# The Pathological Changes Caused by Eimeria falciformis var pragensis in Mice

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## ABSTRACT

Groups of Swiss white mice weighing 25-28 grams were infected orally with 500, 2,000, 5,000 or 20,000 oocysts of Eimeria falciformis var pragensis. Depression, anorexia, weight loss, diarrhea or dysentery, and dehydration were most pronounced at eight to ten days postinfection.

The highest mortality, 31%, occurred in mice infected with 20,000 oocysts. None of the mice infected with 500 oocysts died. The pathological findings were equally severe in mice infected with 5,000 and 20,000 oocysts. The enteric lesions, most pronounced at eight to ten days postinfection, were restricted mainly to the large intestine and consisted initially of both cryptal and absorptive epithelial cell destruction and submucosal edema. These changes were followed in 12 to 24 hours by a transient influx of neutrophils into the lamina propria followed by mononuclear cell infiltration which lasted for five to ten days. As the infective dose decreased, the inflammatory response occurred later and was less extensive. When seen, hemorrhage occurred seven to 11 days postinfection.

In 50% of the mice infected with 5,000 and 20,000 oocysts, varying degrees of a nonselective mucosal necrosis were seen at eight to 12 days postinfection. In mice infected with 500 oocysts, mucosal destruction was restricted to the epithelium. Neutrophils predominated when necrosis was extensive, otherwise, mononuclear cells were the main inflammatory cells. Two to three days following necrosis, crypt hyperplasia was marked and mucosal integrity was restored. Ulcers, some of which extended into the submucosa, healed by days 14 to 20. Localized granulomatous colitis, induced by trapped oocysts within the lamina propria, was seen until the experiment was terminated at 25 days postinfection. Infection was followed by lymphoid hyperplasia in the lymph nodes and the spleen.



## RÉSUMÉ

Cette expérience visait à faire ingérer à différents groupes de souris blanches suisses, dont le poids variait de 25 à 28 grammes, 500, 2,000, 5,000 ou 20,000 oocystes de la coccidie Eimeria falciformis var. pragensis. Au bout de huit à dix jours, ces souris manifestaient beaucoup de dépression, d'anorexie, de perte de poids, de diarrhée ou de dysenterie et de déshydratation. Le taux de mortalité le plus élevé (31%) se produisit chez les souris auxquelles on avait administré 20,000 oocystes. Aucune mortalité ne survint chez celles qui avaient reçu seulement 500 oocystes. La gravité des lésions se révéla identique chez les souris auxquelles on avait donné 5,000 ou 20,000 oocystes. Les lésions intestinales atteignirent leur paroxysme au bout de huit à dix jours; elles affectaient surtout le côlon et débutèrent par la destruction des cellules épithéliales absorbantes de la muqueuse et de celles des cryptes de Lieberkühn, ainsi que par un œdème de la sous-muqueuse. De 12 à 24 heures plus tard, il se produisit une infiltration transitoire de la lamina propria par des neutrophiles; les mononucléaires remplacèrent graduellement les neutrophiles et cette nouvelle infiltration dura de cinq à dix jours. Les doses moins fortes d'oocystes provoquèrent une réaction inflammatoire plus tardive et moins marquée. Les hémorragies qui se produisirent, apparurent au bout de 7 à 11 jours.

Chez 50% des souris auxquelles on avait administré 5,000 ou 20,000 oocystes, on nota une nécrose non sélective et d'intensité variable, au sein de la muqueuse, au bout de 8 à 12 jours. La destruction de la muqueuse se limitait cependant à l'épithélium, chez les souris qui


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**avaient reçu seulement 500 oocystes. Les foyers de nécrose marquée contenaient surtout des neutrophiles; ailleurs, les mononucléaires constituaient la majeure partie de l'exsudat. De deux à trois jours après le début de la nécrose, les cryptes de Lieberkühn présentaient une hyperplasie marquée et la muqueuse avait retrouvé son intégrité. Les ulcères, dont un certain nombre avaient progressé jusque dans la sous-muqueuse, guérirent au bout de 14 à 20 jours. Une colite granulomateuse focale, consécutive à l'emprisonnement d'oocystes au sein de la lamina propria, persista jusqu'à la fin de l'expérience qui dura 25 jours. À la suite de cette infection, les ganglions lymphatiques et la rate développèrent une hyperplasie lymphoïde.**

## INTRODUCTION

The pathological effects of murine coccidia have been described in only one of the 15 Eimerian species reported to infect mice (25). Reimer described epithelial cell destruction, neutrophil exudation, hemorrhage and crypt hyperplasia in mice presumably infected with *E. falciformis* (26). The life cycle of *E. falciformis* var *pragensis*, a variation of *E. falciformis* (4) has been studied (4, 20) but the clinical signs and pathogenicity have not been reported.

This report describes the clinical signs and pathological changes associated with *E. falciformis* var *pragensis* infection in mice and compares the effects of varying infective dose on the pathological changes.

## MATERIALS AND METHODS

### Experimental mice

A total of 372 randomly bred coccidia-free Swiss white mice weighing 25-28 grams obtained from the Animal Resources Center, University of Saskatchewan, Saskatoon, was used in this study.

### Coccidia

A mouse coccidium characterized as *E. falciformis* var *pragensis* (20) was maintained by frequent passage through coccidia-free mice, sporulated in 2.5% potassium dichromate and stored at 4°C for not more than eight weeks before it was used.

### Experimental design

One hundred and five mice were assigned randomly to each of the three test groups and infected orally with 500, 5,000 or 20,000 oocysts as described previously (20). The fourth test group of 65 mice was infected similarly with 2,000 oocysts. Twenty-two mice were used as an uninfected control group.

Six male mice from each group used for oocyst counting and weight recording were kept in individual wire-netted cages. The rest were kept in groups of four to five in shoebox plastic cages that were replaced with clean cages every other day. The day of inoculation was designated as day 0 and weights were recorded on days 0, 5 to 12, 14, 16, 20 and 25. Daily oocyst output was determined using the methods of Long and Rowell (16). When there were too few oocysts to count with a McMaster chamber, feces were examined by the sugar flotation method.

### Histopathological Methods

On days 2, 4 to 12, 14, 15, 20 and 25, four mice from infected groups and on days 0, 2, 8, 12 and 30, two mice from the uninfected group were selected randomly and killed by atlanto-occipital separation and necropsied immediately. Tissues from four equidistant points along the length of the small intestine, the last one to three cm of the ileum, the cecum, the colon, the mesenteric lymph nodes, spleen, liver, and kidney were fixed in Bouin's fluid for 24 hours, postfixed in 70% alcohol, dehydrated, paraffin embedded, sectioned at 5 μm and stained with hematoxylin-eosin (H & E). Selected tissues were also stained with Periodic-Acid-Schiff (PAS), Gram and Masson's trichrome. Tissues were examined histologically and mucosal destruction, submucosal edema and neutrophilic infiltration were graded blindly on a scale from 0 to +4. Hemorrhage and mononuclear infiltration were graded on a scale from 0 to +3. Crypt

hyperplasia was evaluated by determining the mitotic index and crypt depth at about 5 cm distal to the ceco-colic junction. At ten and 11 days PI, crypt cells in mitosis were counted and, using an ocular micrometer, crypt depths were measured. The mean values for 20 to 40 longitudinally sectioned crypts in each of the six mice infected with 5,000 or 20,000 oocysts were obtained and compared to 50 longitudinally sectioned crypts in each of six uninfected mice.

### Bacteriological Methods

Colonic content of four uninfected mice and five mice infected with 20,000 oocysts and killed on days 9 or 10 were streaked on blood agar plates and incubated both aerobically and anaerobically[1] at 37°C. After 48 hours, aerobic organisms were characterized, anaerobes were subcultured aerobically and colonies that failed to grow were considered to be strict anaerobes. The anaerobes were observed for features of *Clostridium perfringens* and further identified using Gram's stain.

### Statistical Methods

Mortality rates, mitotic indices, crypt depths and weight changes were compared using Student's t-test. The means for oocyst production in the various groups were compared using Duncan's multiple range test.

## RESULTS

### Clinical Signs

Diarrhea which began on the sixth day PI became dysentery on days 7, 8 and 9 in mice infected with 5,000 and 20,000 oocysts. The mice stopped eating, became dehydrated, were severely depressed, were reluctant to move and had ruffled hair coats and hunched backs during the acute phase of the disease, seven to 11 days PI. Diarrhea began a day later in mice infected with 2,000 oocysts and dysentery observed on days 8 and 9 was inconsistent and less severe than in the more heavily infected mice. Dehydration and depression was less severe in most mice infected with 2,000 oocysts. Mice infected with 500 oocysts where bright and active except for slightly loose feces and moderate anorexia and depression on day 9.

The weight loss of mice infected with different doses of *E. falciformis* var *pragensis* is shown in Fig. 1. As the infective dose was increased, weight loss began earlier, became progressively more severe and mice took longer to regain their normal weight. By day 7 PI, the weights of mice infected with 2,000 to 20,000 oocysts were significantly lower ($P < 0.01$) than that of the noninfected mice. Significant acute weight loss ($P < 0.001$) on days 8, 9 and 10 was followed by a progressive increase in weight and increased consumption of food and water. The weights of mice infected with 500 and 2,000 were not significantly different from those of the controls by days 12 and 20, respectively. The weight of mice infected with 5,000 or 20,000 oocysts, however, were still significantly lower than those of the controls ($P < 0.05$) at 25 days PI.

### Oocyst Production

Oocyst discharge began on the seventh day PI regardless of the infective dose. Eighty to ninety-five percent of the oocysts were discharged on days 8 and 9. The patent period of the infection was increased from ten (range 9-11) days to 13 (range 11-16 days) as the infective dose increased from 500 to 20,000 oocysts. Higher infective doses resulted in fewer total oocysts being discharged (Table I).

### Mortality

The mortality increased as the infective dose increased (Table II). The mortality in mice infected with 20,000 oocysts, however, was not different ($P < 0.01$) from that seen in mice infected with 5,000 oocysts. Mice died between eight and 11 days PI, with 98% of the mortality occurring on days 8, 9 and 10. Mice that died or were killed when moribund were markedly dehydrated. The mice that did not regain their appetite by the end of the ninth day rarely recovered.

[1]Gas pak anaerobic systems, BBL., Div. Becton, Dickinson & Co., Cockeyville, Maryland 21030.

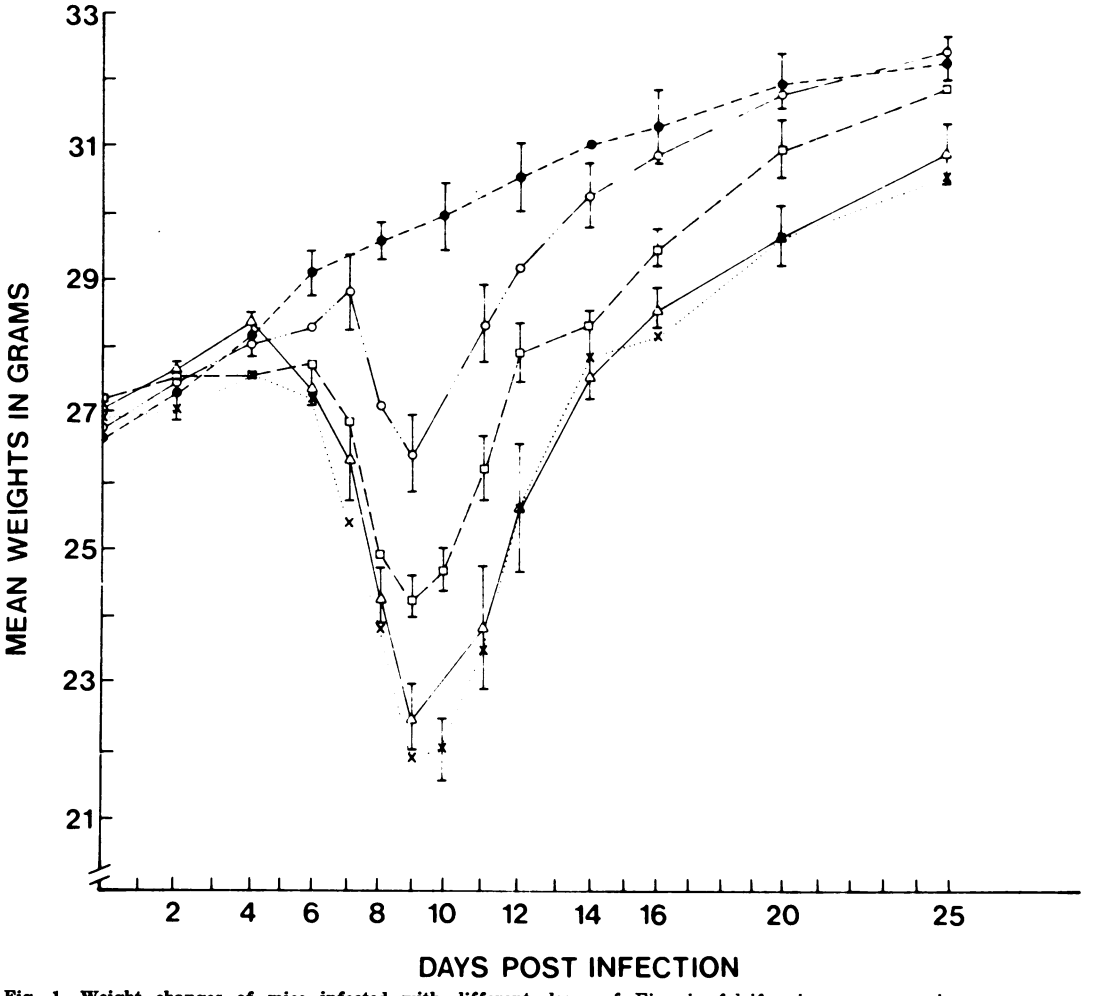


**Fig. 1. Weight changes of mice infected with different doses of** Eimeria falciformis var pragensis. ●———● **Control, o—..—o 500 oocysts, □— — — —□ 2,000 oocysts, x.....x 5,000 oocysts, △———△ 20,000 oocysts. Each point represents the mean ± standard error of six mice.**

TABLE I. Total Oocyst Discharge from Mice Infected with Different Doses of *Eimeria falciformis* var *pragensis*

| Infective Dose (Oocysts) | Oocyst Discharge ($\times 10^6$)[a] |
|---|---|
| 500 | 12.6 ± 1.3[b] |
| 2000 | 9.6 ± 2.6[c] |
| 5000 | 6.8 ± 1.6[d] |
| 20000 | 5.5 ± 1.3[d] |

[a]Values represent the mean ± standard deviation of six mice. The means with different superscripts are significantly ($P < 0.05$) different

## Gross Pathological Findings

Mice infected with either 5,000 or 20,000 oocysts showed similar changes. By the seventh to tenth day PI, the mice were dehydrated and their perineal areas were soiled with bloodstained feces. The small intestines of mice killed during the acute phase of the disease (eight to ten days PI) were empty and atrophic and the liver of some mice was pale. In four mice killed during the acute stage of the disease and in six mice that died, the large and small intestinal serosa was hyperemic. As early as five days PI, gelatinous fluid distended the colonic submucosa, distinctly separating the mucosa from the underlying tunica muscularis. Colonic contents were scanty and blood tinged. Petechial hemorrhages were scattered over the colonic mucosa which was thick and flattened with poorly

TABLE II. Mortality of Mice Infected with Different Doses of *Eimeria falciformis* var *pragensis*

| Infective Dose (Oocysts) | Number of Deaths on Specific Days Postinfection | | | | |
|---|---|---|---|---|---|
| | 8 | 9 | 10 | 11 | Mortality (%)[a] |
| 500 | – | – | – | – | 0[b] |
| 2000 | – | – | 2 | – | 5.6[b] |
| 5000 | 3 | 8 | 9 | 2 | 27.3[c] |
| 20000 | 6 | 10 | 7 | 1 | 30.8[c] |

[a]Sixty-five mice were used in the groups infected with 2000 oocysts and 105 mice were used in each of the other groups. Mice sacrificed before the eighth day postinfection were not included in expressing percentage mortality. Values with different superscripts are significantly different ($p < 0.05$).

defined rugae. In a few cases, ecchymotic hemorrhages were present and the mucosal surface of the mid colon were irregular, roughened and necrotic (Fig. 2). In two mice that died, the cecum and the entire colon including the rectum were diffusely hemorrhagic and necrotic.

Mice infected with 2,000 oocysts had less extensive changes that were also restricted to the cecum and colon. Submucosal edema was a consistent feature at days 6 to 10 and mucosal petechiation was seen irregularly at eight to ten days PI.

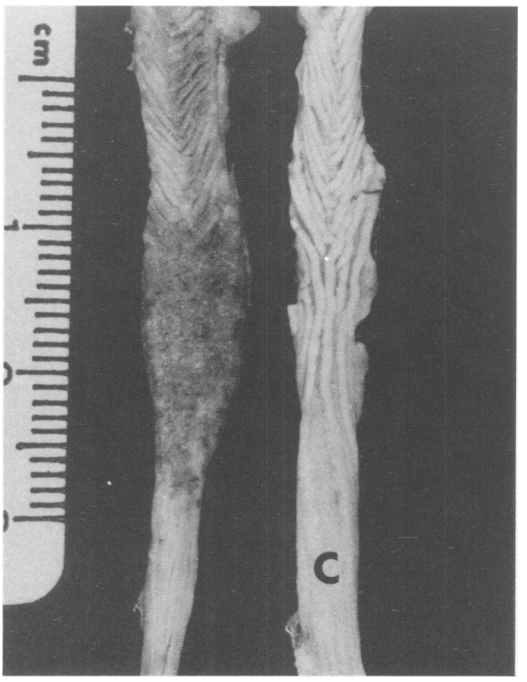


**Fig. 2. Colons from an uninfected mouse (C) and from a mouse infected with 5,000 oocysts of Eimeria falciformia var pragensis on the tenth day PI. The colon of the infected mouse, especially the mid colon, is necrotic.**

Colonic contents were either fluid or consisted of poorly formed pellets covered with a mucohemorrhagic exudate.

In mice infected with 500 oocysts the only changes observed were slightly loose feces in the rectum and a moderately thickened colonic mucosa on days 8 and 9.

## Histopathological Changes in the Intestine

Regardless of the infective dose, the histopathological findings were most marked in the colon. The pathological changes in mice infected with 20,000 and 5,000 oocysts were very similar and will be described together. References to the specific infective dose will be made when pathological changes differed. (For convenience, the two dose levels will be referred to as "higher doses".)

## Infection with 20,000 and 5,000 oocysts

As each coccidial endogenous stage developed within a parasitophorous vacuole, nucleus of the host epithelial cell became indented, enlarged and more vesicular. Maturing endogenous stages displaced host cell nuclei to one corner of the cell, usually towards the basement membrane.

Submucosal distention with clear fluid (Fig. 3A), especially in the midcolon, coinciding with the maturation of the third generation schizonts (Fig. 3B), was prominent in most mice killed as early as five days PI. The edema became progressively more marked, stained more intensely with eosin, involved the tunica muscularis and contained more neutrophils and macrophages from the seventh to the tenth day PI. Edema of the lamina propria was not seen at any stage of the disease.

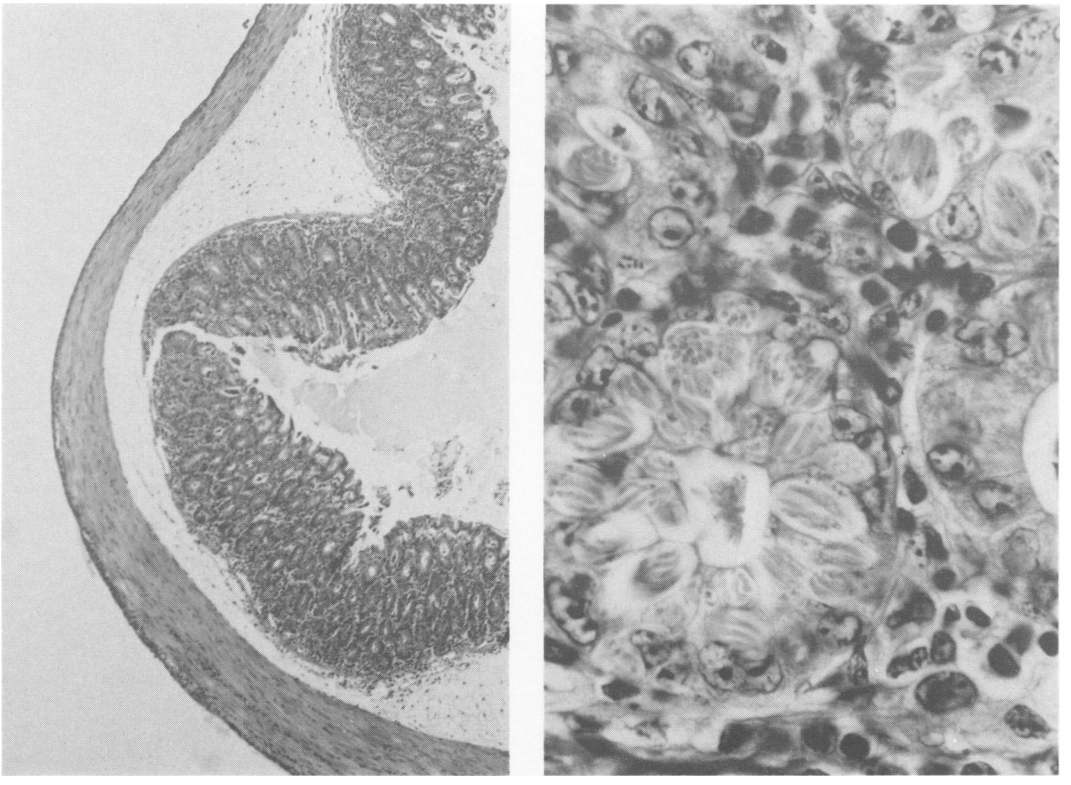

**Fig. 3. Colonic section of a mouse infected with 20,000 oocysts of Eimeria falciformis var pragensis on the fifth day PI.**

**A. Clear acellular edema fluid separates the mucosa from the tunica muscularis. H & E. x50.**

**B. Most crypt epithelial cells contain one or more mature 3rd generation schizonts. H & E. x800.**

Degenerate desquamated epithelial cells were first observed on the fifth day and were more numerous and accompanied by neutrophils on the sixth day, at which time the absorptive epithelium was either flattened or focally disrupted. Neutrophils migrated into the colonic lumen through mucosal defects and often formed a dense deposit over the exposed lamina propria (Fig. 4). In some mice infected with 20,000 oocysts, extensive epithelial destruction resulted in a virtually crypt-free but cellular mucosa. Immature looking mononuclear cells with large hypochromatic round to ovoid nuclei, occasionally seen in mitosis, formed the major cellular component (Fig. 5). At least some of these cells appeared to be remnants of crypt epithelial cells trapped within collapsed crypts (see Discussion).

By the seventh day PI, existing crypts were either occluded with intraepithelial maturing oocysts or were filled with extruded oocysts and debris. Progressive destruction of crypts through days 8 and 9 exposed lamina propria which in some areas was covered with a hemorrhagic and neutrophilic exudate. The nonnecrotic lamina propria was markedly cellular due to the presence of some neutrophils, macrophages and what appeared to be fibroblasts and displaced crypt epithelial cells.

All of the mice that died and 50% of those killed on days 9 and 10 had various degrees of nonselective colonic coagulative necrosis. The necrotizing lesion was limited either to the mucosa or extended into the submucosa. In a few mice the necrotizing process extended right through the tunica muscularis almost resulting in perforation of the colon (Fig. 6). Serosal mesothelial cells over such areas were prominent or necrotic and the adjoining edematous mesenteric fat was infiltrated with lymphocytes. The necrotizing lesion occasionally involved the entire circumference of the co-

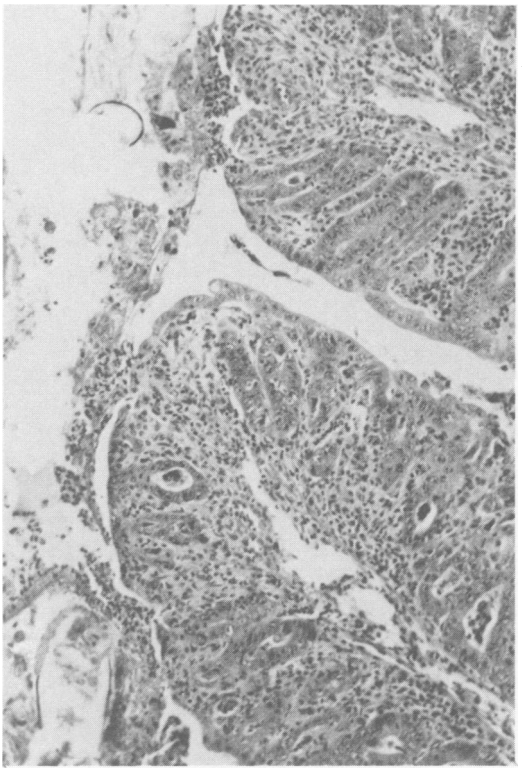

Fig. 4. Colonic section of a mouse infected with 20,000 oocysts of Eimeria falciformis var pragensis on the sixth day PI. Neutrophils emigrating into the lumen through multiple surface epithelial defects form a deposit over the eroded mucosa. Note flattened intact surface epithelia. H & E x120.

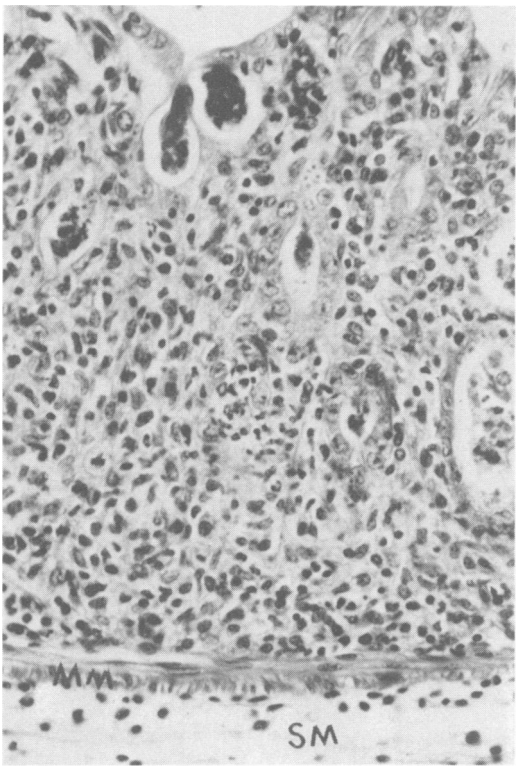


Fig. 5. Colonic section of a mouse infected with 20,000 oocysts of Eimeria falciformis var pragensis on the sixth day PI. Neutrophils emigrating into the the presence of neutrophils, macrophages, lymphocytes and "mononuclear cells". The submucosa (SM), muscularis mucosa (MM) and remnants of 6 crypts containing debris are shown. H & E. x300.

lonic wall. Dense accumulations of degenerating neutrophils and some macrophages were situated along the border between the necrotic tissue and the edematous, neutrophil-containing underlying structures. Most of the necrotic tissue contained massive numbers of both Gram-positive and Gram-negative bacteria. Submucosal vessels were either congested, thrombosed or necrotic, depending upon the extent of necrosis. In some areas, muscle fibres widely separated by edema fluid were disrupted and permeated with neutrophils.

By day 11, diffuse necrotizing colitis was seen in only one mouse. Lesions were seen as mucosal ulcers, some of which penetrated the muscularis mucosae. Ulcers had bases of what appeared to be fibroblasts covered with fibrin, neutrophils and macrophages and the epithelial margins were hyperplastic. Regenerating surface epithelial cells changed from columnar to cuboidal as they migrated from the margins to the center of the ulcers (Fig. 7). Resolved superficial ulcers were represented by epithelialized, contracted, crypt-free areas of mesenchymal cells at 12 to 14 days PI. Cuboidal, vesiculated epithelial cells appeared to be migrating under the superficial inflammatory exudate in some resolving ulcers as late as 20 days PI (Fig. 8).

In most resolving superficial ulcers, the muscularis mucosa was intact although it was occasionally included within the inflammatory exudate. In some ulcers, the gap between the ends of the disrupted muscularis mucosae was filled with macrophages.

Mucosal destruction was followed immediately by crypt regeneration on day 9 in mice which did not have extensive necrotizing lesions. Crypt hyperplasia was more uniform on days 10 and 11 except in the ulcerated areas. Regenerating crypt cells were hyperchromatic with prominent

Fig 6. Colonic section of a mouse infected with 5,000 oocysts of Eimeria falciformis var pragensis on the tenth day PI. There is extensive necrosis and the colon is nearly perforated. Tunica muscularis is fragmented (arrows) and the mesentery is infiltrated with inflammatory cells. H & E. x75.

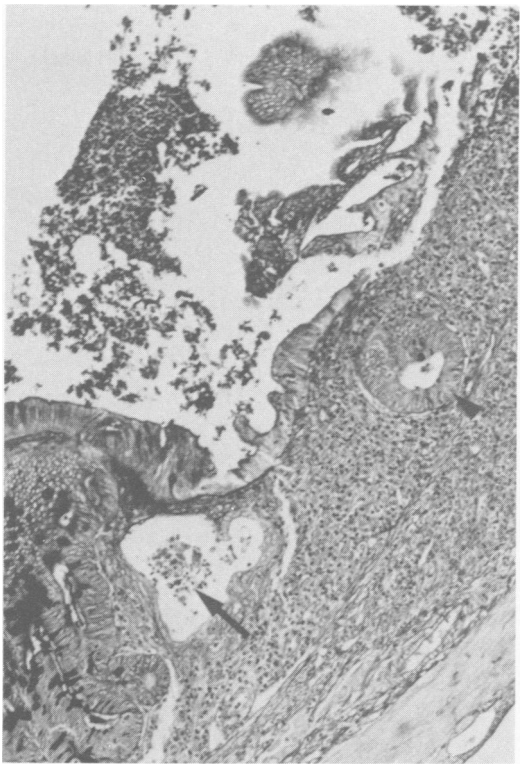

Fig. 7. Colonic section of a mouse infected with 20,000 oocysts of Eimeria falciformis var pragensis on the tenth day PI. Regenerative surface epithelial cells change from columnar to cuboidal as they migrate from the margin to the center of the ulcerated mucosa. Note a regenerating crypt (arrow head) and a dilated atrophic crpt containing some debris (arrow). PAS. x120.

nuclei and increased mitotic activity. The crypts were longer and almost devoid of goblet cells (Fig. 9B). Some regenerating crypts appeared more tortuous than normal and absorptive epithelial cells, especially at the margins of mucosal defects, were often arranged in finger-like projections. A comparison of mitotic index and crypt depth between uninfected mice and mice infected with 5,000 or 20,000 oocysts at days 10 and 11 is presented in Table III. Mitotic activity and crypt depth were significantly increased ($P < 0.05$ and $P < 0.01$ respectively) in the infected mice indicating active epithelial regeneration. Dilated crypts with flattened epithelial cells were observed regularly among the regenerating crypts (Fig. 7). The dilated crypts sometimes contained neutrophils, a few lymphocytes, degenerate epithelial cells, oocysts and cell debris.

The hyperplastic response regressed from days 12 to 16 except at the margins of mucosal ulcers. Crypts became narrower and shorter and the numbers of goblet cells increased progressively. The numbers of immature epithelial cells (seen at six to eight days PI) in the mucosa decreased as crypt hyperplasia became less evident. However, the lamina propria was irregularly broadened by an increased number of macrophages, epithelioid cells, giant cells and a few lymphocytes and neutrophils at 12 to 25 days PI (Fig. 10). The neutrophils accumulated focally, occasionally assuming a rosette formation or a "microabscess", around retained oocysts within the lamina propria. By days 20 to 25, the colonic mucosa returned to normal except for localized crypt-free areas which often contained retained oocysts surrounded by granulomatous tissue. Some of the defects in the muscularis mucosa were bridged by what appeared to be fibroblasts. Dense populations of normal intraluminal fusiform bacteria, seldom seen during the acute

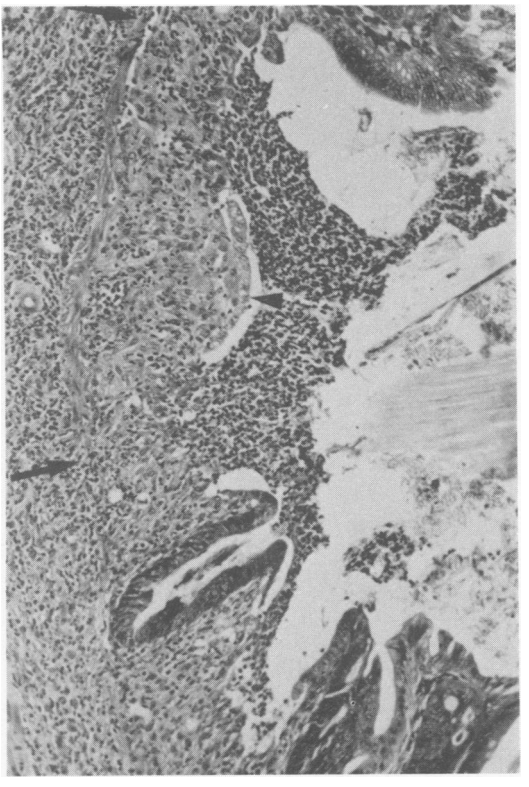

**Fig. 8. Colonic section of a mouse infected with 5,000 oocysts of Eimeria falciformis var pragensis on the 20th day PI. Cuboidal epithelial cells (arrow head) under an inflammatory exudate. The muscularis mucosa is disrupted at two sites (arrows). H & E. x120.**

stages of the disease, became evident again in the mice that recovered from the infection.

Although the parasite infected mainly the colon and cecum, a few coccidial stages, primarily gamonts, were seen in the terminal ileum of 68% of the mice infected with the higher doses. Only five percent of the mice infected with 20,000 oocysts had extensive destruction of ileal crypts. In the other 95% destruction was very focal and limited to the occasional group of crypt epithelial cells.

### Infection with 2,000 oocysts

The histopathological findings in mice infected with 2,000 oocysts were essentially similar to the descriptions given for the higher doses. However, epithelial destruction, edema, neutrophilic infiltration and hemorrhage appeared later, were of shorter duration and were less severe.

Involvement of the lower ileum occurred in only 30% of the infected mice. Necrotizing colitis occurred less often and did not involve the entire circumference of the colon except in one of the mice that died. Mucosal defects consisted usually of small focal ulcers, a few of which penetrated through the muscularis mucosae. Unresolved colonic ulcers were also seen as late as 20 days PI. A localized granulomatous response in association with trapped oocysts was observed until the experiment was terminated at day 25 PI.

### Infection with 500 Oocysts

Histopathological findings in mice infected with 500 oocysts were of the same general pattern as in the higher doses but were strikingly less severe. Mucosal necrosis was limited to the epithelium and consisted of focal epithelial destruction. Submucosal edema was less severe than in more heavily infected mice but edema of the lamina propria was observed occasionnally. Focal accumulations of neutrophils, restricted to surface epithelial defects, were not seen before the seventh day PI. The inflammatory cellular response in mice infected with 500 oocysts was primarily mononuclear. Necrotizing and ulcerative colitis was not seen in any of the mice and a few macrogamonts were observed in the terminal ileum of two mice. The colon returned to normal by days 14 to 16 except for a few trapped oocysts with an accompanying focal granulomatous response.

The histopathological features observed in mice infected with the different doses of oocysts are summarized in Fig. 11.

### Histopathological Changes in the Mesenteric Lymph Nodes and Spleen

The changes seen in the mesenteric lymph nodes and spleen were the same regardless of the infective dose of oocysts. Lymph nodes were moderately edematous at six to ten days PI, especially in mice infected with the higher doses. Although there was variation in individual mice with all infective doses, progressive hyperplasia of the lymphoid tissue in the lymph nodes and spleen was evident through days 8 to 16. Reactive germinal centers of lymphatic nodules of both the mesenteric lymph nodes and the spleen were surrounded by promi-

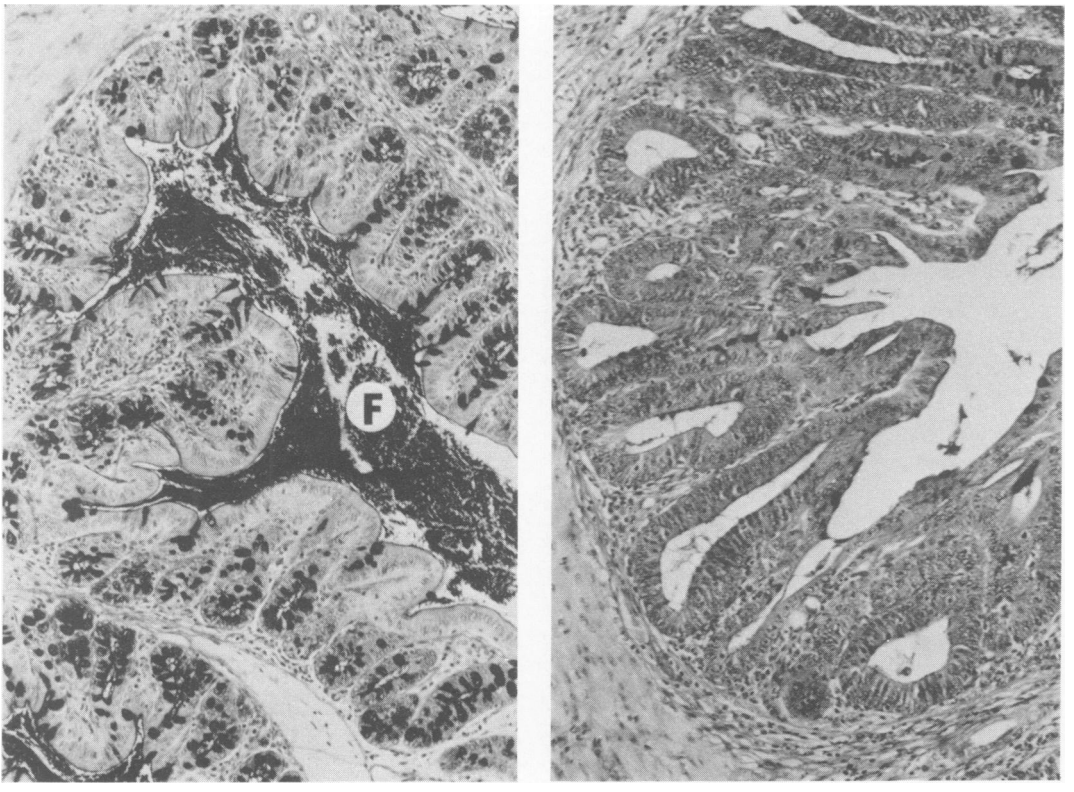


**Fig. 9. A. Colonic section of an uninfected mouse. Crypts are uniform and have a high concentration of goblet cells. Note the dense populations of fusiform bacteria (F) within the lumen. PAS. x150.**

**B. Colonic section of a mouse infected with 20,000 oocysts of Eimeria falciformis var pragensis on the 11th day PI. Crypts are widened, elongated and irregular. Note the reduction in the goblet cell concentration of the crypts and the absence of intraluminal fusiform bacteria. PAS. x150.**

**TABLE III. Mitotic Index and Crypt Depth in Uninfected Mice and Mice Infected with 5,000 or 20,000 Oocysts of *Eimeria falciformis* var *pragensis***

| | Mitotic Index[a] | Crypt Depth (μm)[a] |
|---|---|---|
| Uninfected | 0.7 ± 0.3 | 181.3 ± 41.2 |
| Infected | 1.8 ± 1.0[b] | 320.3 ± 98.9[c] |

[a]Fifty crypts in uninfected mice and 20 to 40 crypts in infected mice at days 10 and 11 were evaluated. Values represent the mean ± standard deviation of six mice
[b]Significantly different ($P < 0.05$) from uninfected group
[c]Significantly different ($P < 0.01$) from uninfected group

nent mantles of mature lymphocytes. In most mice, at days 12 to 25, mature plasma cells were the predominant cell population of the medullary cords.

### Bacteriological Findings

The bacterial species isolated from the colon of uninfected and infected (20,000 oocysts) mice are listed in Table IV. *Yersinia enterocolitica* was isolated from some of the infected and uninfected mice. The number of C. *perfringens* increased during the acute stage of the disease.

## DISCUSSION

*Eimeria falciformis* var *pragensis* caused

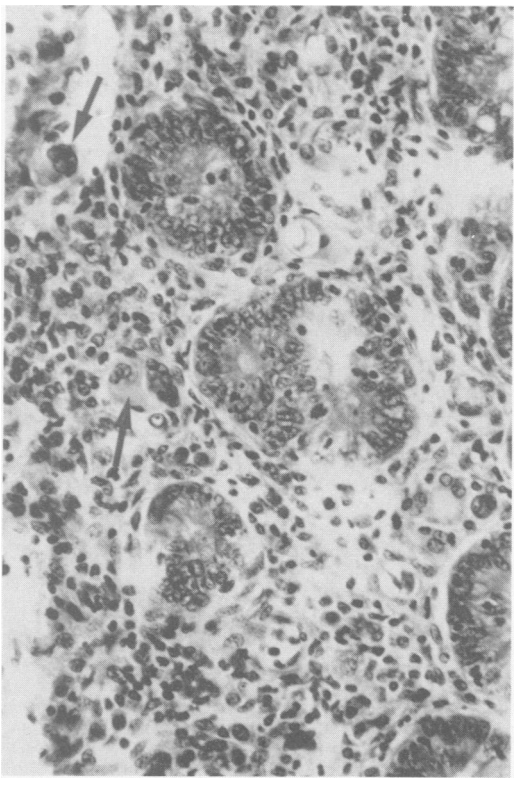

**Fig. 10. Colonic section of a mouse infected with 20,000 oocysts of Eimeria falciformis var pragensis on the 25th day PI. Oocysts trapped within the lamina propria have induced a granulomatous reaction. Note the giant cells (arrows). H & E. x300.**

pathological changes in mice comparable to those reported in other intestinal mammalian or avian coccidial infections of the intestine (17, 31, 32), with the possible exception of the necrotic enteritis seen in the mice. The pathological processes included destruction of epithelial cells by developing endogenous stages, a concommitant inflammatory response and regeneration of injured tissue.

It is difficult to compare Reimer's (26) findings, the only brief description of pathological lesions in murine coccidiosis and the findings in the present study since the time when the pathological lesions occurred (except for crypt regeneration) were not indicated in the former report. It is even uncertain whether the coccidium used in the previous study (26) was *E. falciformis*. All mouse coccidia at that time were considered to be *"E. falciformis"*. At present, fifteen species of *Eimeria* are reported to parasitize mice (25). A comparison of the results of the present study to those reported by Reimer (26) suggests that the same or closely related Eimerian species were used in both studies. The pathological changes associated with *E. falciformis* var *pragensis*, a variant of *E. falciformis* (4) would not be expected to be different since the two coccidia have similar life cycles (4, 20).

Immediately after extensive destruction of crypt epithelium from six to eight days PI, the mucosa became cellular, primarily due to an increase in immature appearing mononuclear cells. Condensation of existing mesenchymal cells resulting from lamina proprial collapse would not fully account for the absolute increase in the mononuclear cell population. The identity of most of these cells could not be established but the decrease in the number of mononuclear cells as crypt regeneration occurred suggests that at least some of the "mononuclear cells" were morphologically altered remnants of crypt epithelial cells. Displacement and morphological alteration of parasitized crypt cells have been demonstrated in avian cocciodiosis (1, 6). Some of the mononuclear cells were thought to be pericryptal fibroblasts which became prominent as the crypts collapsed. Electron microscopic studies may help to determine the identity of the mononuclear cells.

The pathogenicity of *E. falciformis* var *pragensis*, evaluated both clinically and pathoanatomically was more severe as the infective dose was increased from 500 to 5,000 oocysts. The mortality and the pathological changes were equally severe in mice infected with 5,000 and 20,000 oocysts, suggesting that a single massive dose would tend to saturate host cells and further infective parasites would have no additional effect. In most mice infected with 20,000 oocysts, the colonic epithelium was almost completely destroyed by day 6. It is possible that many of the fourth generation merozoites that would have developed normally into sexual stages (4, 20) were discharged in the feces. This hypothesis of insufficient epithelial cells to host the parasite was supported by the progressively fewer oocysts discharged as the infective dose was increased from 500 to 20,000 oocysts. The ileum was involved more often as the infective dose was increased. This apparent attempt at increasing the surface area for oocyst production was not effective, however, in increasing the output of oocysts. Similar reduced reproductive potential with increasing infective doses

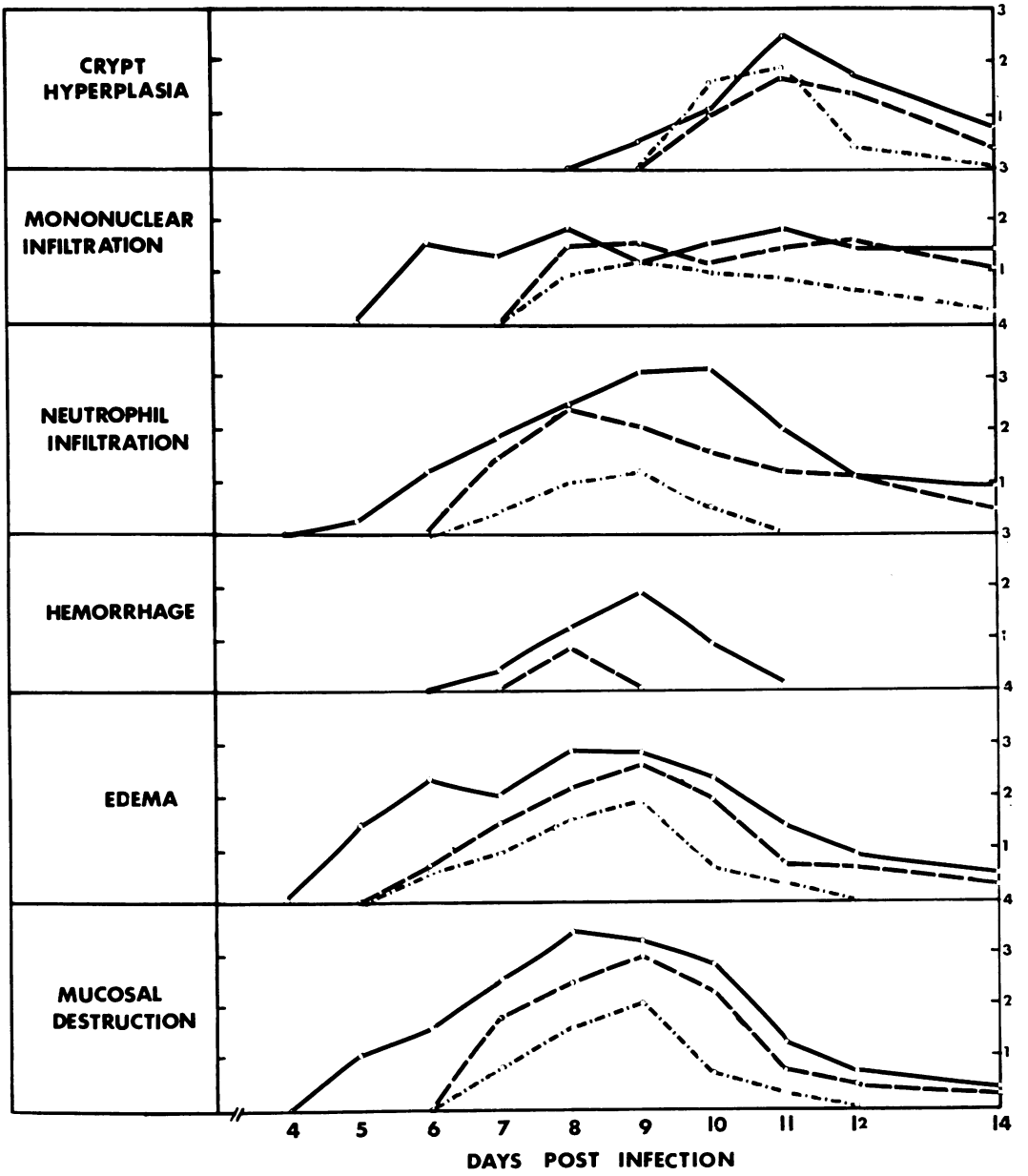


Fig. 11. The main histological findings (graded) in the colon of mice infected with different doses of Eimeria falciformis var pragensis, o—.—.—o 500 oocysts, o— — — —o 2,000 oocysts, o————o the mean of 5,000 and 20,000 oocysts.

observed in other studies (8, 10, 14) indicates that output of oocyst does not necessarily reflect the degree of coccidial burden in an animal.

The marked weight loss within a period of 24-48 hours, occurred when mice completely refused to eat and drink and when the cecocolic destruction was most severe. The acute weight loss over such a short period of time was probably due to dehydration which likely contributed to the death of most of the mice. A few of the mice died of acute peritonitis.

Necrotizing colitis, a feature in most of

TABLE IV. Colonic Bacterial Species Cultured from Uninfected Mice and Mice Infected with 20,000 Oocysts of *Eimeria falciformis* var *pragensis*

| Bacteria | Uninfected | | | | Infected | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mouse # | | | | Mouse # | | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 5 |
| *E. coli* | 5 | 5 | 3 | 2 | 5 | 5 | 5 | 2 | 5 |
| *C. perfringens* | 1 | 2 | 1 | 1 | 5 | 5 | 4 | 5 | 5 |
| *Y. enterocolitica* | 2 | 3 | — | — | 4 | 2 | — | — | — |
| *Staph. epidermidis* | — | — | 5 | 5 | — | — | 5 | — | — |
| *Lactobacillus* spp | 2 | — | — | 1 | 3 | — | — | — | — |
| Streptococcal spp | 1 | — | 5 | 5 | 2 | 4 | 5 | 3 | — |
| *Corynebacterium* spp | — | 1 | 5 | — | — | — | — | 4 | — |
| *Bacillus* spp | — | 1 | — | — | 2 | — | — | — | — |
| Gram neg. anaerobes | 1 | — | — | — | — | 2 | — | — | — |
| Gram pos. anaerobes[a] | — | — | 5 | 5 | — | — | — | — | — |

[a]Other than *C. perfringens*
5 Very heavy growth
4 Heavy growth
3 Moderate growth
2 Light growth
1 Sparse growth
— No growth

the mice that died and in a few mice infected with higher doses, seems to be related to massive epithelial destruction by the coccidia. However, the pathogenesis for such nonselective necrosis, occasionally extending right through the colonic wall, is not clear. *E. faciformis* var *pragnesis* develops intraepithelially (20) and the few oocysts within the lamina propria could not have caused the extensive necrosis observed. Similar nonselective colonic coagulation necrosis progressing from mucosal to serosal surfaces occurs in amoebiasis and was thought to be caused by an unrecognized extracellular product elaborated by the amoeba (12). Attempts to demonstrate a toxic principle in avian coccidia have been unsuccessful. Birds inoculated intravenously with oocyst material of *E. tenella* were not affected although the same material was fatal to rabbits (3, 28, 29).

The normal murine colon is inhabited by large numbers of aerobic and anaerobic bacteria (27) which have the potential to invade a devitalized mucosa and complicate existing lesions. It is tempting, therefore, to speculate that exposure of the lamina propria could either enhance bacterial attachment or allow for diffusion of free intraluminal toxin causing nonselective mucosal necrosis. Massive bacterial deposits within the necrotic tissue, an increase in the number of *C. perfrigens* and the reduction in colonic fusiform bacteria during the acute stage of the disease indicate a disturbance in the colonic bacterial population. A marked increase in *C. perfringens* and a decrease in lactic acid-bacteria were found in birds infected with *E. tenella* (13). Although the toxins of *C. perfringens* types B anl C cause a necrotizing enteritis (11), an increased number of *C. perfringens* does not necessarily mean that the bacteria were involved in the development of the necrotizing lesions. Necrotic enteritis often associated with *E. brunetti* of the chicken was considered to be due to *C. perfringens* type C (23). However, Hedge (9) observed similar necrotizing lesions in both gnotobiotic and conventional birds and concluded that the necrotic enteritis was due to *E. brunetti.*

The use of germ-free mice would resolve the role of normal intestinal microflora in the pathogenesis of necrotizing colitis in mice infected with *E. falciformis* var *pragensis.* Owen's (22) report that germ-free mice were refractory to *E. falciformis* was interesting but requires confirmation in view of the fact that gnotobiotic birds were found to be susceptible to coccidial infections (9, 33).

The pathogenicity of *Yersinia enterocolitica* is unoknown in mice (30). The necrotizing enteric lesions observed in the present study are similar to those observed in Yersiniosis of man (2). The isolation of *Y. enterocolitica* from both the infected and uninfected mice however, raises some doubt as to its possible role

in the pathogenesis of the necrotizing lesions.

The crypt hyperplasia which followed the mucosal destruction was similar to that observed in various diseases that cause crypt or villus epithelial injury (12). Hyperplastic crypts have been observed histologically in avian (5) and ovine (24) coccidiosis during the stage of peak parasitization of the villus epithelium. Fernando and McCraw (7) have provided evidence that crypt hyperplasia occurred before villus or crypt epithelial necrosis, suggesting that the developing parasites had a direct stimulatory effect on the progenitor cells of the intestine. The mucosal ulcers in murine coccidiosis resolved in a manner similar to colonic ulcers induced by surgical excision or cauterization in mice, dogs and man (18, 19, 21). It was difficult to determine whether the muscularis mucosa was regenerating or was merely included within an inflammatory process. However, some muscularis mucosal defects were bridged with what appeared to be fibrocytes. O'Conner (21) has reported that the muscularis mucosa in mice was incapable of regeneration.

Oocysts trapped within the colonic lamina propria appeared to be either within disrupted crypts that had failed to regenerate and establish patency with the colonic lumen or within displaced parasitized epithelial cells. Second generation schizonts of *E. necatrix*, believed to develop within the fibroblasts of the lamina propria (15) were considered to be within epithelial cells displaced into the lamina propria (6).

In summary, the pathological lesions of *E. falciformis* var *pragensis* in the mouse included epithelial destruction, edema, inflammation and occasionally necrotizing or ulcerative colitis followed by crypt regeneration and resolution. The lesions were dose-dependent but reached a maximum at an infective dose of 5,000 oocysts.

## ACKNOWLEDGMENTS

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## BOOK REVIEW

PROCEEDINGS OF THE TWENTIETH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS. *Obtainable from Dr. M. W. Vorhies, Secretary-Treasurer, AAVLD, 6101 Mineral Point Road, Madison, Wisconsin 53705. 390 pages. Price $10.00.*

These proceedings are a report of the most recent annual meeting of the AAVLD which was held in Minneapolis, Minnesota on October 16, 17 and 18, 1977. The editors are to be congratulated on the speed of publication and the quality of this presentation.

The majority of the thirty-seven papers in this publication describe the etiology, pathology and the laboratory diagnoses of a wide variety of infectious disease conditions in cattle, swine, goats, sheep, cats, dogs, chickens, psittacine birds and fish.

As a reflection of some of the current concerns in animal production in the U.S.A. there are five papers dealing with pseudorabies, three with bluetongue and three with bovine abortion. A few examples of other disease conditions discussed include channel catfish virus disease, canine histoplasmosis, *Cytauxzoon felis* infection in a cat, porcine parvovirus-induced reproductive failure, acute bovine pulmonary emphysema and malignant catarrhal fever.

Noninfectious diseases have received appropriate attention in papers discussing such topics as the toxicological and residual aspects of pentachlorophenol, the diagnostic problems of anticoagulant rodenticide toxicoses and the toxicity of plants containing perilla ketone.

Those persons interested in diseases of wildlife will find the article discussing the problems in diagnoses and control of wildlife diseases of particular interest.

Diagnostic methods and techniques are constantly being improved. The application of some of these advancements in pseudorabies diagnoses are described in considerable detail in papers outlining (a) a serological test for this disease using a cell-bound antigen and a peroxidase enzyme labelled assay method and (b) an agar-gel immunodiffusion test. A third paper prepared by the Pseudorabies Diagnostic Standardization Committee of the AAVLD discusses the recommended minimum standards for tests employed in diagnosing this disease.

The constitution and by-laws of the AAVLD have been included in these proceedings. Space limitations do not permit further description of its contents.

Membership in this association is open to any laboratory worker engaged in the field of disease diagnoses in animals and applications for membership should be forwarded to the Secretary-Treasurer.

Readers of the Canadian Journal of Comparative Medicine will be pleased to know that Dr. Julius Frank, Director, Animal Pathology Division, Agriculture Canada, is the 1977-78 President of the AAVLD.

It has been stated that receipt of the Proceedings each year is, by itself, well worth the membership fee for the AAVLD. This reviewer agrees with that statement. — *C. C. Stewart.*