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Recent Developments in Viral Gastroenteritis

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In recent years impressive advances have been made in understanding viral gastroenteritis. At last there is proof for the practitioner's long-held assumption that such an entity exists. There really is a "virus going around" in that a specific causative agent has been identified in human infants and young children. Furthermore, studies of a similar but distinct enteritis in piglets have given us new insight into the pathogenesis of viral diarrhea. This article discusses these recent findings in veterinary and human medicine. They open up intriguing prospects for future research that should have a real impact on a devastating worldwide disease.

VIRAL GASTROENTERITIS IN THE PIG

Transmissible gastroenteritis is a specific viral enteritis highly contagious among pigs which can be produced experimentally in young animals.[9] Suckling pigs are resistant to the infection. The causative agent is a corona virus[24] with an RNA genome and a diameter of approximately 80 to 90 nm. It infects the proximal portion of the small bowel although it may extend to more distal portions and possibly to regional

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nodes. The virus has been identified by electron microscopy and by immunofluorescence in the enterocytes on villi rather than in the crypts.[20] Infected cells are shed and the migration of epithelial cells up from the crypts is accelerated in infected bowel.[19] A lesion may or may not be seen in the mucosa. The most severe damage, diffuse blunting of villi and changes in the epithelial cells from a columnar to cuboidal configuration, occurs when large doses of virus are administered to young pigs. The commonest electron microscopic finding is a spotty shortening and sparsity of microvilli. Severe clinical disease may occur in the absence of any measurable microscopic lesion of the mucosa.[13] Although young pigs usually die quickly from dehydration and acidosis, those that survive seem to recover completely within a week.

Information on the timing of the epithelial events after controlled infection of pigs with virus has led us to propose that when gut function is most deranged in transmissible gastroenteritis, the villi are covered with relatively immature crypt-type epithelial cells containing little or no virus. Pensaert has demonstrated virus antigen in the jejunal epithelial within 10 hours of infection; infected cells then desquamate or disrupt and are replaced by cells from the crypts.[20] Within 16 hours, probably few infected cells remain in the jejunal mucosa and yet diarrhea, which usually begins at about 12 hours, does not peak in severity until between 16 and 40 hours after infection.

Our experiments have been carried out in pigs two to three weeks of age, infecting the animals with a constant dose of virus which produces severe but self-limited diarrhea and little or no structural damage in the mucosa.[3, 13] Diarrhea occurs within 18 hours and is massive by 40 hours after infection. The watery stools contain increased concentrations of sodium, potassium, and chloride but no significant excess of sugar or fat (Table 1). Mucosal disaccharidases, alkaline phosphatase, and sodium-potassium-dependent adenosine triphosphatase are significantly depressed in activity in the small intestine,[13] but adenyl cyclase activity is normal throughout the gut.[3]

These findings point to disordered electrolyte transport as an important factor in the pathogenesis of transmissible gastroenteritis diarrhea. We used a marker perfusion technique to study electrolyte and water absorption at different levels of the gut and found a net secretion of water, sodium, and chloride in the jejunum of infected pigs compared with absorption in controls studied 40 hours after infection.[3] Segments distal to the infected region showed a trend toward increased absorption, as if to compensate, but no significant differences were detected. Using ^{22}Na in the same model we found the net secretion of sodium was due to a decreased flux from lumen to extracellular fluid and an increased flux from extracellular fluid to lumen.[21]

When jejunal epithelium from infected pigs was studied in vitro, either in short-circuited chambers or in suspensions of epithelial cells, the most obvious defect was the failure of sodium transport to respond to glucose.[14, 17] This particular abnormality was most marked at the stage when diarrhea was most severe. Infected tissue in short-circuited chambers responded normally to the addition of theophylline with brisk

increments in electrical activity and in chloride secretion.[17] These in vitro experiments suggest a mechanism for the defective lumen to extracellular fluid flux of sodium observed in intact pigs, but fail to explain the increased extracellular fluid to lumen flux. The normal response to theophylline certainly mitigates against the possibility that cyclic adenosine monophosphate is the mediator of this secretory flux. Whether factors outside the epithelium are involved or whether our in vitro techniques have masked important transport defects that determine the secretory flow of sodium and chloride remains to be seen.

Recently we have studied the nature of the enterocytes that cover the villi at the time mucosal transport function is most deranged.[14] These cells contain little sucrase activity but are rich in thymidine kinase. Thymidine kinase is involved in DNA replication and normally is confined to the crypts. The fascinating implication from these data is that 25 to 40 hours after injection when diarrhea is severe the epithelium clothing the jejunal villi consists of immature cells that have migrated up from the crypts at an accelerated rate.

The studies described above clearly indicate that disordered sodium transport is central to the production of viral diarrhea. However the mechanisms causing viral diarrhea differ from those causing enterotoxigenic diarrhea as seen, for example, in cholera.[6, 15] The hallmarks of enterotoxigenic diarrhea are a secretory response mediated by excessive concentrations of cyclic adenosine monophosphate in the epithelium while sodium absorption responds normally to glucose. In viral diarrhea cyclic adenosine monophosphate appears not to be an important mediator of secretion and sodium absorption does not respond normally to glucose.

Now we shall consider human viral gastroenteritis in the light of what we know about transmissible gastroenteritis.

Table 1. *Stool Constituents in Viral Diarrhea: Piglets 18 to 42 Hours after Infection with Transmissible Gastroenteritis Virus*

	CONTROL (5)*	INFECTED (5)*
Stool Weight		
gm/24 hours	36 ± 9	262 ± 74†
Fat		
per cent of intake	3.9 ± 1.0	5.1 ± 1.5
Sodium		
mEq/24 hours	0.9 ± 0.6	22.0 ± 6.1†
mEq/kg stool	18.2 ± 7.5	82.0 ± 9.9†
Potassium		
mEq/24 hours	1.5 ± 0.5	14.2 ± 3.1†
mEq/kg stool	28.0 ± 6.5	64.0 ± 11.8†
Chloride		
mEq/24 hours	0.4 ± 0.3	19.0 ± 5.3†
mEq/kg stool	10.5 ± 5.3	66.0 ± 4.4†

*Values represent mean ± standard error. Number of animals is in parenthesis.
†Differ significantly from control, $p < .01$.

INFANTILE VIRAL GASTROENTERITIS

Although its name has not been decided, a specific virus has been identified which seems to cause much of the acute nonbacterial gastroenteritis among infants and young children throughout the world.[1, 4, 8, 10, 12, 18, 22, 25] Different workers have applied a variety of names to what is certainly the same agent—orbi,[1] reo-like,[8] rota,[7] and duovirus.[5] Until more information becomes available on the structure and properties of this virus we prefer the term infantile gastroenteritis virus. It was the long overdue application of electron microscopic techniques to stool examination that led to the identification of infantile gastroenteritis virus. Electron photomicrographs of infantile gastroenteritis virus obtained in many centers show remarkably similar virus particles with an inner core of about 38 nm and a capsid diameter of 60 to 70 nm (Fig. 1). Empty and broken particles are frequently seen. Morphologically similar viruses that cause diarrhea in young calves and infant mice share certain antigens with one another and with infantile gastroenteritis virus.[7] The human virus has not been fully characterized but it is thought to be a double stranded RNA virus. It may replicate slowly in human fetal gut organ cultures.[26]

Proof that this agent actually causes gastroenteritis comes from a series of observations made in various centers over the past 18 months. The virus is seen in stools, often in massive quantities,[8, 12, 18] in upper intestinal juice from acute cases,[11, 18] but almost never in controls or in convalescent cases. It is seen in the duodenal mucosal epithelium of children with acute enteritis.[18] Antibodies have been identified in convalescent sera[7] and an adult volunteer has been infected.[18]

The human virus described above differs from the transmissible gastroenteritis virus which does not infect man, however pigs can be infected with the human agent. Other candidate viruses have been proposed as causes of human gastroenteritis. Small 22 nm diameter parvoviruses have been identified in stools from patients with gastroenteritis and also in normal controls. Adult volunteers have been infected with parvo or picorna-like particles detected during a gastroenteritis epidemic in Norwalk, Ohio. Probably more viruses will be recognized as causes of human gastroenteritis but to date there is no conclusive proof that any of these candidates, apart from infantile gastroenteritis virus, is a widespread human pathogen.

Infantile gastroenteritis virus has been identified in stools of children with acute diarrhea in Australia, Britain, Canada, Africa, Europe, Asia, and the United States in Caucasians, Aborigines, Blacks, Orientals, and Indians. A recent epidemiological study in Melbourne showed the virus to cause about 50 per cent of the hospitalized acute diarrheal illness in infants and young children.[26] Our experience in Toronto has been similar. The infection is rarely seen in children older than six years and it is particularly prevalent during the colder months of the year. Epidemiological studies have not yet been carried out in countries where malnutrition is prevalent and where morbidity and mortality from infantile diarrhea are so appalling.

Spread of infection probably occurs human to human via stools. The

disease is highly contagious among hospitalized infants. Long-term carriers and animal reservoirs have not been identified. An antibody response occurs in sera of infected infants,[11] and one study has shown complement fixing antibodies to be prevalent among adults.[12] The fact that we have not yet identified a second attack in a patient suggests that an infection may confer lasting immunity. It is difficult to determine whether breast feeding is protective for the young infant in our locality because such a small proportion of the infant population is breast fed.

The virus, like the transmissible gastroenteritis agent, invades the mucosal epithelium of the upper small bowel where it has been identified in the cisternae of the endoplasmic reticulum of villous cells. In postmortem tissue from a patient with fulminant disease, we found virus antigen throughout the small intestine, even involving the crypt regions. It is not known whether the abnormalities of epithelial migration seen in transmissible gastroenteritis occur in infantile gastroenteritis virus in-

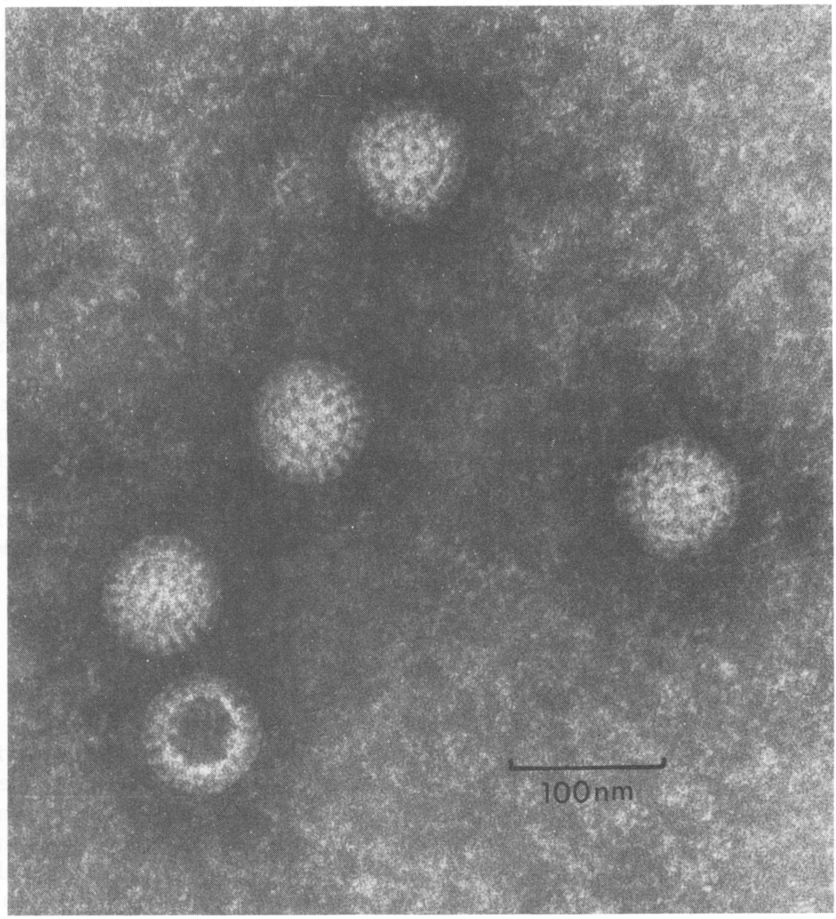

Figure 1. Negative contrast-stain electron micrograph of infantile gastroenteritis virus particles in diarrhea stool—one empty, several complete particles are seen.

fection. In some patients, as in some pigs with transmissible gastroenteritis, marked morphological abnormalities occur with flattening of villi, irregularity and derangement of surface epithelium;[1, 18] in others, no lesion is demonstrable by light microscopy.[18] Disaccharidase activities are diminished in proximal intestinal mucosal biopsies from infected children. Recovery is rapid with disappearance of virus from stools and duodenal juice within five to seven days and the reappearance of normal mucosal structure within four to six weeks.[1] No evidence has emerged to incriminate secondary changes in bacterial flora in the pathogenesis of this disease. To date, studies in Toronto and in Melbourne looking for known bacterial pathogens and for enterotoxin-producing strains of E. coli have been unrewarding. Furthermore, we have been unable to identify any abnormalities in the metabolism or conjugation of bile salts in children with acute disease.[2]

Now that a specific diagnosis can be made it will be possible to define the clinical condition caused by infantile gastroenteritis virus. To date we have had sufficient experience to gain some impression of it. The incubation period is brief, 48 hours or less, and the disease affects the very young; we have diagnosed it as early as four days of life. Usually the first manifestation is vomiting followed by watery diarrhea, cramps, and low-grade fever, a similar sequence to that observed in young piglets after transmissible gastroenteritis infection. Diarrhea is aggravated by feeding and relieved by fasting; it lasts longer than the vomiting but usually settles within four days after which recovery is rapid. The disease may be fulminant with rapid progression to dehydration, collapse and death. There have been at least five fatalities from infantile gastroenteritis virus infection in our community within the past four months in which infants died before they received medical care. If the child survives the acute illness, as in transmissible gastroenteritis, recovery is complete and chronic symptoms are rare.

Our preliminary studies on stools from these infants have shown sodium concentrations of approximately 50 mEq per liter, potassium concentrations of approximately 40 mEq per liter, and insignificant quantities of sugar in the acute phase of the disease. These concentrations are similar to those reported previously in nonspecific infantile gastroenteritis, but less than those occurring in enterotoxigenic diarrhea, caused for example by V. cholerae.

RELATIONSHIP BETWEEN RECENT RESEARCH AND CURRENT CLINICAL PRACTICE

Although caused by a different virus there is circumstantial evidence to support the view that transmissible gastroenteritis in the piglet is an appropriate model of human infantile gastroenteritis caused by infantile gastroenteritis virus. A comparison of the two diseases is seen in Table 2, suggesting that the impact of these two infections on the small intestines of two different species is remarkably similar. Perhaps the small intestine responds similarly to a variety of invasive infections both viral and nonviral. We suggest that the piglet data can guide us in

arriving at certain predictions about the human disease. The infant with gastroenteritis should have a limited capacity to handle dietary loads of sugar and sodium since mucosal disaccharidase activities are diminished and impaired sodium transport is a major factor in the pathogenesis of viral diarrhea. Since the major defect in viral diarrhea appears to involve transport processes we would have no hope for constipating drugs that alter intestinal motility. Because mucosal epithelial maturation and migration are potentially important determinants of the severity and duration of illness, factors that disturb mucosal dynamics, nutrition for example, should be important to the infant with gastroenteritis. Our practical experience with the disease has been consistent with these predictions. In infantile gastroenteritis dietary lactose or high solute loads may prolong diarrhea,[23] antidiarrheal medications are not efficacious and provision of good nutrition to the very ill infant may be extremely beneficial in reducing the severity and duration of diarrhea.[16] These and other concepts need further study, but now that we have specific diagnostic methods and an adequate animal model, rational questions can at last be asked and answered.

Clinical studies of the human disease are really just beginning. Already there are some lessons to be learned. For example, the disease is highly contagious, the incubation period is short, and the quantity of virus excreted in stool can be massive. Clearly there is a need for protec-

Table 2. *Comparison of Two Types of Acute Viral Enteritis*

	INFANTILE GASTROENTERITIS VIRUS (HUMAN)	TRANSMISSIBLE GASTROENTERITIS (PIG)
Characteristics of Infection		
Incubation period	< 48 hours	6 to 12 hours
Site of infection	villous epithelium upper small bowel crypts in severe cases	villous epithelium upper small bowel
Epithelial localization	cytoplasm of enterocyte	cytoplasm of enterocyte
Characteristics of Disease		
Duration	4 to 5 days	4 to 5 days
Vomiting	+	+
Watery diarrhea	+	+
Frequent and severe in young	+	+
Characteristics of Mucosa		
Structure (light microscopy)	variable normal to villous flattening	variable normal to villous flattening
Mucosal enzymes:		
disaccharidases	↓	↓
Na-K-ATPase	*	↓
thymidine kinase	*	↑
Characteristics of Stools		
Na (mEq/1)	~ 50	~ 80
K (mEq/1)	~ 35	~ 65
Cl (mEq/1)	~ 40	~ 65
Fat (% intake)	nil	5
Sugar (gm/dl)	nil†	nil

*Unknown.

†An occasional patient has up to 1 gm/dl stool.

testinal or systemic disorders. The speculative concepts suggesting that clinical disease states may be associated with altered mucosal permeability have been discussed.

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