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Histopathology of Fasting and Bluecomb Disease
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Experimentally Infected with
Bluecomb Disease Coronavirus

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SUMMARY

The histopathology of fasting and bluecomb disease in one-day-old turkey poults inoculated with bluecomb disease coronavirus (BCDCV) was studied. Uninoculated fasting poults produced clinical signs similar to those observed in BCDCV-inoculated poults. No histological changes in the intestines were observed in the fasted poults whereas definite lesions were observed in the BCDCV-inoculated poults. The lesions did not differ significantly with whether they were fed or fasted. The severity of the lesions in the intestinal epithelium was in decreasing order in the jejunum, ileum, and cecum.

The lesions first appeared 24 hours postinoculation (PI) and progressed through 96 hours PI, as marked shortening of the villi, loss of microvilli, granular appearance of the cytoplasm of epithelial cells with nuclear margination of chromatin, and accentuation of the nucleolus. Similar lesions were observed in the jejunum, ileum, and cecum of turkey embryos inoculated at 24 days old as well as poults from these embryos. Signs of healing were first seen at 120 hours PI. No histopathological changes were observed in the pancreas, brain, kidneys, liver, adrenal, and bursa of Fabricius.

The intestinal lesions observed should be a useful histological technique for differentiating fasting from bluecomb disease in turkey poults.

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INTRODUCTION

Bluecomb disease of turkeys is characterized by depression, anorexia, dehydration, watery diarrhea, rapid loss of body weight, high morbidity, and a high mortality in younger poults. Some of the pathological changes have been reported by Hilton (5). His studies involved turkeys infected both naturally and experimentally. The inoculum was a crude 20% homogenate prepared from the small intestine of turkeys with clinical signs of bluecomb disease. Nonspecific lesions were observed in the liver, pancreas, kidneys, and adrenal. The only significant lesion observed was intraluminal exudate of mononuclear cells in the duodeno-jejunal area. Hilton concluded from those lesions that bluecomb disease of turkeys is not characterized by any pathognomonic lesions.

In 1970, Adams *et al.* (1) reported on histopathological lesions in eighteen-day-old poults inoculated with 220-nm Millipore filtrates of homogenates prepared from the intestines of turkeys infected with the Minnesota strain of bluecomb disease. Their significant findings were decreases in the length of microvilli and in the number of goblet cells and a separation of the epithelium from the lamina propria.

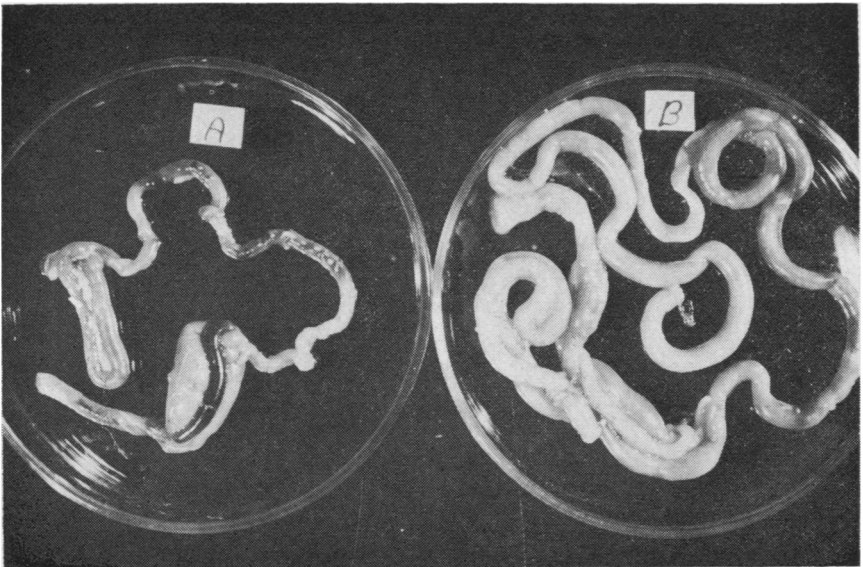


Fig. 1. Intestine A (infected fed) and B (uninfected fed) poults at 72 hours postinoculation. Note the reduction of intestinal mass in A.

In 1969, Dziuk *et al.* (4) reported that uninfected-fasted and bluecomb-infected turkeys appeared similar clinically and physiologically. The inoculum used was a crude 10% homogenate prepared from the intestines of turkeys infected with bluecomb disease.

The present studies were done to determine the clinical and pathological manifestations produced in one-day-old turkey poults inoculated with Minnesota strain of BCDCV isolated in embryonated turkey eggs and in uninoculated-fasted poults of the same age. Also determined were clinical and histopathological changes in the BCDCV-infected turkey embryos and the poults hatched from these embryos.

MATERIALS AND METHODS

Turkey poults. Three hundred one-day-old turkey poults were obtained from a commercial hatchery from flocks with no previous history of bluecomb disease. Poults from this hatchery had been used in our previous studies on bluecomb disease (8) and were shown to be consistently susceptible to the disease. Three hundred poults were divided into 4 groups of infected (I), uninfected (U), fed (Fe), and fasted (Fa), as follows: 50 UFe, 50 UFa, 100 IFe, and 100 IFa. Feeding was with a commercial turkey starter, whereas fasted poults were kept without feed until death or sacrifice but given water *ad lib.* I poults were inoculated with 1000 PID's (poult infectivity dose) of BCDCV (3) by the oral route at one day old. The poults were observed for clinical signs, and individual weights were recorded each day for four days to correlate clinical signs and weight loss with lesions.

On PI days 1, 2, 3, 4, 5, 6, 7, 9, 14, and 21, four poults from each group were sacrificed, necropsied, and examined for gross lesions. The liver, spleen, kidneys, adrenal, heart, yolk sac, duodenum, pancreas, jejunum, ileum, cecal tonsils, ceca, and bursa were placed in neutral buffered 10% formalin. Tissues were sectioned at 6 μ m, stained with hematoxylin-eosin, mucicarmine, or periodic-acid-Schiff (PAS) stains.

Embryonated turkey eggs. Eighty 24-day-old embryonated turkey eggs were obtained from the commercial hatchery which provided the experimental turkey poults. Forty embryonated eggs were inoculated by the amnionic cavity route with 100 PID's of BCDCV per embryo. The remaining 40 embryonated eggs were

Table 1. Weight differential in uninfected (fed and fasted) and infected (fed and fasted) poult.

Group	No. of poult weighed for 4 days	Av. wt. (g)		Change in wt. gain or loss	
		on day 1	on day 4	(g)	(%)
Uninfected					
Fed	12	50	72	+22.0	+44.0
Fasted	13	51	45	-6.0	-11.8
Infected					
Fed	16	49	36	-13.0	-26.5
Fasted	17	50	38	-13.0	-24.0

used as uninoculated controls. The embryos were reincubated. On PI days 1, 2, 3, and 4, live embryos were sacrificed from each group and their tissues were collected and stained by the procedures described in the section on turkey poult. Similarly, poult were hatched from the infected and uninfected embryos and kept under observation in separate Horsfall-Bauer units until 4 days old. Their individual weights were recorded daily. Two poult from each group were sacrificed and necropsied, and their tissues were collected and stained in the same manner.

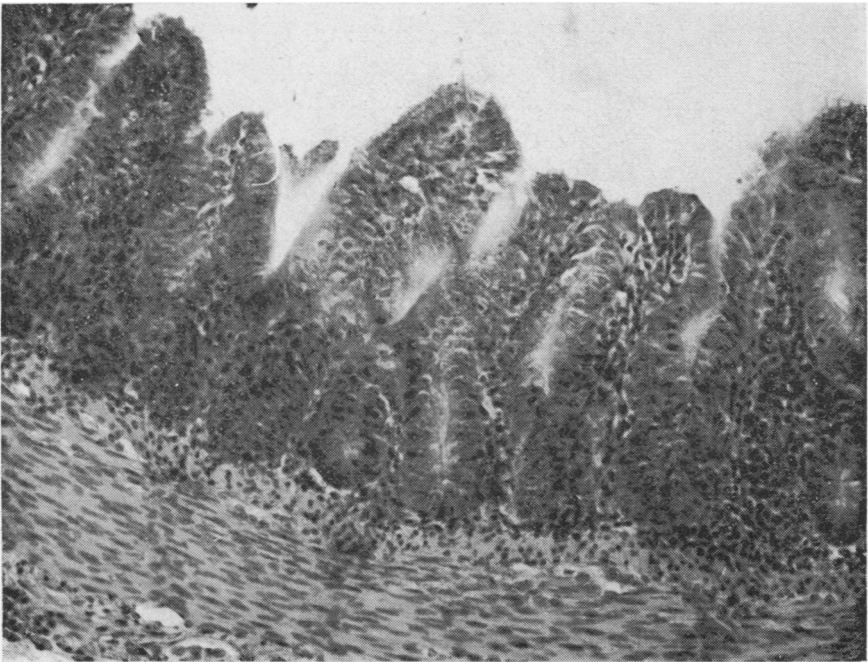


Fig. 2. Jejunum of infected-fed poult at 48 hours postinoculation. The villi are shortened, with uneven margins, granular cytoplasm, and prominent nuclei as compared with uninfected-fasted poult in Fig. 3. H & E, $\times 150$.

RESULTS

Turkey poults. The differences between I and U turkeys were grossly obvious at 24 hours PI. I poults at necropsy were dehydrated and the ceca contained brown-colored fluid and gas, compared with the normal appearance of ceca of U poults. At 48 and 72 hours PI the gross difference in the mass of the intestinal tracts (Fig. 1) was remarkable. This comparison also reflected a difference in overall carcass appearance and weights (Table 1). The brain, heart, pancreas, liver, adrenal, and kidneys, examined microscopically, gave negative findings. Tissues examined from the intestinal tract were selected from the duodenum, jejunum orally and aborally to the yolk sac attachment, cecal tonsil areas, mid-cecal areas, and bursa.

The first change (24 hours PI) was a marked shortening of the jejunal villi in I poults (Fig. 2) from that in U poults, both Fe and Fa (Fig. 3). The lamina propria became more cellular and



Fig. 3. Jejunum of an uninfected-fasted poult at 48 hours, showing the normal appearance of the mucosa. H & E, $\times 170$.

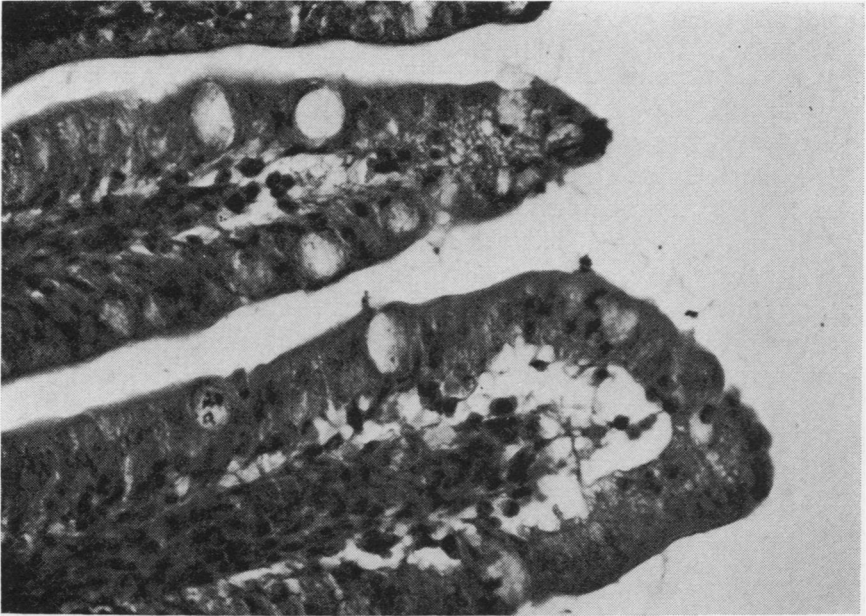


Fig. 4. Jejunum of an infected-fed poult at 24 hours postinoculation, showing mucosal separation, hypercellular lamina, and enlarged goblet cells. H & E, $\times 340$.

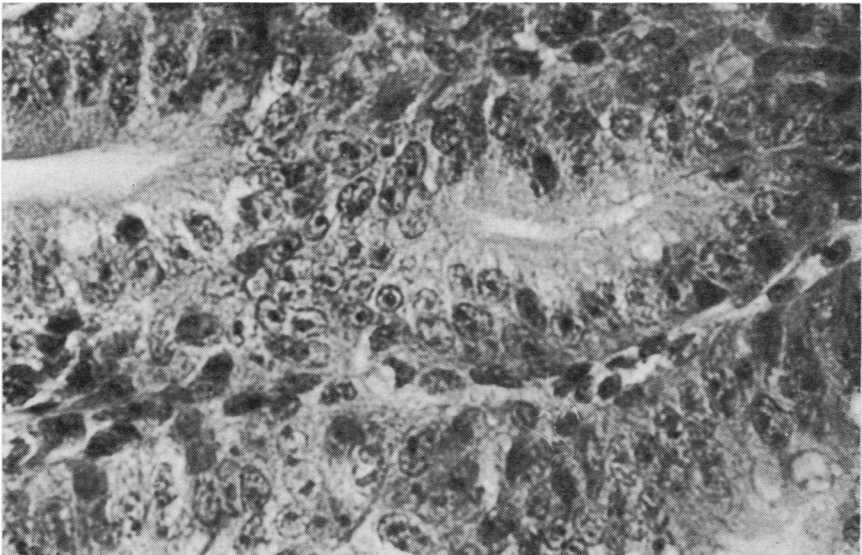


Fig. 5. Jejunum of an infected-fed poult at 48 hours postinoculation, showing mucosa cells with granular cytoplasm, nuclear margination, and prominent nucleoli. H & E, $\times 450$.

vascular with its separation from the epithelium (Fig. 4). The cells in the lamina propria that accounted for the cellular increase were lymphoid cells. Changes in the epithelium were the loss of microvilli, granular appearance of the cytoplasm, nuclear margination of chromatin, and accentuation of the nucleolus (Fig. 5). There was no appreciable increase in mucous cells at 24 hours PI. No such changes were observed in tissues from U poult (Fig. 6).

Changes at 48 hours PI were similar to those at 24 hours but more accentuated. There were a few mucous cells and a few heterophils in the lamina propria of some of the specimens. The enterochromaffin cells had decreased slightly in numbers. Numbers of mitotic figures had increased in the epithelial cells at the bottom of the crypts at this time and became more noticeable at 72 hours PI.

At 72 hours PI the sections from the cecal tonsillar area and midcecum did show, to a lesser extent, the changes in the jejunum that were found at 24 and 48 hours PI. The cecal tonsillar areas appeared to be more reactive, although variations due to the plane from which they were cut may have been responsible for some of these changes. The mucosa in both midcecal and tonsillar areas showed the shorter villi and granularity. Sections through the duodenum appeared to be the least affected and showed changes to

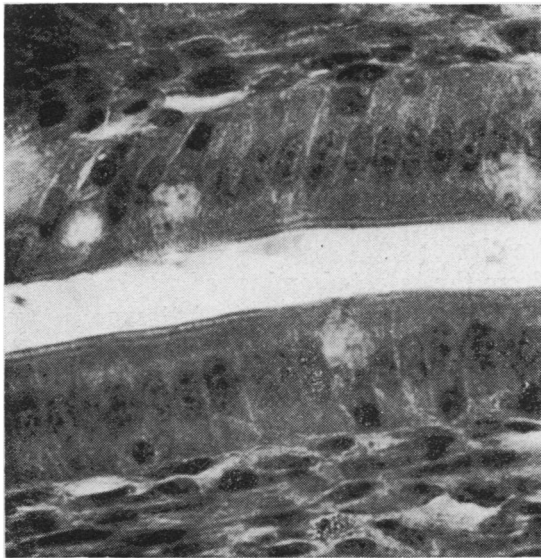


Fig. 6. Jejunum of an uninfected-fed poult at 48 hours, showing even rows of nuclei, well-defined border with microvilli, and normal goblet cells. H & E, $\times 450$.

a much lesser extent. No lesions were seen in the pancreas, heart, brain, kidneys, liver, adrenal, and the bursa.

Tissues collected at 120 hours PI contained more heterophils scattered throughout the villi as well as debris-laden macrophages in the lumina, especially in the cecal sections.

The jejunal sections appeared to be the first and the most acutely affected by the infection and therefore began to show signs of healing first such as increased mitosis in the 120 hours PI groups. Signs of healing in the jejunum increased progressively through 120, 144, and 216 hours PI. These changes were followed by similar alterations in the cecal areas.

At 144 and 216 hours PI the most noticeable activity was mitosis in the bottom of the crypts. Lymphocytic aggregations were more obvious in the cecal tonsillar and midcecal areas than in the jejunum. At no time postinoculation did IFe and IFa poult differ significantly from each other in average weight gains or microscopic changes in the intestines.

No microscopic changes were observed in any tissues of UFa or UFe poult.

Embryonated turkey eggs. The changes observed in the tissues from the embryonated turkey eggs inoculated on the 24th day of incubation did not differ significantly from those described above for one-day-old poult. On 1, 2, and 3 days PI the jejunal villi of the infected embryos showed less differentiation between the lamina propria and epithelium. Also both appeared more cellular, resembling typical embryonic tissues. The epithelial cells did show the granular appearance of the cytoplasm, nuclear margination, and prominent nucleoli similar to those described earlier for the turkey poult inoculated at one day old.

The yolk sacs from the infected embryos were examined also for histopathological alterations, but did not differ significantly from those of uninfected embryos.

The poult hatched from the infected embryos had characteristic clinical signs of bluecomb, and their intestinal tissues showed changes similar to those described for the 1-to-4-day-old poult. No such lesions were observed in any poult hatched from uninoculated embryos.

DISCUSSION

After inoculation of BCDCV, changes occur rapidly in the mucosa of the intestinal tract of turkey poults. The alterations are accompanied by drastic weight loss. Observed morphologically are marked changes in the mucosa (such as granularity of the epithelial cells, loss of microvilli, margination of chromatin in the nucleus, and increased cellularity of the lamina propria). These morphological changes only in the epithelium may be associated with the epithelial cells as observed by the fluorescent antibody technique (6).

The altered physical condition of the mucosal cells does cause failure of absorption, loss of appetite, and the accumulation of fluid and gases in the intestinal tract. The increase in fluid due to the osmotic load (7) results in a diarrhea and eventual loss of fluid, reflected in the marked weight loss in I poults, in contrast to U poults.

The morphological alterations in the gastrointestinal tract are similar to those recorded by Adams and co-workers (1,2), who used crude inoculum. Their poults were older and thus may have been resistant to severe bluecomb infection. Moreover, they did not evaluate the effect of fasting on U and I poults. The changes observed in the present study from inoculation of a BCDCV isolated in turkey embryos had no features which we feel can be pathognomonic of bluecomb disease, since numerous other conditions may produce similar changes in the intestinal tract.

Certain interesting features of the infection warrant further mention. First, once the poults are infected the survivors fail to grow and develop normally, although they become immune to reinfection by BCDCV. The second interesting feature is that as the disease progresses there is a proliferation of lymphocytic aggregates in the cecal tonsillar areas and the cecal walls, while the bursal lymph tissue appears to remain unchanged.

The lack of pathological alterations in the bursa may be due to the lack of multiplication of BCDCV in this organ. This hypothesis is further supported by our recent findings of the presence of immunofluorescent cells in the epithelium of jejunum, ileum, and cecum but not bursa (6). The lack of any histopathological changes in UFa poults suggests that although these poults closely resemble IFa poults (4), they may be differentiated by histopathological techniques.

The morphological changes from BCDCV infection observed in the intestines were similar in the poults and the embryos but were more severe in the latter.

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