ULTRASTRUCTURAL CHANGES IN THE INTESTINES OF TURKEY POULTS AND EMBRYOS AFFECTED WITH TRANSMISSIBLE ENTERITIS

By

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INTRODUCTION

Transmissible enteritis is a turkey disease of viral aetiology. The disease has been described in the United States and Canada, and a similar clinical syndrome has been observed in Australia. Histological changes in the intestine have been described (Adams, Ball and Hofstad, 1970), including shortened microvilli, slight oedema, a decrease in argentaffin cells and an increase followed by a decrease in goblet cells depending on the stage of infection. Adams and Hofstad (1971) have shown that it is possible to propagate the agent in the intestine of turkey embryos.

Studies on the disease have been hampered by the inability to cultivate the causative agent outside its host. Enteritis of viral origin is probably important in many species, but few viral enteritides have been well characterized. Electron microscopic observations were made to obtain a better understanding of this disease and to attempt to demonstrate the aetiological agent.

MATERIALS AND METHODS

One-day-old turkey poults were obtained from a commercial hatchery which was free of detectable enteritis. The turkey embryos used were from the Veterinary Medical Research Institute mycoplasma-free turkey flock. Turkeys were inoculated orally with intestinal contents of birds infected with the “Minnesota” strain of the transmissible enteritis agent (Hofstad, Adams and Frey, 1969). Embryos were inoculated by the amniotic sac with a 0.22 μm filtrate of infective intestinal material, or with supernatant fluid from homogenized intestine from embryos of a previous passage using the “Minnesota” strain of transmissible enteritis. The filtered material was shown to be free of detectable bacteria and mycoplasmas. Two blind passages, each of 7 days duration, were made in turkey kidney cells, using infective embryo material as initial inoculum. No cytopathogenic effects were seen.

Upper small intestine from poults or 27-day embryos was minced and fixed in cold glutaraldehyde in Millonig’s buffer, and post-fixed in osmium tetroxide. Tissues were embedded in Epon and stained in uranyl acetate and lead citrate. Grids were examined with either a Hitachi HS-6 or a Hitachi HS-8 electron microscope.

A total of 42 turkey poults, aged between 2 and 5 weeks, were examined between 0 and 7 days after inoculation. Twenty-six embryos were examined at various intervals, including 0, 19, 30, 44, 72 and 96 hours, and 7 days after inoculation. Specimens taken before inoculation served as controls.
Poults

In infected birds, the findings were consistent with those of light microscopy. A reduction in length of the microvilli was noted on the second day after infection, persisting to at least the fifth day. Microvilli became shorter and more irregular in number and distribution. In several birds and some embryos, abnormalities of the microvilli, including swelling of the tip, and diverticulation of the limiting membrane, were observed (Fig. 1). This was often associated with free, empty membrane-bound vesicles which appeared to be degenerating microvilli. The terminal web was less dense than normal and was invaded by other cellular organelles (Fig. 2).

By the third day, goblet cells were less numerous than in control birds. The mucigen present in goblet cells stained irregularly. Some parts within a globule were less electron-dense than normal, while other parts were quite dense. This appearance persisted until at least day 7. Mucin appeared to be discharged from goblet cells while still having this non-homogeneous appearance. In two birds at 4 days after infection, many epithelial cells contained small irregularly shaped masses of mucigen scattered throughout the cytoplasm. The Golgi apparatus in these cells was well developed.

In epithelial cells, mitochondrial damage was noted early in infection. Mitochondria became swollen, rounded and fewer in number. By the fifth day mitochondria in most cells had returned to normal. Cells in the lamina propria seemed relatively unaffected. Intercellular oedema was seen between epithelial cells 21 hours after infection. This was accompanied by bud-like protuberances from the sides of cells (Fig. 3) which contained many small round vesicles which were found loose in the intercellular space by 25 hours. They did not appear to be microorganisms, but contained a pale amorphous substance. Oedema in the lamina propria was observed occasionally but was not prominent.

In the early stages of the disease, many cells had rarefied cytoplasm—depleted of organelles. Intercellular interdigitations were more numerous than normal. Dilation of the endoplasmic reticulum was often marked. There was a slight increase in autophagic vacuoles as compared with controls. By the fifth day, recovery was occurring and there was an excess of free ribosomes.

The vasculature of the lamina propria had no structural abnormalities. It was thought that the reduction in feed and water intake which accompanies infection might contribute to lesions; in consequence 3 control birds were examined after 48 hours of feed and water deprivation. They appeared normal (Fig. 4) except for a slight increase in autophagic vacuoles.

Embryos

Intestinal epithelial cells of normal embryos were shorter than in hatched poults and tended to have a larger space between them. Microvilli were shorter and less numerous, and lysosomes seemed to be more common. Mucin was of uniform density. Small pinocytotic vesicles, close to the lumen, contained amorphous material and occasionally membrane-bound structures (Fig. 5).

Ultrastructural lesions in late-term infected embryos were similar to those in...
hatched poults, but were less severe. Alterations were observed in the mitochondria, microvilli and mucin secretion (Fig. 6). Large lipid inclusions, seen in epithelial cells of some infected embryos, were probably derived from lipid of yolk-sac origin. Lipid was uncommon in epithelial cells of infected poults because of reduced feed intake during infection.

Degenerating cells were observed in the intestine of embryos which had been infected for several days. These were rounded, and lay between epithelial cells or in the lumen. Some contained mucin and all had heavy concentrations of ribosomes. Inclusions presumed to be degenerating nuclei were seen in some. No virus-like particles were seen in these cells, nor were such cells commonly seen in poults.

**Virus**

Small enveloped virus-like particles were observed budding into membrane-lined cysternae in the cytoplasm of epithelial cells from both poults and embryos (Figs. 7 and 8). Most particles were in cysternae derived from the Golgi apparatus, but some were in the perinuclear space and others were free in the lumen. They were observed as early as 20 hours after infection, but were most commonly seen at 36 hours in poults and at 72 hours in embryos. They were seen as late as 5 days after inoculation, but were never observed in controls. Such particles were not seen in embryos inoculated at 14 days of incubation, a time when the embryo is refractory to transmissible enteritis, although of undiminished susceptibility to most other viruses. Most cells containing virus particles had some degree of structural abnormality. No crystalloids of viral material were seen, but some cells with active budding had abnormal amounts of fibrillar material in the cytoplasm. The fibrils were about 8 nm. in diameter and were often associated in pairs.

The diameter of the enveloped virus-like particles varied widely between 50 and 120 nm. Many particles appeared ellipsoid and the axis of flattening was not always in the plane of section. Some particles appeared to have projections from the outer envelope, but it was not possible to distinguish these clearly. In most particles, the envelope was closely apposed to an inner shell of circular sub-units, although in some particles a small clear area was seen between the two. The inner core had no distinct structures (Fig. 7).

It is postulated that the earliest stage of viral development appeared as a crescent in the triplet membrane lining the lumen of the Golgi apparatus. The membrane in this area was increased in electron density and a band of small ring-shaped particles lay approximately 10 nm. below it (Fig. 8a). The crescent bulged into the lumen (Fig. 8b). The core of the particle at this stage appeared continuous with the cell cytoplasm and occasionally contained one or more circular structures. The layer of capsid sub-units was completed first, and then the outer envelope was pinched off (Fig. 8c). This route of morphogenesis appeared most common, but may not have been the only route, as an occasional unenveloped, preassembled particle was seen in the cell cytoplasm.

Particles typical of the avian leucosis viruses were not seen in either infected or control tissue. The virus-like particles seen could be distinguished by their structure and heavier staining from empty membrane-bound vesicles of various sizes which occurred in cysternae in both infected and control poults and embryos.
Transmissible enteritis is marked by malabsorption (Dziuk, Duke, Evanson, Nelson and Schultz, 1969) which is often associated with shortened microvilli. Padykula, Strauss, Ladman and Gardner (1961) suggested that the abnormal microvilli seen in non-tropical sprue result from a failure of cells to mature as they move more rapidly up the villus. The latter phenomenon is also implied by Abrams, Schneider, Formal and Sprinz (1963), who state that increased epithelial cell turnover occurs in most enteritides. Microvillus damage similar to that observed in transmissible enteritis was seen by Curran and Creamer (1963) in malabsorption syndromes in man, suggesting that direct damage also may contribute to the reduction in microvilli in malabsorption syndromes.

Results indicate that transmissible enteritis affects mainly the intestinal epithelium. In contrast, most intestinal infections with Gram-negative bacteria involve the lamina propria to some extent, particularly the blood vasculature (Takeuchi and Sprinz, 1967; Kenworthy, 1970). The changes observed in transmissible enteritis are of a non-specific nature and are closely similar to those seen in transmissible gastroenteritis of pigs (Chandler, Derbyshire and Smith, 1969). However, the lesions are not like those seen in epizootic diarrhoea of infant mice (Adams and Kraft, 1967), which is another viral disease of the intestinal epithelium. In this disease, dilation of the endoplasmic reticulum is most important, and cells appear vacuolated. Several other suspected intestinal viruses have proven recalcitrant to cultivation outside the living host. Electron-microscopic studies have been undertaken in several syndromes, with varying success. The observation of a virus-like particle does not necessarily mean that it is associated with the disease being studied, but it does warrant its careful investigation. Electron microscopy has been a useful tool in the study of other viruses which cannot be grown or quantitated in cell culture.

Working with the Minnesota strain of the transmissible enteritis agent, both Deshmukh, Larsen, Dutta and Pomeroy (1969) and Hofstad et al. (1969) concluded that the agent was sensitive to chloroform, suggesting that it has an external layer containing lipid. Desmukh et al. (1969) found that the agent would pass a 100 nm. average pore diameter filter, but Hofstad et al. (1969) were unable to pass the agent through a filter smaller than 220 nm. Using the conversion figure of Black (1958), this would suggest a diameter of between 64 and 140 nm. for the transmissible enteritis agent. Thus, the virus observed in this study fulfills the known criteria of the transmissible enteritis agent. Morphologically, the particles observed resemble several unclassified cytoplasmic enveloped viruses described as myxo-like. Since the particles bud into cytoplasmic vesicles, rather than from the cell surface, they are not typical of the myxovirus or paramyxovirus group, and must remain unclassified. The small, empty, membrane-bound vesicles which occur in cisternae and which may occasionally be mistaken for virus, have been seen in many intestinal conditions (Brown, Richter and Bloomer, 1969). They play no aetiological role in any of these.

Adams et al. (1970) suggested that transmissible enteritis infection acts as a strong stimulus for mucin production. It is possible, therefore, that the cellular protuberances seen at 21 hours were associated with excessive mucin production.
They appeared to form as a collection of intracellular vesicles containing a thin, pale staining material, possibly mucigen. Bennett and LeBlond (1970) demonstrated radiographically that cell coat material is transported in small vesicles and is excreted at all cell surfaces. The appearance of cells with hyperdeveloped Golgi apparatus and disorganized mucigen globules would suggest that there is alteration in mucin production. The presence and discharge of unevenly staining mucigen in many cell supports this idea, since Freeman (1962) showed that mucigen changes in electron density as it matures. Thus, it would appear that mucus is being discharged while still in an immature form.

The role played by the mucin response in body defense mechanisms is still not clear. Infection in the turkey embryo provides a simple laboratory system, free from extraneous influences, where factors concerned in the mucin productive response could be easily manipulated.

**SUMMARY**

Electron microscopic observations were made on 42 poults and 26 turkey embryos in various stages of infection with transmissible enteritis infection. Primary lesions included damaged microvilli and mitochondria and a disruption of mucin production. Lesions were similar in both poults and embryos indicating that the presence of secondary bacteria had no specific structural effect. Enveloped virus-like particles were observed in the intestinal epithelium of infected poults and embryos. These particles fulfill all known criteria for the agent of transmissible enteritis. Their morphogenesis is described. It is suggested that the turkey embryo may serve as an inexpensive and readily available model for the study of mucin production during infection, and other general phenomena associated with injury.

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**REFERENCES**


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N. R. ADAMS et al.

All micrographs are of jejunal epithelial cells.

Fig 1. Microvilli of an infected embryonic cell, showing a diverticulation of the limiting membrane (arrow) and other empty vesicles. × 44,000.

Fig. 2. Infected poult. 4 days post inoculation. The microvilli are shortened, and the terminal web is diminished. Both cells contain small discrete droplets of mucin (arrows), and the mitochondria in one cell are swollen. × 13,100.

To face page 192
Fig. 3. Cross section through the basal parts of epithelial cells from a poult at 21 hours post inoculation, showing intercellular oedema. One cell has a typical multi-vesiculated protuberance. × 24,500.

Fig. 4. Epithelial cells from a 48 hour feed and water deprived poult. Except for an increased number of autophagic vacuoles (arrows), the appearance is normal. × 6,000.
Fig. 5. Epithelial cells from a normal 27-day turkey embryo. Arrow indicates a pinocytotic vesicle.

Fig. 6. Epithelial cells from an infected 27-day turkey embryo. The mucin is of uneven density, and the mitochondria in one cell are swollen (arrow). The microvilli are not markedly abnormal. × 8,900.
Fig. 7. Virus-like particles in a vesicle in the cytoplasm of an epithelial cell from an infected poult.  
× 129,000.

Fig. 8. Morphogenesis of the virus-like particle.  
(a) Crescent-shaped bulge in the cell membrane lining a cytoplasmic vacuole, with a dense layer forming beneath it (arrow)  × 55,000.  
(b) Bulging of the thickened membrane and dense layer (arrows)  × 45,000.  
(c) Particle with inner shell completed and outer envelope starting to pinch off (arrow).  
× 41,500.