Intestinal Disaccharidase and Peroxidase Deficiencies during *Eimeria nieschulzi* Infections in Rats*

DONALD W. DUSZYNSKI,† SHIRLEY A. ROY, and GILBERT A. CASTRO‡

Department of Physiology, University of Texas Medical School, Houston, Texas 77030

SYNOPSIS. Experiments were designed to study intestinal pathophysiologic changes associated with coccidial infections in mammalian hosts. Pairs of male Sprague-Dawley rats were killed at various times postinoculation (PI) with 10⁶ or 10⁷ sporulated oocysts of *Eimeria nieschulzi*. The small intestine from each rat was removed, weighed, measured, and divided into thirds. From the middle 11 cm of each third, one cm was fixed for histologic examination. Mucosa was scraped from the remaining 10 cm and was assayed for protein content and for peroxidase, sucrase and trehalase activities. Infection with *E. nieschulzi* was associated with increased mass of the small bowel. Histologically, crypt depth throughout the small bowel was significantly greater (P < 0.005) in infected rats than in non-infected ones on PI days 8 and 16. Villus height did not change drastically during low-dose infections (10⁶ oocysts) and varied during high-dose infections (10⁷ oocysts). As a result of these morphologic changes in the mucosa, crypt villus ratios were usually significantly greater (P < 0.005) in all infected rats throughout the small bowel. In general, increased gut weight and changes in crypt and villus dimensions became evident by PI day 2, were most pronounced at PI day 8, and began to return to control values by PI day 16. Peroxidase, sucrase, and trehalase levels equalled or were slightly higher in controls on PI day 2, dropped significantly below controls (P < 0.05) by PI day 8, and returned to, or exceeded control levels by PI day 16. The intensity of all changes was directly dose-dependent.

Index Key Words: *Eimeria nieschulzi*: rats; intestinal pathophysiology; peroxidase; sucrase; trehalase; villus height; crypt depth.

It is generally accepted that mechanical damage to cells and tissues invaded by sporozoites and merozoites is probably the major cause of pathologic changes in coccidial infections. Thus, the degree of such changes induced by these parasites is directly related to the number of oocysts ingested. For example, in rats the endogenous development of *Eimeria nieschulzi* Diiben is accompanied by diarrhea, reduced weight, hemorrhage, and (presumably) some inflammation in heavy infections (1, 2, 23). Slightly reduced weight gain, but no overt signs of disease are noted in light infections (10, 16). Such descriptive parameters, along with mortality, lesion scores, and oocyst production have traditionally been used to evaluate the disease caused by coccidia in vertebrate hosts (see Refs. 17, 19 for reviews). In recent years researchers in England (20-24) and Germany (21) have begun to quantify the pathologic changes attending coccidiosis in chickens, but similar studies have not been done on mammalian model systems.

In this report we summarize experiments designed to determine (a) whether changes in brush border enzyme activity accompany coccidiosis in rats; (b) the dose dependency and timing of any changes; and (c) whether such changes are associated with acute inflammatory responses in the intestinal mucosa. Parallel studies were performed during light and heavy infections with *E. nieschulzi*.

MATERIALS AND METHODS

**Experimental Animals.**—Male Sprague-Dawley rats Sprague Dawley, Madison, W1) weighing 200-300 g, were anesthetized with ether and inoculated by oral intubation with either 10⁶ or 10⁷ sporulated oocysts of *E. nieschulzi*. The oocysts were 3-4 months-old and had been stored at 4-6°C in 3% (w/v) aqueous K₂Cr₂O₇. Infected rats were maintained in pairs, as were uninfected controls, in hanging wire cages over porcelain pans containing K₂Cr₂O₇ (10). All rats received stock diet and water ad libitum.

**Collection and Preparation of Intestines.**—On PI days 2, 4, 7, 8, 9 and 16, respectively, 2 rats infected with each dose and uninfected controls were killed. The abdomen was then opened and the small intestine was cut at its junctions with the stomach and cecum. A glass cannula was inserted into the duodenum and the intestine was perfused with 50 ml of 0.85% (w/v) NaCl saline. The intestine was blotted gently, weighed, and then measured while hanging under its own weight from a hook attached through the duodenum. On PI days 2 (early schizogony), 8 (peak parasitemia) and 16 (host recovery), the intestines were divided into thirds and the middle 11 cm were removed from each third. One cm was fixed in ice-cold 10% (v/v) formalin in saline for histologic examination. The remaining 10 cm of each third were kept in ice-cold saline and used for enzyme studies. After each third had been weighed the mucosal surface was exposed and the mucosa was scraped with a glass microscope slide. The scrapings were homogenized in a prechilled Potter-Elvehjem homogenizer in 4 ml of ice-cold saline; aliquots of homogenate were used for the assay of enzymes and protein.

**Enzyme Assay.**—Peroxidase activity was measured by the method of Mahrly & Chase (19) with slight modifications. The reaction mixture contained: 20 mm aqueous guaiacol, 1 ml; 10 mm phosphate buffer (pH 6.0), 2 ml; 25-30 µl mucosal homogenate; and 100 µm H₂O₂, 20 µl. The increase in absorbance at 470 nm at ambient temperature was measured in a B & L Spectronic 20 spectrophotometer. One U of peroxidase activity is defined as the quantity catalyzing the decomposition of 1.0 µmole of H₂O₂/min. The relationship between moles of H₂O₂ decomposed and change in optical density (OD) was determined by measuring the change in absorbancy using varying amounts of H₂O₂ in the presence of excess standard horseradish peroxidase (EC 1.11.1.7, Sigma Chemical Co., St. Louis, MO). Protein concentration was determined by the Folin phenol method (18).

Disaccharidase activity was measured in mucosal homogenates. 

---

* This investigation was supported by Research Grant Al 11361-04 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service (to GAC).

† Present address: Department of Biology, The University of New Mexico, Albuquerque NM 87131.

‡ Recipient of Research Career Development Award Al 00087, from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.
by the method of Dahlqvist (7). One unit (U) of disaccharidase activity is defined as that amount which hydrolyzes 1 mole of sucrose or trehalose/min. The rate of hydrolysis was determined by measuring the rate of glucose formation by means of the glucose oxidase procedure (glucose oxidase, EC 1.1.3.4, obtained from Worthington Biochemical Corp., Freehold, NJ).

Histologic Examinations.—The middle 1 cm of each third of the small bowel was kept in ice-cold formalin-saline for at least 1 hr, then allowed to come to ambient temperature. Tissues were stored in the fixative until needed. They were then washed in distilled water, trimmed, dehydrated in a graded series of ethanol, and either embedded in 56-58 C Paraplast-Plus® cut into 5-7 μm cross-sections, and stained with Erlich's hematoxylin and eosin (H & E) (15), or embedded in Epon 812, stained with methylene blue-azure II or Paragon, and cut into 1-3 μm sections. In each specimen, 20 measurements were made of the height of the villi and the depth of the crypts, using a calibrated eyepiece micrometer and a 10X objective. The mean crypt/villus ratio was then calculated for each intestinal segment for each animal.

Statistical Analysis.—Data were analyzed by Student's t-test or the t-test for paired data (30).

RESULTS

Gross Changes of Tissues (Table 1).—Infection with E. nieschulzi was associated with increased weight, but not length, of the small bowel. This increase, evident as early as PI day 2 and 4, was most pronounced on PI day 7-9, when the most oocysts were being produced and discharged. It was still seen on PI day 16, 2-3 days after infection was over (as judged by the absence of oocysts in the feces). Increase in intestinal weight, presumably due to E. nieschulzi, was largest in rats infected with 10⁶ oocysts. This was evident from the data in which intestinal weight was expressed as a function of body weight or gut length.

Histopathologic Changes (Figs. 1, 2).—When cross-sections of intestines from infected rats were compared to those from uninfected controls, changes in crypt/villus ratios were seen as early as PI day 2, were most pronounced on PI day 8 (peak oocyst discharge), and began to return to normal values by PI day 16 in rats infected with 10⁴ oocysts, but not in those receiving 10⁶ oocysts (Fig. 2). In rats infected with 10⁴ oocysts the crypt/villus ratios in the proximal 1/3 of the intestine decreased on PI day 2. This decrease was due to slightly increased villus height in both regions during early schizogony (Fig. 1). In all other sections measured, crypt/villus ratios were larger than the corresponding control values. These increases were most often the result of highly significant increases in crypt depth (P < 0.005) although in rats infected with 10⁶ oocysts, villus height was seen to fluctuate significantly in the proximal intestine on PI day 8 and in the distal bowel on PI days 2, 8 and 16 (Fig. 1). The height of villi in the mid intestine of infected rats was rather homogeneous both during and after patency.

Peroxidase Activity (Fig. 3).—Basal levels of mucosal peroxidase were of the same magnitude in the proximal, mid, and distal segments of the small intestine. Therefore, the activities measured in these intestinal segments were pooled. It is evident from the results shown in Fig. 3 that the level of activity is a function of days PI. The same general patterns are obtained regardless of the size of the infective dose. Infection with E. nieschulzi caused peroxidase activity to rise over that of controls on PI day 2. On PI day 8, peroxidase activity decreased significantly in infected intestines. By PI day 16, this enzyme activity in the entire small bowel was significantly higher in infected rats.

Sucrase and Trehalase Activities (Figs. 4, 5).—Distinct differences in the basal activity of the 2 disaccharidases were seen along the length of the intestine. Activities of sucrase and trehalase were considerably higher in the proximal and mid bowel than in the distal part of the intestine (Figs. 4, 5). Eimeria nieschulzi caused a significant decrease in disaccharidase activity by PI day 8, this effect being most pronounced in the more heavily infected hosts. By PI day 16, levels of these enzymes were significantly higher than those recorded from control animals.

DISCUSSION

Our objective was to investigate structural and functional changes in the small bowel of rats infected with E. nieschulzi and to determine, if possible, how such changes might contribute to the symptoms associated with coccidial infections. Evidence is presented that infection causes: (a) an increase in mass of the infected intestines, (b) changes in mucosal structure, especially increased crypt depth, (c) a decrease in the levels of peroxidase in the lamina propria, and (d) a reduction of brush border enzyme activity. The degree of the changes induced was directly related to the infective dose of oocysts and the PI day.

Clarke (5) studied the relationship between cell production rate, villus height and crypt depth from 13 sites along the small intestine of 15 normal albino rats. Interpolated from his data, crypt/villus ratios at the midpoint of each third of the intestine were, proximal = 0.3, middle = 0.4 and distal = 0.6. Although we used different fixation methods, Clarke's data correspond well to our control values (Fig. 2) and help support the contention...
that changes in mucosal structure in infected rats were caused by E. nieschulzi. The greatest changes in crypt depth were seen during peak gametogony (PI day 8), but these changes were not confined to the area of greatest parasitization, as reported for chickens infected with coccidia (13, 20, 21). Nor was recovery of the intestinal mucosa as rapid in rats after infection with E. nieschulzi as in chickens recovering from a single infection with E. acervulina (13, 20, 21). The increases in crypt depth along the length of the small bowel of rats during E. nieschulzi gametogony (PI day 8) and following infection with this parasite (PI day 16) are similar to changes noted by Fernando & McCraw (13) in E. acervulina-infected chickens. These authors (13) also noted a decrease in villus height in the area of greatest parasite development (distal duodenum), whereas in 2 areas distal to the site of E. acervulina development (~ mid and distal small intestine) an increase in villus height was observed after patency ended. We never saw a decrease in villus height in the area of greatest parasitization (mid small intestine) by E. nieschulzi, although the distal gut had significantly longer villi at peak parasitemia (10⁶ oocysts) as did the proximal gut after patency (10⁴ oocysts). We also observed significantly shorter villi in the distal and proximal gut at PI days 2 and 8, respectively (10⁶ oocysts). The specific mechanisms by which coccidia alter gut morphology (other than by cell destruction) are not understood. Symons (31) saw similar increases in crypt depth in rats infected with Nippostrongylus brasiliensis and attributed these changes to a direct stimulation of progenitor cells in the crypts by an unspecified factor of parasite origin which caused an increased
proliferation of these cells and thus crypt elongation. Perhaps similar events occur in the gut during coccidial infections.

Reports of reduced enzyme activity in enterocytes during heavy coccidial infections in lambs (24) and chickens (11, 21), and of impaired glucose assimilation by the intestine of *E. nieschulzi*-infected rats (27) indicate that structural derangements in the mucosa induced by coccidia are accompanied by functional alterations. This view is supported by our finding that *E. nieschulzi* decreased the activities of the gut to hydrolyze disaccharidases (Figs. 4, 5).

Of the various disaccharidases, we chose sucrase and trehalase because they are specific brush border enzymes that attack a single substrate, whereas some of the other disaccharidas (e.g., maltase, isomaltase) attack more than a single disaccharide (11). Thus, any change in disaccharidase activity that we measured could be presumed to be due to alteration in a single enzyme. We know that disaccharidase activity varies greatly depending upon factors such as dietary intake, feeding patterns, and other environmental factors (3, 8). Therefore, all experiments were conducted with paired groups so that conditions within animal quarters which might contribute to day-to-day fluctuations in enzyme levels were experienced by both infected and uninfected rats. The pattern of slightly increased disaccharidase activity during early schizogony (PI day 2), a significant decrease in activity at peak parasitemia (PI day 8), and recovery and apparent over-compensation of enzyme activity after infection (PI day 16) closely parallel the response in the intestinal mucosa of chickens infected with *E. necatrix* (11).

Despite the possibility that altered cell turnover may contribute to the observed changes in enzyme levels, it is reasonable to assume that specific functions associated with enterocytes might be impaired due to the invasion of these cells by coccidia. Sheppard (26), for example, noted reduced height in the microvilli of enterocytes from the middle third of the bowel in rats infected with $4 \times 10^4$ *E. nieschulzi* oocysts (PI day 8). Allegedly, *E. nieschulzi* does not inhabit the anterior or posterior sections of the small bowel. Our findings, however, suggest the parasite may not be as site-specific as previously suspected, because its developmental stages were seen in each third of the intestine on each PI day in infections with $10^4$ and $10^6$ oocysts. This suggests that brush-border enzyme deficiencies as well as structural changes in the mucosa are related to the presence of the parasite within cells, particularly on PI day 8.

Alterations in enterocyte functions are known to be associated with inflammation in the underlying mucosa in some enteric helminth infections (4, 31). Smith & Castro (28, 29) found that
in rats infected with *T. spiralis* peroxidase activity in mucosal scrapings correlated directly with the spatial and temporal distribution of the worms in the small bowel and with the appearance of polymorphonuclear leukocytes (myeloid derived cells) in the mucosa of the same regions. Similar studies on the infiltration of immune cells into the lamina propria of rats infected with *E. nieschulzi* will be presented elsewhere.

The relationship between peroxidase levels and disaccharidase activity in the small bowel mucosa of rats infected with *E. nieschulzi* differed from the findings with regard to these enzymes in rats infected with *T. spiralis* (28, 29). During coccidiosis mucosal peroxidase levels increased early (PI day 2), but by PI day 8 (when most of the gametes were mature and fertilization and oocyst discharge were occurring) the concentration of the enzyme dropped well below levels seen in controls. In contrast, during primary trichinosis peroxidase levels continue to rise significantly above control values until about PI day 14 when the worm population in the bowel begins to decrease in numbers. During both infections PI day 8 was about the time when disaccharidase activities were at their lowest levels.

The quantitative relationship between peroxidase and disaccharidase activities at PI day 16 in *E. nieschulzi*-infected rats was also at variance with that observed in rats that recovered recently from primary infection with *T. spiralis*. In primary trichinosis, peroxidase activity dropped toward preinfection levels, and disaccharidase activity, which was suppressed during early infection, rose to normal levels following elimination of the worms. During infection with *E. nieschulzi* elimination of the parasite from the gut is not only followed by a significant rise in disaccharidase activity, but also by a significant elevation in mucosal peroxidase activity. Thus, it appears that peroxidase and disaccharidase activity are regulated by different mechanisms; it cannot be concluded that elevated peroxidase activity is associated with brush-border enzyme deficiency.

Fig. 4. Total sucrase activity (U cm) in each third of the small intestine of paired groups of uninfected rats and rats on PI days 2, 8 and 16 with 10⁶ and 10⁷ oocysts of *E. nieschulzi*.

Fig. 5. Total trehalase activity (U cm) in each third of the small intestine of paired groups of uninfected rats and rats on PI days 2, 8 and 16 with 10⁶ and 10⁷ oocysts of *E. nieschulzi*.

The rise in mucosal peroxidase associated with certain stages of *E. nieschulzi* development can be attributed to mucosal cell damage; however, the exact mechanism(s) by which lowered peroxidase activity is brought about in the intestine by *E. nieschulzi* remains unknown. Despite the lack of a rational explanation for the marked suppression of peroxidase activity, this observation is interesting in view of recent findings by Fayer (12) and others (for review see Ref. 9). These workers reported that *Sarcocystis* spp. do not cause an immune or inflammatory response in the bowel of carnivores. The point of coincidence is that only gametogony is known to occur in the carnivore gut. Based on this evidence, we propose that *E. nieschulzi* can regulate the inflammatory state of the host's intestine during primary infection. This capacity might retard the development of immunity and assure the parasite sufficient time to complete the intestinal phase of its life cycle. Although this contention is speculative, it is evident that following recovery from primary infection, which is immediately preceded by and concomitant with high mucosal peroxidase levels, the rat develops solid immunity to subsequent challenge (6).
ACKNOWLEDGEMENT

Thanks are due Mr. Alan A. Marchiondo, Department of Biology, The University of New Mexico, Albuquerque, for embedding, sectioning, and staining intestinal tissues for histologic study.

REFERENCES


BOOK REVIEW


This is a commendable exposition of the principles of biological nomenclature as established by the international codes of zoological, botanical, bacterial, and viral nomenclature. Since these codes do not always agree with each other, the poor protozoologist or protistologist is left in something of a quandary. If he says that the organism he is working with is a plant, its name is subject to one set of rules; if he says that it is an animal, its name is subject to another. I gather, too, that the generic name Nuttallia (Protozoa) is homonymous with but not a homonym of Nuttallia (Mollusca). If both were plants and so widely separated, they would be both homonymous and homonyms. These divergent codes were developed for the 2-kingdom, plant-animal system. How they would work in the 5-kingdom or some other system is subject to discussion. Obviously, they need to be coordinated and consolidated. However, there are no international codes of prokaryote or protistan nomenclature so we must make do with what we have.—NORMAN D. LEVINE, College of Veterinary Medicine, Univ. of Illinois, Urbana, IL 61801, USA.