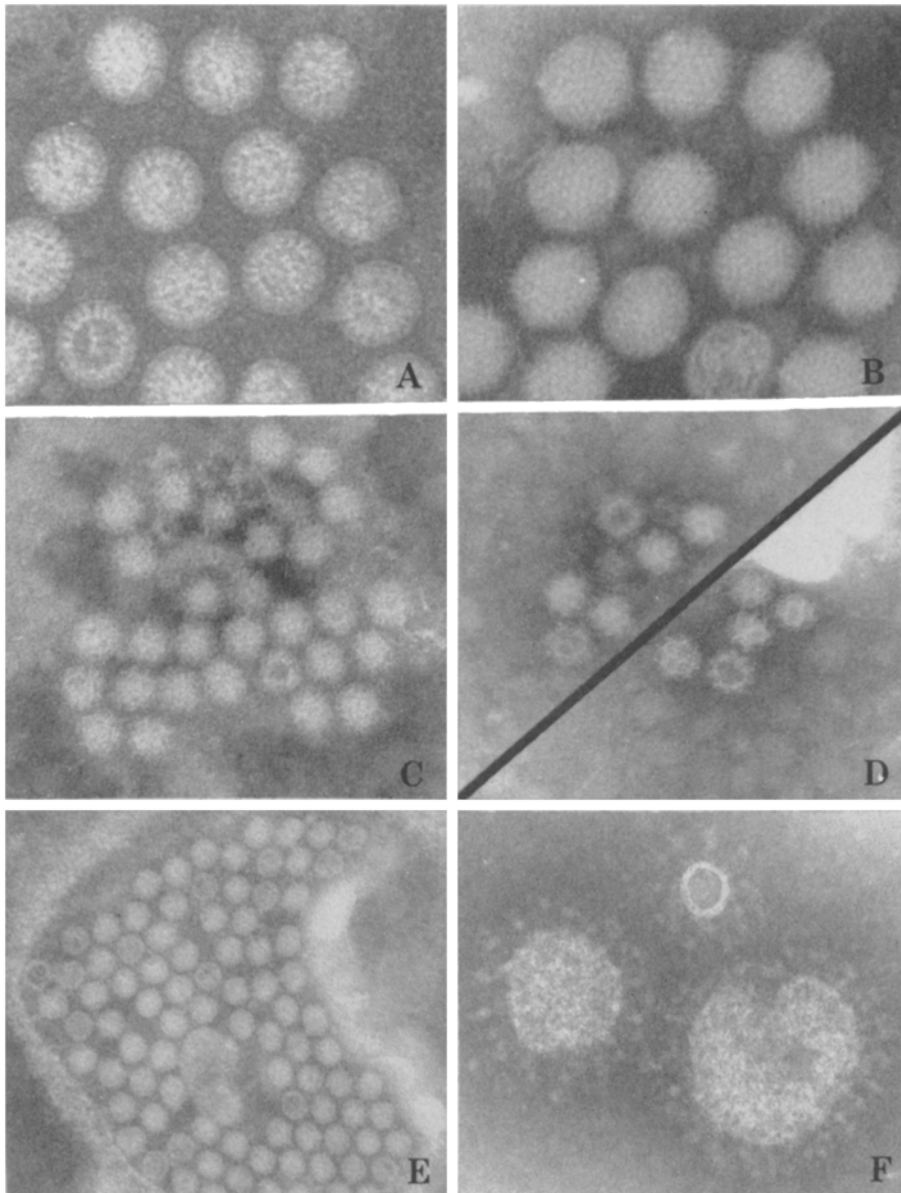
Figure 1: Electronmicrographs of human enteritis associated virus-like particles (magnification 200,000 times): A) rotavirus; B) adenovirus; C) minirotavirus; D) calicivirus; E) picorna-parvovirus; F) coronavirus.

Rotavirus (also called reovirus-like agent, infantile gastroenteritis virus, duovirus or orbivirus)
Adenovirus
Coronavirus
Minirotavirus (also called minireovirus)
Calicivirus
Astrovirus
Small featureless particles

Rotavirus

Calf rotavirus was identified in 1969, and serial propagation of two strains was reported two years later (17). Rotavirus diarrhoea is now recognised in numerous animal species; and although isolates possess species specific antigenic determinants, they also bear one or more common group antigens (18). Cross antigenicity between calf and human rotaviruses has been utilised in many studies of human disease (19).

Human rotavirus (Figure 1a) was first identified in Melbourne (20), Birmingham (21) and Toronto (22), and has subsequently been found worldwide (2). Prospective controlled epidemiological studies from several centres have demonstrated the prevalence of rotavirus gastroenteritis, especially in wintertime, accounting for half or more of cases admitted to hospital (23, 24, 25). Humoral antibody is present in up to 90% of children (26). In addition to childhood gastroenteritis, rotavirus has been associated with intestinal intussusception in children (27), and adult gastroenteritis (28). It has not been convincingly associated with chronic diarrhoeal states, or extra-intestinal disease. Identification of rotavirus-like particles in Crohn's tissue is unconfirmed (29).

The pathology of rotavirus gastroenteritis appears to be limited to the small intestine. Virus particles have been demonstrated in duodenal mucosa (20), and apparently transient abnormalities of D-xylose absorption have been

documented in six infants shown to have rotavirus in duodenal aspirate during acute gastroenteritis (30). Viral cytotoxicity is undoubtedly contributory, but the exact pathogenesis of disease is unclear (31).

Clinically, rotavirus gastroenteritis is most severe in children under two years of age. Disease is characterised by a short incubation period of less than three days, followed by the sudden onset of vomiting and diarrhoea (32, 33). Fever is usual and core temperatures ranging from 39–40° C are common. Dehydration is often marked in young infants and in cases of intractable vomiting. Isotonic dehydration is most common (33); but in at least one large series of unselected patients, 47 of 75 (63%) clinically dehydrated infants had raised serum sodium concentrations (34). Also at least 16 of 21 fatal cases of rotavirus gastroenteritis studied in Toronto had hypernatraemic dehydration, and in many there was a paucity of clinical signs of impending danger (35).

The clinical course is variable, and symptoms may be especially mild in neonates (36). Vomiting usually precedes diarrhoea and both are invariably present in cases admitted to hospital. In such presumably more severely affected children, vomiting and fever are short-lived and usually last for less than three days, whilst diarrhoea persists for an average of five days (33). Stools are watery and especially frequent during the initial diarrhoea; blood, mucus and inflammatory cells are sometimes present in bacterial diarrhoeas. Faecal losses are significantly increased, and stool concentrations of sodium and chloride are increased 2–4 times (37).

Diagnosis is most often made by negative contrast electronmicroscopy of stool suspension showing characteristic virus particles (38). Parenthically, this technique may reveal the presence of other virus-like particles in stool. Several immunological means of diagnosis have been proposed, of which the enzyme linked immunosorbent assay (ELISA) seems most promising (39). A novel culture technique has been developed by *Bryden* et al. (40), but it may be less suitable for routine diagnosis.

Treatment is symptomatic with attention to oral hydration. The use of clear fluids is traditional; but although there is evidence of reduced mucosal disaccharidase activity (20), lactose intolerance seems to have little clinical importance in infants fed milk preparations (37). Uncommonly dehydration is severe enough to necessitate intravenous rehydration; and in such infants fluids, electrolyte and acid-base requirements are of first importance. Complications such as lung aspiration, encephalopathy, cardiac arrhythmias and transient renal impairment are rare. About one third of patients have concurrent respiratory symptoms which have not been shown to be due to rotavirus (37).

The prognosis for uncomplicated gastroenteritis is excellent. But in underdeveloped countries the clinical course is often complicated by preexisting malnutrition and chronic infection, and this underlies the massive mortality due to diarrhoeal disease in the Third World (41).

Data concerning the communicability of rotavirus disease is sparse, but studies of spread in neonatal populations, hospital wards, and families have emphasised the mild or asymptomatic nature of disease in many close contacts (36, 37, 42–44). These data raise questions about the epidemiology of rotavirus acquisition, and the mechanisms of immunity and breast milk protection. These complex problems are now receiving the attention of several investigators. *Simhon* and *Mata* (45) have identified anti-rotavirus factor(s) in human colostrum, which they believe may be IgA antibody. However, the confident hypothesis that breast feeding provides immunological protection to the young infant has been questioned by the data of *Schaub* et al. (46), who showed no fluorescent antibody in human colostrum from seropositive mothers against the antigenically related simian rotavirus, despite the presence of normal total IgA levels.

Lecce et al. (47), have described the epidemiology of rotavirus acquisition in an artificial neonatal pig unit. Their data provide strong inferential support for the view that protection of young piglets is afforded by sow colostrum, and that the crucial factor in infection is the concentration of virus inoculum delivered to the young animal. This invokes the concept of an epidemiological threshold which must be breached within a susceptible population in order to produce epidemic diarrhoea, and is in part consistent with the reports of *Murphy* et al. (36), and *Totterdall* et al. (42), showing endemicity of rotavirus colonisation in infant nurseries in London and Sydney.

Thus far attempts to serially propagate human rotavirus have failed. *Wyatt* et al. (48), have demonstrated propagation of viral antigen in human foetal intestinal organ culture and were able to pass an apparently defective virus in human embryonic kidney monolayer culture (49). But they were unable to sustain virus growth, or reproduce their success with the latter system. *Bryden* et al. (40), have used a novel technique of cell inoculation to demonstrate propagation of virus antigen in a single generation system. This method has been adapted to provide a neutralisation test for rotavirus antibody, and preliminary data suggest the existence of several serotypes (50). The clinical significance of serotype differences is unclear, but second episodes of rotavirus gastroenteritis have been observed in hospitalised children (*Middleton, P. J.,* personal communication, Montreal, 1978).

Adenovirus

The standing of adenovirus (Figure 1b) as enteric pathogens is obscured by conflicting data from culture and electronmicroscopic studies (1, 16, 25, 51). A significant association of adenoviruses and diarrhoea was found in two early studies of sporadic diarrhoea (5, 6), but others failed to substantiate these findings (9). More recently it has been recognised that some enteritis-associated strains of adenovirus cannot be cultured by routine laboratory methods, and that many grow poorly and cannot be

sustained by serial passage (16, 25, 51). These findings question the validity of tissue culture as a solitary investigational method.

Adenoviruses are seen with some frequency in diarrhoeal stool examined by electronmicroscopy (16, 25), and have been detected in duodenal aspirate in association with transient abnormalities of D-xylose absorption (30). An unidentified adenovirus was the presumed cause of an epidemic of nosocomial diarrhoea, suggesting the potential for lateral transmission and a correlation with symptoms in close contacts (51). Confirmation of the significance of these data has been hindered by the difficulties of virus identification mentioned above, and the lack of histological evidence of enteritis, and/or an animal model of disease. Moreover, the interpretation of data is complicated by the frequent association of respiratory disease and diarrhoea, and the finding of virus in stool does not necessarily imply enteric replication.

It must be concluded that evidence supporting the aetiological role of adenoviruses in infantile gastroenteritis remains largely circumstantial, and that a great deal of basic information is not yet available. The authors believe that at least some strains of adenovirus are potential enteric pathogens; but that patient age, conditions and route of exposure, inoculum size and the status of host respiratory defences are likely to influence the expression of disease.

Coronavirus

Coronaviruses are pleomorphic fringed particles, and have been identified in association with the human common cold syndrome, enteritis in a number of domestic and farm animals, and a variety of other diseases (52). Coronavirus-like particles (Figure 1f) have been associated with human enteritis in two uncontrolled studies (53, 54); and have been found in stools from normal children and adults in India, where their presence was tentatively linked to sub-clinical intestinal disease (55). Propagation of a human enteric coronavirus has been reported, but attempts to reproduce this finding with a second isolate were unsuccessful (56). Because of inconsistent epidemiological data and morphological variations of putative particles, it seems best to suspend judgement of the aetiological claims of these particles, and the authors are unconvinced of the viral nature of some fringed structures.

Small viruses

Small round viruses were found in diarrhoeal stool in a number of early investigations (57). Further studies have enabled characterisation of four particle types, which show consistent morphological differences and breed true in small epidemics of gastroenteritis (16, 44, 58).

Minirotavirus

Minirotavirus (Figure 1c) is the name we have chosen for a distinct 32 nm particle identified in infants in Montreal (44). A morphologically indistinguishable particle has been identified in Toronto ("minireovirus"), where the incidence of associated gastroenteritis ranked second only to rotavirus disease in the year 1975/76 (16). Unnamed particles identified in Glasgow (25), and Melbourne (59), closely resemble the Canadian viruses.

Preliminary data suggest that minirotavirus associated gastroenteritis is more prevalent in wintertime, and tends to be a greater threat in closed populations (16, 44, 59). We have observed a minimum incubation period of two days in close contacts, and in a small series of 20 affected infants vomiting and/or diarrhoea occurred in almost all, usually without fever. In most cases diarrhoea did not last more than five days, and viral shedding was not demonstrable after the first week of illness (44). These clinical features are similar to those associated with rotavirus infection (32, 33), except in the absence of fever in most of our patients. *Cameron* et al. (59), have commented on the apparent lack of serious symptoms in affected newborn infants, but the significance of this is unclear.

Calicivirus

Caliciviruses (Figure 1d) are morphologically distinct 28 nm particles, belonging to a sub-group of picornaviruses (60), and only recently recognised as potential human pathogens (61). They have been associated with infantile gastroenteritis in at least four centres (16, 44, 61, 62), with a single reported fatality (62). Preliminary data suggest that they share most of the epidemiological and clinical characteristics described for minirotavirus infection, and show the same propensity for nosocomial spread (44).

Astrovirus

Astroviruses (Figure 2) have been identified in British centres (15, 58), but particles fulfilling the strict morphological criteria of *Madely* and *Cosgrove* (63), have seldom been encountered in other countries. Based on the Glasgow experience, astroviruses may be an important cause of sporadic gastroenteritis (25), but the frequency of dual viral infections and symptomatic variations make interpretation difficult. Moreover, data on communicability and clinical features are lacking. The description of a modified tissue culture system (64), holds out the prospect of early advances in our knowledge of the epidemiology and patient's response to astrovirus infection.
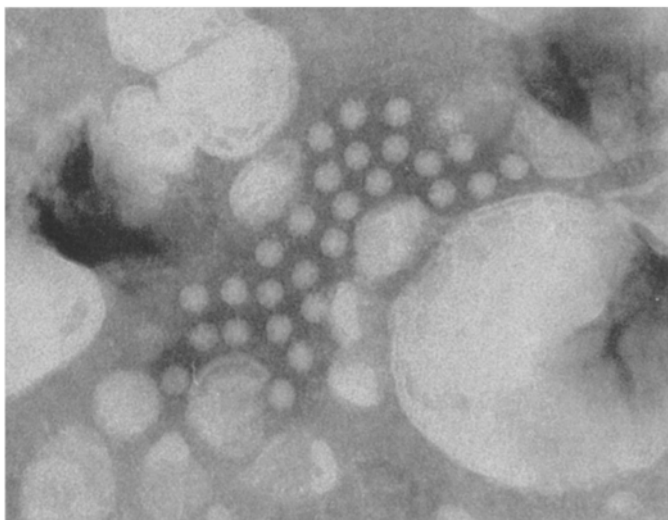
Figure 2: Electronmicrograph of human astroviruses (magnification 200,000 times).

Small featureless particles

Small featureless particles (Figure 1e) sometimes called "picorna-parvoviruses", are a common finding in stool from ill and well children (16, 14, 44). They probably represent a heterogeneous group of viruses; and there are many uncertainties about the exact nature of these particles, and their claim as enteric pathogens. Some particles (e.g. Norwalk agent) have caused outbreaks of gastroenteritis in confined populations (14, 65, 66). But prospective studies of sporadic and nosocomial gastroenteritis have not shown a convincing association between small featureless viruses and diarrhoea (16, 24, 25, 44). The clinical features of the Norwalk outbreak (65) resemble those observed in our patients with minirotavirus and calicivirus.

In considering the standing of particles other than rotavirus as aetiological agents, it must be stated that there is as yet insufficient data to prove cause and effect, or to understand the epidemiology of virus distribution in the community. Drawing upon the Norwalk experience and the preliminary data reviewed above, it seems likely that small viruses play a limited role in sporadic diarrhoea; but that certain particles are true enteric pathogens and are more likely to produce disease in closed populations. Asymptomatic carriage has been documented in each virus group; a finding consistent with studies of rotavirus acquisition, especially in young infants (36), and family contacts (37).

Increased awareness of small viruses in stool has already led to more detailed reporting of their association with gastroenteritis, and the development of serological techniques and investigations with animal model systems is presently under consideration. These methods served to establish the accepted aetiological role of human rotavirus, and pending advances in tissue culture techniques are the best immediate hope for expansion of our knowledge of other candidate particles.

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