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Astrovirus Infection in Hatchling Turkeys: Alterations in Intestinal Maltase Activity

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SUMMARY. Two experiments were conducted to determine intestinal disaccharidase activity in 1-day-old commercial turkey poults inoculated with astrovirus. Small intestinal samples were collected on days 0.5, 1, 3, and 7 postinoculation (PI) in Expt. 1 and on days 7, 10, and 14 PI in Expt. 2 and evaluated for specific maltase activity (SMA). Astrovirus infection was verified on day 7 PI by immune electron microscopy of intestinal contents. Inoculated poults developed diarrhea and a transient, significant decrease in intestinal SMA. SMA was significantly ($P < 0.05$) lower in astrovirus-inoculated poults than in control poults throughout the entire small intestine from day 3 through day 7 PI. However, SMA had returned to normal in inoculated poults by day 10 PI and was significantly higher than control values ($P < 0.05$) in all sections of the small intestine, except in the proximal jejunum, by day 14 PI.

Decreased SMA caused by astrovirus infection resulted in disaccharide maldigestion, malabsorption, and subsequent osmotic diarrhea. As astrovirus was cleared from the intestinal tract, SMA was restored and diarrhea was resolved.

RESUMEN. Infección por Astrovirus en pavitos recién nacidos: Alteración de la actividad de la maltasa intestinal.

Se realizaron dos experimentos para determinar la actividad de la disacaridasa intestinal en pavitos comerciales de un día de edad inoculados con Astrovirus. Para evaluar la actividad específica de la maltasa, después de la inoculación se tomaron muestras de intestino delgado los días 0.5, 1, 3 y 7 en el experimento 1, y los días 7, 10 y 14 en el experimento 2. La infección por Astrovirus en los contenidos intestinales fue verificada al día 7 después de la inoculación por medio de la prueba inmune con el microscopio electrónico. Los pavitos inoculados presentaron diarrea y una disminución transitoria pero significativa de la actividad específica de la maltasa intestinal. La diferencia fue más significante ($P < 0.05$) en los pavitos inoculados con Astrovirus que en los pavitos usados como controles desde el día 3 hasta el día 7 después de la inoculación. Sin embargo, la actividad específica de la maltasa retornó a la normalidad en los pavitos inoculados a partir del día 10, y fue significativamente mayor que los valores de los controles ($P < 0.05$) en todas las secciones del intestino delgado, excepto en la parte proximal del yeyuno al día 14 después de la inoculación.

La disminución de la actividad específica de la maltasa causada por la infección por Astrovirus se manifestó por una mala digestión de este disacárido, mala absorción y subsecuente diarrea osmótica. A medida que los Astrovirus fueron eliminados del tracto intestinal, se observó el reestablecimiento de la actividad específica de la maltasa y la resolución de la diarrea.

Astrovirus, along with other enteric viruses, has been incriminated as a possible cause of turkey viral enteritis. Other viruses identified in association with this enteric syndrome include reovirus, adenovirus, coronavirus, groups A and D rotavirus, enterovirus, parvovirus, and pseudopicornavirus (1,10,15,17). The respective roles of these viruses in the pathogenesis of turkey viral enteritis remains to be fully explained.

The enteropathogenicity of astrovirus has been documented by experimental infection of specific-pathogen-free (SPF) turkey poults and

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commercial hatchling poults (9,16). Infection of these poults with astrovirus consistently resulted in diarrhea, large dilated ceca filled with frothy yellow-brown fluid, loss of intestinal tone, and significant decrease in weight gain. Furthermore, malabsorption was substantiated by a significant decrease in D-xylose absorption (9). However, the only consistent morphological change in astrovirus-infected poults was mild crypt hyperplasia; villous atrophy was not a feature of astrovirus infection in these studies (16). Therefore, diarrhea that occurs in astrovirus-infected poults may be due to a mechanism different from malabsorption and maldigestion secondary to villous atrophy.

Complete digestion of oligosaccharides and disaccharides is primarily dependent on the membrane-bound disaccharidases located on the microvilli of enterocytes (13). Maldigestion due to a decrease in lactase activity occurs in gnotobiotic lambs experimentally infected with astrovirus (14). It was hypothesized that lactase activity was decreased secondary to astrovirus-induced injury associated with viral replication in the mature enterocytes on the apical two-thirds of villi in the small intestine (5,14). Although malabsorption has been documented in experimental astroviral infection of poults, the possibility of concurrent maldigestion has not yet been investigated (9). Recent experimental studies of "stunting syndrome" in young turkeys documented decreased activities of intestinal disaccharidases in the jejunum and ileum (2,3).

The purpose of the present study was to quantify intestinal mucosal specific maltase activity (SMA) in astrovirus-inoculated turkey poults in an effort to help delineate the mechanism by which astrovirus causes diarrhea in turkeys.

MATERIALS AND METHODS

Poults and housing. Day-old turkey poults were obtained from a commercial source and housed in separate, pre-sterilized positive-pressure plastic isolators equipped with intake and exhaust air filters and maintained at approximately 30 C. All poults were provided with feed and water *ad libitum*.

Bacteriology. Cloacal swabs for bacterial culture were taken from all poults before placement in isolators to check for enteropathogenic bacterial infections. Swabs were incubated for 24 and 48 hr in selenite enrichment media and then streaked on brilliant green agar. Suspect colonies were placed on triple-sugar-iron agar and enterotubes (7).

Virus inoculum. Virus for the inoculum was derived from an intestinal sample collected from diarrheic poults in Wisconsin (9). The original sample contained both rotavirus and astrovirus before it was purified by sonication and serial filtration to 0.05 μm with disposable filters (Millipore Corp., Bedford, Mass.). This preparation was evaluated by immune electron microscopy (IEM) to verify the presence of astrovirus and ensure that no other viruses were present. To increase the pool of astrovirus, SPF poults were inoculated with this preparation, and their intestinal contents were collected at day 6 postinoculation (PI). The intestinal contents were mixed with phosphate-buffered saline, filtered, examined by IEM, and found to contain astrovirus only. This filtered pool of intestinal contents was used as the inoculum in Expts. 1 and 2.

Experimental design. Poults were randomly allotted into two equal groups and placed in separate sterile isolators. At 1 day of age, poults in one group were each inoculated orally with 0.2 ml of bacteria-free preparation containing only astrovirus, as previously determined by IEM. The inoculum was administered with a sterile plastic tuberculin syringe and teat canula. The second group of poults served as controls and was not inoculated. Poults were observed twice daily for clinical signs of disease.

At various intervals PI, several poults from each group were selected at random and euthanatized by intraperitoneal or intravenous injection with 5% pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, Ill.). The intestinal tract was exteriorized, removed immediately, and flushed with cold saline. It was divided into four segments: duodenum (from the curve of the duodenal loop to the pancreobiliary ducts), proximal jejunum (from the pancreobiliary ducts to the yolk stalk), distal jejunum (distal from the yolk stalk to 2 cm proximal to the cecal tips), and ileum (remainder of the small intestine terminating at the ileocecal junction). The intestinal contents and ceca were evaluated for the presence of astrovirus by IEM, and the small intestine was evaluated for intestinal SMA, as described below.

Data were analyzed statistically by analysis of variance for a completely randomized design.

Expt. 1. Three inoculated poults and three control poults were euthanatized on days 0.5, 1, 3, and 7 PI. Twenty-four birds in all were evaluated.

Expt. 2. The second experiment differed from the first only in collection times. Collections were made from each group on days 7, 10, and 14 PI. Eighteen birds in all were evaluated.

IEM. Intestinal contents were evaluated for the presence of astrovirus by negative-stain IEM. The method used was a modified version of the method described by Saif *et al.* (11). Intestinal and cecal con-

tents were obtained by flushing the entire intestine with cold saline. Intestinal contents were pooled by treatment group and by day PI, weighed, diluted 1:2 in phosphate-buffered saline, and frozen and thawed three times. Contents were then homogenized, sonicated, and clarified by centrifuging at 1000 × *g* for 20 minutes. The supernatant was serially filtered through 0.8-μm and 0.45-μm disposable filters (Millipore) and then incubated overnight at 4 C with diluted convalescent antiserum prepared against astrovirus. Following the incubation, samples were pelleted through 33% sucrose by ultracentrifugation (55,000 × *g*) for 45 minutes at 10 C. The pellet was resuspended in 1 ml double-distilled water and recentrifuged as described above. The resulting pellet was resuspended in 10 μl double-distilled water and 10 μl of 4% phosphotungstic acid (pH 7.1) to yield a final concentration of 2%. Finally, samples were applied to 200-mesh carbon-coated copper grids and evaluated at 75 kV in a Hitachi H500 transmission electron microscope (Nissei Sanjyo Co., Ltd., Hitachi division, Tokyo, Japan) for the presence of astrovirus. For each sample evaluated, five grid squares were scanned. Any sample having at least one aggregate of astrovirus was considered positive.

Convalescent antisera used in IEM identification of astroviruses were prepared by inoculating commercial poults 1 day after hatch with astrovirus inoculum. Sera were collected 3 to 4 weeks after the initial inoculation, incubated with astrovirus, and evaluated by IEM for formations of astrovirus aggregates.

Intestinal disaccharidase and protein concentration. The intestinal segments were opened longitudinally, minced, and placed in pre-weighed vials. The vials were immediately frozen in liquid nitrogen to preserve mucosal disaccharidases. Net weights of the samples were recorded, and samples were kept at −20 C until analyzed. Just before analysis, intestinal samples were thawed and homogenized with quantities of cold deionized water to yield a final concentration of 50 mg tissue per ml of homogenate. A VirTis Hi-Speed 23 homogenizer (VirTis Co., Gardiner, N.Y.) was used for homogenization at a speed setting of 30% to 50% (moderate) for 30 to 60 seconds. Aliquots of the homogenates were processed to determine protein concentration by the Lowry method and maltase activity (4,6,12). To determine SMA, a sample of intestinal homogenate was incubated in a 0.056-M solution of maltose at 37 C. Next, the glucose released by the action of maltase on the substrate was oxidized with glucose oxidase to allow coupling to dianisidine. The amount of glucose was then measured spectrophotometrically. Measurements were corrected as needed for initial glucose contained in homogenates and for glucose detected in the substrate. The SMA was expressed as μM of substrate hydrolyzed per mg of mucosal protein per hour (μM substrate/mg mucosal protein·hr).

RESULTS

Clinical signs and gross lesions were first noted on day 2 PI and persisted through day 10 PI. Similar clinical signs and gross lesions were noted in astrovirus-inoculated poults in both experiments. The predominant clinical sign was diarrhea. Gross lesions included dilated ceca containing light-yellow fluid feces and gas, variable hyperemia of the intestinal mucosa, and thinning or loss of tone of the jejunal and ileal walls.

Astrovirus was found by IEM in pooled samples of intestinal contents from inoculated poults but not in control poults. No other viruses or recognized enteropathogens, including *Salmonella* species, were detected during evaluation of intestinal contents from inoculated or control poults by IEM or bacterial culture.

Figs. 1 and 2 show SMA levels of small intestinal sections from poults at various sampling intervals. The highest levels of SMA in control poults occurred in the proximal jejunum (Figs. 1B and 2B), and the lowest levels occurred in the ileum (Figs. 1D and 2D), regardless of age. In Expt. 1, there were no significant differences between SMA levels of control and inoculated poults on days 0.5 and 1 PI in any of the four areas sampled. However, SMA levels were significantly ($P < 0.05$) lower in inoculated poults than in controls in all areas of the small intestine on days 3 and 7 PI (Fig. 1). In Expt. 2, SMA levels in inoculated poults were also significantly lower than in controls in all areas of the small intestine on day 7 PI, but there were no significant differences in SMA levels on day 10 PI (Fig. 2). On day 14 PI, SMA levels in inoculated poults were significantly ($P < 0.05$) higher than SMA levels in control poults in all sections except the proximal jejunum.

DISCUSSION

These experiments were designed to determine the effect of astrovirus infection on the level of membrane-bound disaccharidase (maltase) activity in the small intestine of hatchling turkey poults. Our results document that astrovirus infection causes clinical enteric disease and significantly reduces maltase activity throughout the small intestine, even though it causes only subtle microscopic lesions, as reported elsewhere (16). Maltase activity de-

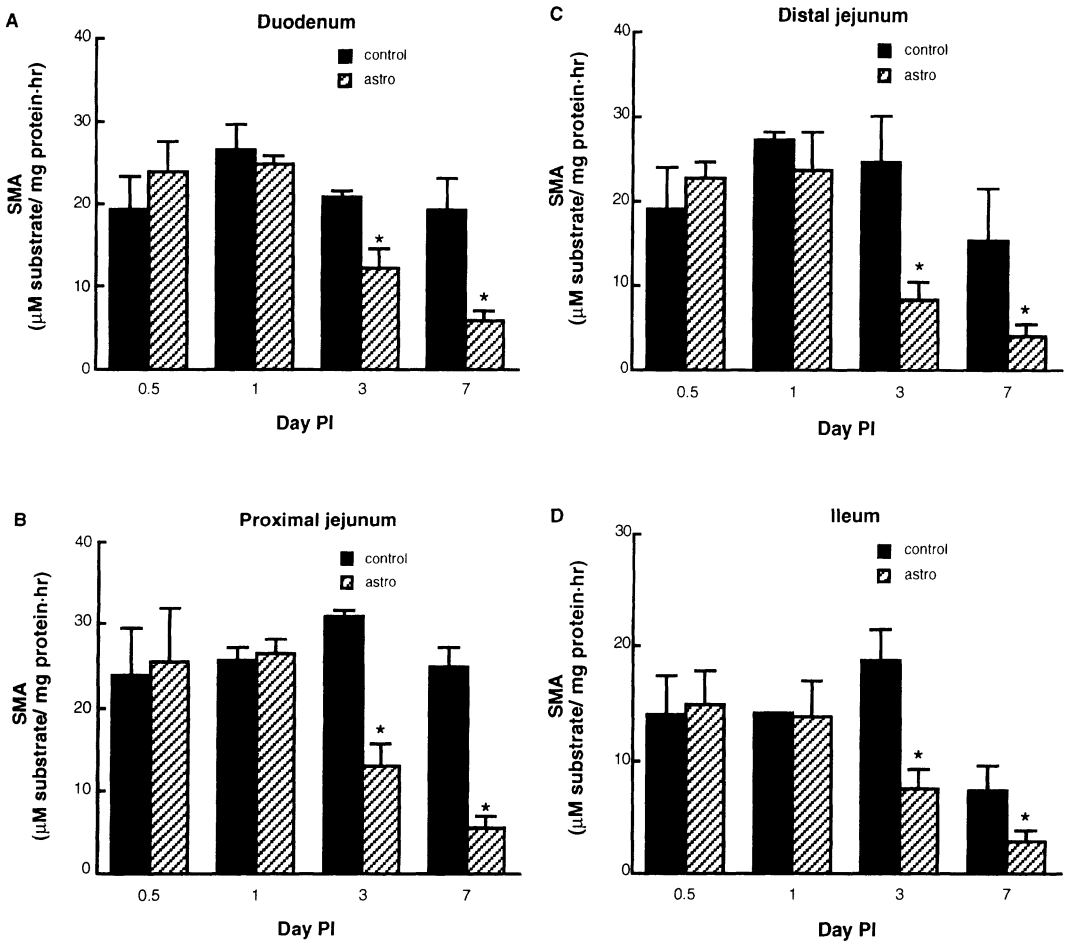

Fig. 1. Expt. 1. Specific maltase activity of the small intestine in control and inoculated poults. Error bars = S.E. of mean. Asterisks indicate significant differences from control values at each interval ($P < 0.05$).

creased initially at day 3 PI and remained low until day 10 PI. These findings coincide with initial reductions (day 3 PI) in both average body weights and 90-minute D-xylose absorption values in astrovirus-infected SPF poults reported by Reynolds and Saif (9). Additionally, the present study revealed that maltase levels in inoculated poults return to normal by day 10 PI. Therefore, maltase levels seemed to follow a trend similar to that seen with D-xylose absorption in astrovirus-infected SPF poults, in which no significant difference occurred in D-xylose absorption in infected SPF poults on days 9 and 13 PI (9). These results indicate that a transient period of maldigestion occurs concurrently with malabsorption and that in combination the two produce osmotic diarrhea and depressed weight gain. It is interesting to note that on day 14 PI in the present study, SMA was significantly higher ($P < 0.05$) in inoculated poults than in controls. This finding demonstrates the "hyper-regenerative" nature of intestinal disaccharidase activity as described by Michael (8) in an earlier study of intestinal coccidiosis in chickens. In that study, the "hyper-regenerative" nature of the small intestine was based on patterns of histochemical staining specific for various intracellular and extracellular enzymes. Another noteworthy observation by Michael (8) was the rapidity of recovery in the avian intestine. Michael noted that the enzymes returned to normal or higher levels before normal mucosal structure was regained. Our observations concur with this rapid recovery of the intestinal mucosa, as evidenced by the return to normal enzyme levels in astrovirus-inoculat-

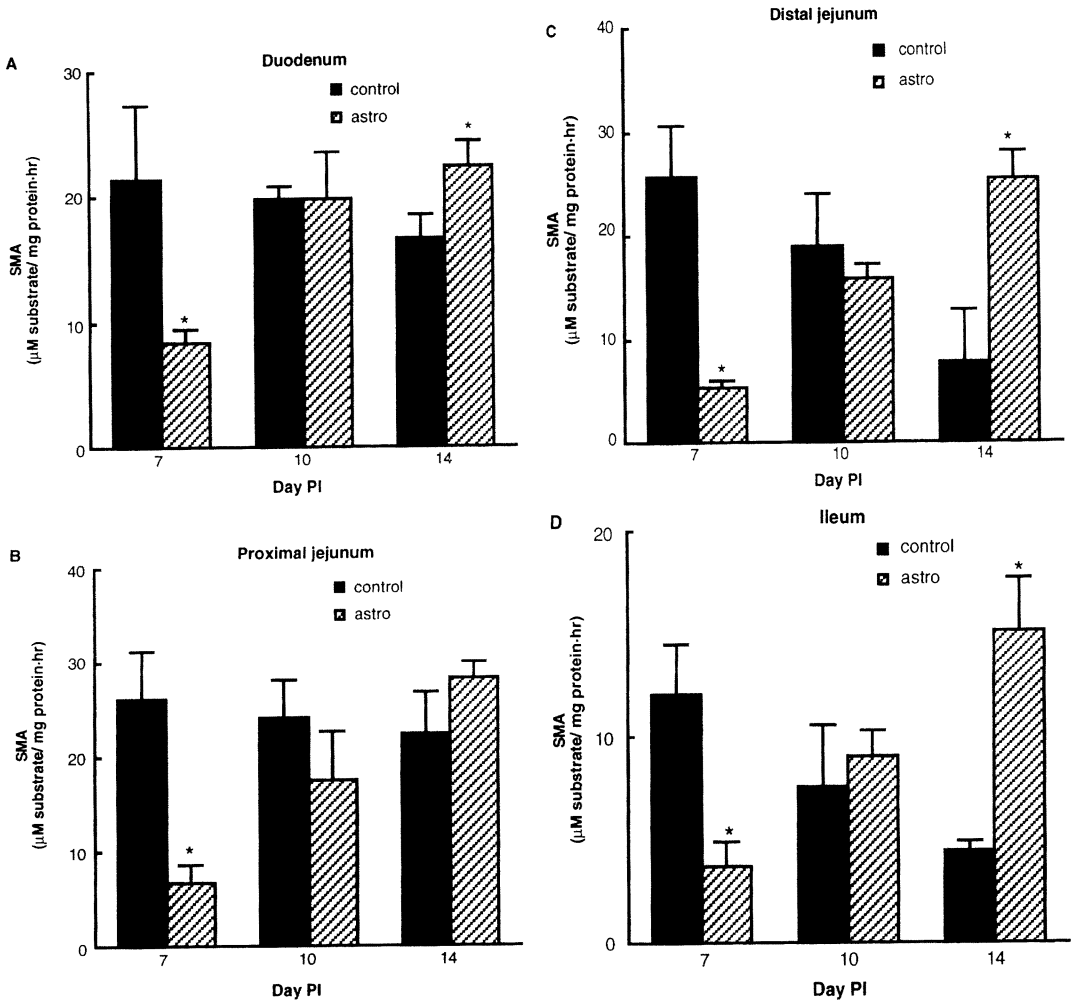

Fig. 2. Expt. 2. Specific maltase activity of the small intestine in control and inoculated poults. Error bars = S.E. of mean. Asterisks indicate significant differences from control values at each interval ($P < 0.05$).

ed poults 10 days after inoculation. Clinical signs and post-mortem lesions were consistent with those seen in astrovirus-infected SPF poults (9).

The pathogenesis of diarrhea in the astrovirus-inoculated poults is most likely due to an astrovirus-induced decrease in intestinal disaccharidase activity, which results in maldigestion of disaccharides, their subsequent malabsorption, and an osmotic attraction for water. A deficit in a particular disaccharidase results in osmotic diarrhea when the corresponding disaccharide is administered (13). How astrovirus caused the decrease in SMA in the present study is not clear. Viruses that cause loss of mature enterocytes from the villi (e.g., rotavirus) or destruction of the germinative epithelium of the crypts (e.g., parvovirus) will cause a decrease in intestinal disaccharidase activity and villous atrophy. Turkey astrovirus, however, seems to be able to induce decreased disaccharidase activity and mild crypt hyperplasia without causing significant villous atrophy (16).

In the present study, SMA in controls was greatest in the proximal jejunum at all sampling intervals, a finding that agrees with results of Sell *et al.* (12). However, SMA levels in control poults in the present study were 50% to 75% higher than levels in normal poults in the previous study (12). Sell *et al.* noted that disaccharidase activity was related to location in the small intestine, age, and diet of the poult. The higher SMA levels we noted may be due to dif-

ferences in diet and sample sites in the small intestine.

Although the current study clearly documents that astrovirus is an enteropathogen, it is unusual for astrovirus to occur as the sole enteric virus in diarrheic poults. Consequently, the manifestation of enteric disease may be altered by concurrent infection with other enteric viruses, such as group D rotavirus, the enteric virus most frequently identified in combination with astrovirus in fecal samples taken from diseased flocks (10).

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