REOVIRUS-LIKE CALF ENTERITIS

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Neonatal calf diarrhea, was once believed to be of solely bacterial, viz *E. coli*, etiology. However, in 1969 a reovirus-like agent (1, 2) and in 1972 a coronavirus (3, 4) were isolated from diarrheic calves. These viruses in gnotobiotic calves caused diarrhea typical of that seen in field cases. The recent association of a reovirus-like agent with acute nonbacterial gastroenteritis of infants and young children in many parts of the world (5–23) has stimulated interest in viral enteritis and, in particular, diarrhea caused by reovirus-like agents. In this paper, calf diarrhea caused by a reovirus-like agent will be reviewed.

VIRUS

REO is an abbreviation for respiratory, enteric orphan. The first viruses in this group were isolated from the respiratory and enteric tracts; however, they could not be associated with disease and thus were called orphans.

The calf diarrheal agent belongs to the family Reoviridae (1, 24–27) on the basis of morphology, cytopathogenicity, resistance to lipid solvents, stability at pH 3, and presence of double-stranded RNA. However, it is serologically unrelated to reoviruses 1, 2, and 3 is thus called a reovirus-like agent. An important practical property of the reovirus-like agent is its stability; feces kept at room temperature for 7 months still contained viable virus (14).

As a result of studies by various groups working on the human reovirus-like agent of infantile gastroenteritis, we now recognize a group of morphologically similar enteric viruses: reovirus-like agent of calf diarrhea, human reovirus-like agent of infantile gastroenteritis, reovirus-like agent of porcine diarrhea (28), epizootic diarrhea of infant mice (EDIM) (29), simian SA11 virus, and O agent from sheep and cattle (30, 31). The calf reovirus-like agent has been shown to be related antigenically to the human reovirus-like agent (17, 32, 33), porcine reovirus-like agent, and epizootic diarrhea of infant mice (17, 32, 33). It is serologically unrelated to the reoviruses and to the orbiviruses (33). The human reovirus-like agent was initially thought to be an orbivirus (6); subsequently the names rotavirus (32) and duovirus (12) were suggested.

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EXPERIMENTAL CALVES

Two types of calves, hereafter referred to as colostrum-deprived and gnotobiotic, were used in the calf diarrheal reovirus-like agent studies. Colostrum-deprived calves were obtained by hysterotomy, housed in individual isolation rooms which were scrubbed and fumigated with formaldehyde gas between calves, and fed pasteurized, homogenized milk. These calves were contaminated with *E. coli* and other bacteria. Gnotobiotic calves were delivered by hysterotomy into a sterile plastic surgical isolator glued to the abdominal wall and then transferred to a sterile isolator. The calves were fed autoclaved homogenized milk. Approximately 70% of these calves were bacteriologically sterile during the experimental period and the remainder were contamined with *B. subtilis* and/or a staphylococcus.

CLINICAL SIGNS

The incubation period after oral inoculation was somewhat dependent on the viral titer in the inoculum. When 10 ml of bacteria-free feces containing virulent reovirus-like agent was used, the incubation period was as short as 12 ½–13 hr. Low viral titer inocula caused incubation periods of 20–36 hr. Variations in the incubation period of usually only 1–3 hr occurred among calves inoculated with a constant-size inoculum from the same virulent virus pool.

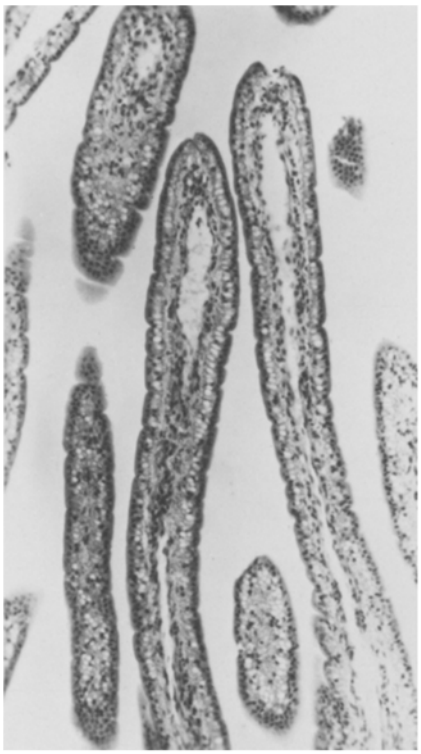

Fig 1. Section from the middle part of the small intestine of a control calf. The villous epithelium consists of tall columnar vacuolated cells in which the nuclei are next to the lumen. There is a minimum cellularity in the lamina propria. (H&E, ×100)

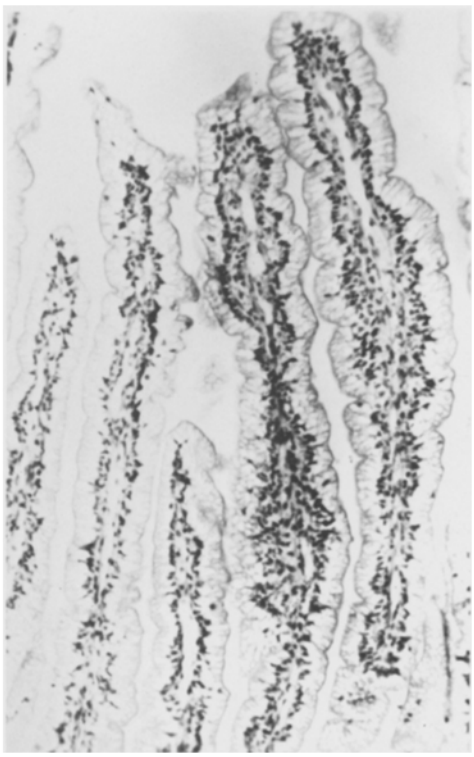

Fig 2. Section from the lower part of the small intestine of a control calf. The villous epithelium consists of tall columnar vacuolated cells; the nuclei are at the base of the cells. There is a minimum cellularity in the lamina propria. (H&E, ×100)

Signs usually progressed in the following order: depression, anorexia, a few strings of thick saliva hanging from the lips, and diarrhea. Onset of depression was rapid; eg, a calf that appeared normal at 8 AM was so depressed that it would not stand at 10 AM. The diarrheic period lasted 5–6 hr and the animal passed about 300 ml of liquid yellow feces. Volume of feces was somewhat dependent on the amount of milk consumed.

Signs during the postdiarrheic period depended on the type of calf inoculated. Gnotobiotic calves appeared normal, suckled, and had pasty feces 24 hr after the onset of diarrhea. Gnotobiotic calves removed from isolation units and placed in isolation rooms 24 hr after the onset of diarrhea remained normal. Up to 50% of the colostrum-deprived calves died shortly after the onset of diarrhea to 6 days postinoculation. Mortality in natural infections in normally born, colostrum-fed calves on ranches ranges from near 0 to 50% or more depending on the secondary bacterial infection.

PATHOLOGY

Changes induced by the calf diarrheal reovirus-like agent were studied using colostrum-deprived calves in isolation rooms and gnotobiotic calves. Infected gnotobiotic calves were killed at two intervals after the onset of diarrhea, 0.5–1.5 hr and at 4.5–6 hr. Control gnotobiotic calves were killed at approximately the same ages as the inoculated calves (34).

At necropsy the only difference observed between the control gnotobiotic and diarrheic gnotobiotic calves was that the diarrheic calves had more liquid in the small and large intestines. Colostrum-

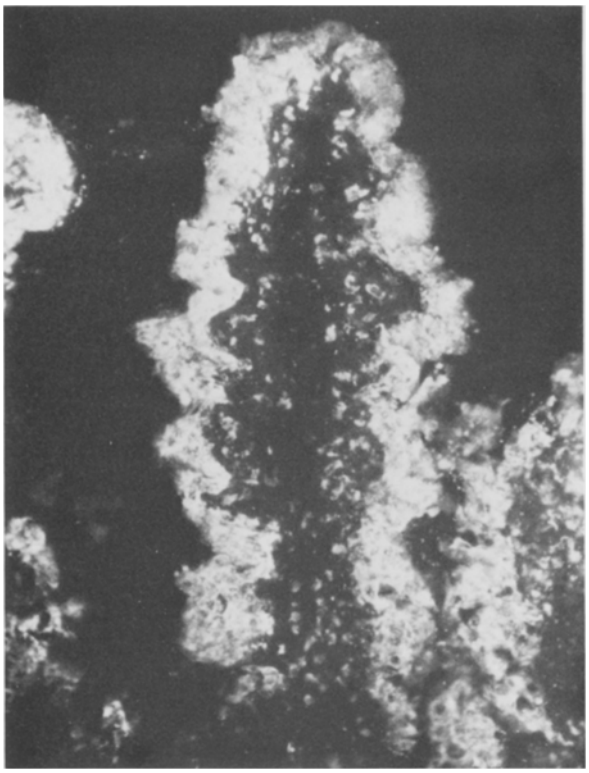

Fig 3. Longitudinal section of a villus from the lower part of the small intestine of a calf killed 0.5 hr after the onset of diarrhea. The epithelial cells on the distal two thirds of the villus fluoresce. (immunofluorescent stain, ×250)

deprived calves that died or were killed when moribund usually had a suppurative arthritis, suppurative meningitis, and occasionally peritonitis. *E. coli* was isolated from these fluids and tissue.

Sections from the upper, middle, and lower parts of the small intestine from the control gnotobiotic calves were immunofluorescent negative for the reovirus-like agent. Histologically, the epithelium on the distal two thirds of the villi in the upper and middle parts of the small intestine was composed of tall columnar cells. The nuclei were located in the apex of the cells next to the lumen, and the basal parts of the cells were vacuolated. In these parts of the small intestine the villous lamina propria consisted mostly of blood vessels, a lacteal and a few reticulum cells (Figure 1). The epithelium over the distal three fourths of the villi in the lower part of the small intestine consisted of tall, columnar cells that had the nucleus at the base and large vacuoles in the cytoplasm. The lamina propria resembled that in the upper parts of the small intestine (Figure 2) (34).

Small intestine from calves killed shortly after the onset of diarrhea had immunofluorescent epithelial cells on the tips of the villi in the upper part of the small intestine, over the distal half to one third of the villi in the middle part of the small intestine, and over the distal three fourths of the villi in the lower part of the small intestine (Figure 3). No immunofluorescence was observed in other organs. Histologically the upper small intestinal villous epithelium was composed of low columnar to cuboidal cells. Intestinal villi in the middle part of the small intestine had low columnar to cuboidal nonvacuolated epithelial cells. Tips of many villi were denuded, and there were free epithelial cells in the lumen. The villous lamina propria in sections of the upper and middle parts of the small intestine had an increase in reticulum-like cells. Lower small-intestinal villi resembled those in the control calf (34). Transmission electron microscopic examination of villous epithelium in sections of intestine adjacent to those which were immunofluorescent positive revealed large numbers of virions in the cytoplasm

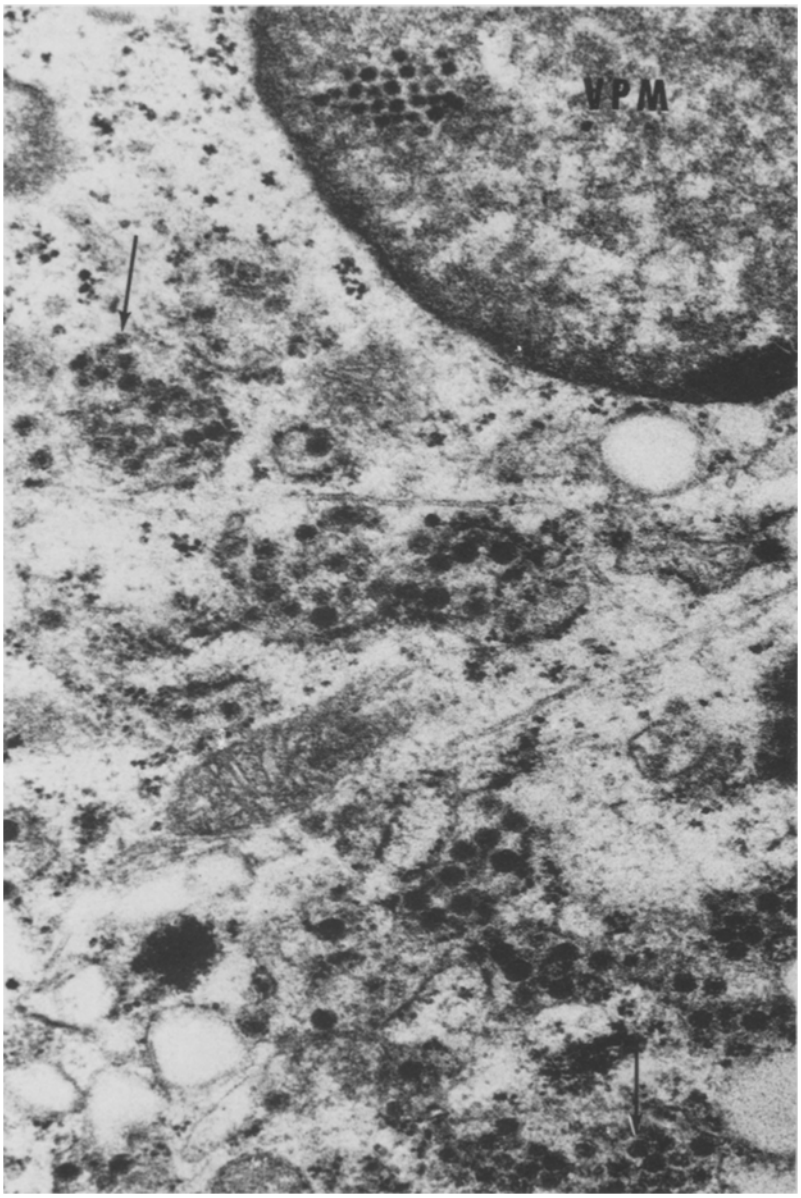

Fig 4. Section of a tall columnar epithelial cell from the ileum of a virus-inoculated calf. Viral precursor material (VPM) is enclosed by a single membrane; ribosomes are present along the membranes enclosing virions; viral nucleoids can be distinguished from the rest of the viral particles (arrows). (uranyl acetate and lead citrate stain, ×34,000)

(Figure 4). The viral particles and electron-dense material which was considered to be precursor viroplasm, since it often contained some viral particles in the beginning of a crystalline array, were present within the cisternae of the rough endoplasmic reticulum of the villous epithelial cells (35).

Calves killed approximately 4–6 hr after the onset of diarrhea had no immunofluorescent villous epithelial cells, but immunofluorescent cells were present in the intestinal contents in the lower small intestine and colon. No immunofluorescence was observed in other organs. Histologically, the epithelial cells over the villi in the upper and middle parts of the small intestine and on the sides of the villi in the lower part of the small intestine had a cuboidal morphology (Figure 5). Squamous epithelial cells

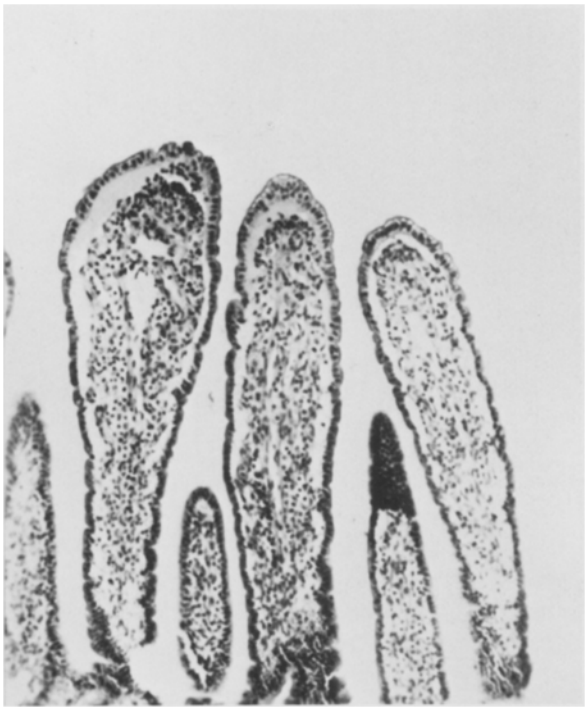

Fig 5. Villi in the middle part of the small intestine of a calf killed 6 hr after the onset of diarrhea. The villous epithelium is composed of cuboidal cells, and there is an increase in reticulum cells in the lamina propria. (H&E, ×100)

covered the tips of some villi in the lower part of the small intestine; tips of other villi were denuded (34) (Figure 6). No virions were observed by electron microscopy in the cuboidal or squamous villous epithelial cells (35). The villi, particularly in the lower part of the small intestine were moderately shortened. Villi in all three levels of the small intestine had an increase in reticulum-like cells in the lamina propria (34).

Two calves inoculated with cell culture-adapted virulent virus and killed 2 and 6 hr after the onset of diarrhea had similar clinical signs and lesions as those inoculated with fecal material. Immunofluorescence was observed only in the small intestinal villous epithelium. In addition, the following fluids and tissues were cultured for the reovirus-like agent: blood plasma, mesenteric lymph nodes draining the ileum and jejunum, spleen, liver, kidney, lung, thymus, and colonic contents. Using tissues from the first calf, virus was detected on the first passage of inoculum prepared from the jejunal mesenteric lymph nodes and on second blind passage of lung inoculum. The viral titer in the colonic contents was 10^6/ml. From tissues of the second calf, virus was isolated from only the jejunal and ileal mesenteric lymph nodes. The viral titer in the colonic contents was 10^8/ml (34).

Immunofluorescent, histologic, and transmission of electron microscopic findings suggested the following pathogenesis for the reovirus-like infection. After oral inoculation the columnar epithelial cells over the distal two thirds of the villi in the upper part of the small intestine became infected and the infection rapidly progressed caudally. When the calf became depressed and diarrhea began, the villous epithelial cells were morphologically normal, but the cells contained a large quantity of viral antigen. It is postulated that the viral infection of the villous epithelial cells redirected cell function from absorption to virus production, and thus the digestive fluids and partially digested milk accumulated in the

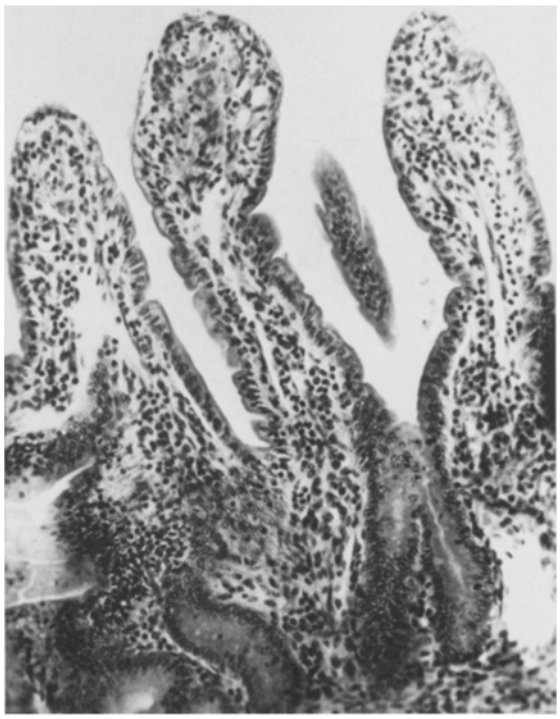

Fig 6. Villi in the lower part of the small intestine of a calf killed 6 hr after the onset of diarrhea. The epithelium on two villi is composed of cuboidal cells. The center villus has cuboidal and squamous epithelial cells on the sides and a denuded tip. There is an increased cellularity in the lamina propria. (H&E, ×160)

intestinal lumen. As the infection proceeded, there was an accelerated migration of the infected epithelial cells toward the tips of the villi; the cells were shed off the ends of the villi and replaced by cuboidal epithelial cells. Thus, calves killed shortly after the onset of diarrhea had immunofluorescent cells at the tips of the villi in the upper part of the small intestine and over most of the villi in the middle and lower parts of the small intestine. Calves killed later had immunofluorescent epithelial cells at the tips of the villi in the middle part of the small intestine and upper half of the villi in the lower part of the small intestine. About 4 hr after the onset of diarrhea all the immunofluorescent epithelial cells had been shed and replaced by cells with a cuboidal to squamous morphology. Calves which had no bacteria or had a nonpathogenic bacterial population during the viral infection recovered. If pathogenic bacteria, viz *E. coli*, were present, the altered epithelium and consequently altered intestinal function permitted an overgrowth of bacteria or bacteremia or both, followed by infection of other tissues. Large populations of bacteria in the intestinal lumen might also delay restoration of normal intestinal epithelium or even cause further injury to the epithelium.

LABORATORY TECHNIQUES

The calf reovirus-like agent has been demonstrated in diarrheic feces using electron microscopy (1, 36) and the fluorescent antibody techniques on fecal smears and culture (37–39).

Electron microscopic examination of feces for virions having a reovirus morphology has several advantages: (1) a higher percentage of infected feces will be detected, (2) virions can be detected in feces collected several days after the onset of diarrhea, and (3) the method will detect virions in putrefied feces. Immunoelectron microscopy permits a more accurate identification of the particles.

Villous epithelial cells which were shed off the ends of the villi can frequently be demonstrated by the immunofluorescent techniques in fecal smears prepared from feces collected early in the diarrheic period. Using electron microscopy, these cells have been shown to contain a large number of virions (31). Successful clinical implementation of the immunofluorescent technique depends on collecting early fecal samples, examining specimens from 5 to 10 animals in the herd, and prevention of autolysis of the cells in the feces after collection. Some laboratories have found this method of diagnosis difficult.

Inoculation of bovine kidney cell cultures with fecal filtrate and examination of the culture by the immunofluorescent technique 24–48 hr after inoculation has been successfully used by other (37–39) for detection of the calf reovirus-like agent.

IMMUNITY

It was observed in the early studies with the virulent calf reovirus-like agent that recovered calves did not develop diarrhea when reinoculated with the virus several days to 4 weeks after the initial infection. Therefore, the possibility of protecting calves by oral inoculation with an attenuated virus seemed feasible.

The calf reovirus-like agent was attenuated by passing it approximately 140 times on fetal bovine kidney (FBK) cells at 37°C and an additional 60 times on FBK cells at 29–30°C. Safety and potency tests were performed in gnotobiotic calves. Twenty-four 6–7-hr-old calves were inoculated orally with the attenuated virus. 20 of these calves were observed for 48–72 hr and then challenge inoculated orally with 10 ml of gnotobiotic calf feces containing virulent virus. These calves remained normal during the postvaccination observation period, and one developed mild diarrhea after challenge inoculation. 5 nonvaccinated challenge control calves developed severe diarrhea. The remaining 4 calves were not challenge inoculated but were observed for 30 days; they remained normal (40).

Prevaccination serums had neutralizing (SN) antibody titers of less than 2. 30 days postvaccination, the vaccinated–challenge inoculated calf SN titers ranged from 32 to 512; SN titers for the 5 challenge–control calves ranged from 256 to 512, and the titers in the calves which received only vaccine ranged from 64 to 256 (40).

The calf reovirus-like vaccine was field tested on ranches from which feces collected from diarrheic calves contained immunofluorescent cells. Calves were vaccinated when less than 24 hr old; prevaccination serums were obtained from about 10% of the calves. The reduction in the morbidity and mortality from diarrhea after vaccination in 12 of 14 herds was statistically significant. Most of the calves on these ranches had prevaccination (colostral) SN antibody titers to the reovirus-like agent (40).

On the basis of the above results, protection against infection appears to be dependent on a local cellular resistance and not circulating antibody. Initially this resistance may be due to an interference phenomenon and later to local antibody production.

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