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Research Note—

Hepatic and Renal Ultrastructural Lesions in Experimental Clostridium perfringens Type A Enterotoxemia in Chickens

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SUMMARY. Hepatic and renal electron microscopic investigations were carried out in 10 chickens that had experimental intraduodenal infection with *Clostridium perfringens* Type A. Fourteen days postinfection, there were ultrastructural changes in hepatocytes and renal tubular epithelial cells; these included mitochondrial lesions (swelling, cristolysis, rarefication of the matrix, myelin figures), glycogen loss, and capillary endothelial cell swelling in both organs, as well as thickening of glomerular basement membrane. The findings are discussed with particular reference to the pathogenesis of *Clostridium perfringens* Type A enterotoxemia.

RESUMEN. *Nota de investigación*—Lesiones ultraestructurales hepáticas y renales debido a una enterotoxemia experimental en pollos con Clostridium perfringens tipo A.

Por microscopía electrónica se realizó una investigación del hígado y riñón en 10 pollos que tenían una infección intraduodenal experimental con *Clostridium perfringens* tipo A. A los 14 días hubo cambios ultraestructurales en los hepatocitos y las células epiteliales de los túbulos renales; estos cambios consistían en lesiones mitocondriales (tumefacción, cristólisis, rarificación de la matriz, figuras mielínicas), pérdida de glicógeno y tumefacción de las células endoteliales capilares de ambos órganos, al igual que engrosamiento de la membrana basal del glomérulo. Los hallazgos son discutidos con particular referencia a la patogenesis de la enterotoxemia con *Clostridium perfringens* tipo A.

Key words: chickens, *Clostridium perfringens* enterotoxemia, liver, kidney, ultrastructure

Clostridium perfringens enterotoxemia in chickens can be fatal both with (5,10,12,14) and without necrotic enteritis (17,18). Morphological findings can include hemorrhagic to necrotic enteritis. The affected mucosa is covered by a diffuse, grey-brown to yellow-green diphtheric membrane. Necropsy findings of cases without necrotic enteritis may reveal diarrhea and liquid intestinal contents, sometimes with gas bubbles. Intraepithelial granulocyte accumulation, lympho-histiocytic infiltration into the villous lamina propria, and "crypt abscesses" have been observed histologically in the intestine. Pulmonary edema may occur. Transmission and scanning electron microscopic investigations detected scattered alterations (mitochondrial damage, altered microvilli, plasma membrane bleb formation, disrupted terminal web, dilated endoplasmic reticulum, and enterocyte detachment) (18). However, these investigations focused on the intestine as the primary target organ of the α-toxin. The purpose of this paper is to describe the hepatic and renal ultrastructural lesions of chickens experimentally infected intraduodenally with *Clostridium perfringens* Type A.

MATERIALS AND METHODS

Ten 26-day-old conventional broiler chickens were intraduodenally infected, according to the procedure described previously (2), with 5×10^8 colony-form-

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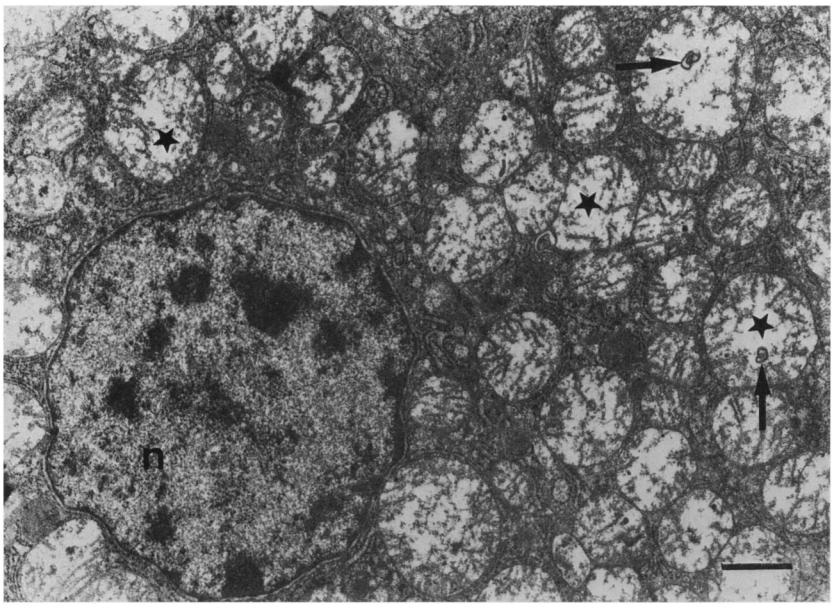

Fig. 1. Liver, chicken. Mitochondria are swollen and show partial cristolysis (★). Note the myelin figures (arrows). n = nucleus. Uranyl acetate, lead citrate. Bar = 1 μm.

ing units of an enterotoxin-negative *Clostridium perfringens* Type A strain (strain R20) that produced α-toxin. Isolated from the intestine of a turkey that died from necrotic enteritis, this strain was passaged and kept at the laboratories for veterinary research and food hygiene (Potsdam, Germany). Fourteen days postinfection, all 10 infected birds and 4 age-matched control birds were killed by cervical dislocation. Small portions of livers and kidneys were freshly fixed in 10% neutral buffered formalin. Paraffin-embedded sections of both organs were stained with hematoxylin and eosin and examined by light microscope. For electron microscopy, small sections of liver and kidney from each bird were immediately fixed in 3% glutaraldehyde in phosphate buffer (pH 7.2) and subsequently in chrome osmic acid, dehydrated in an ethanol series, and embedded in Epon 812. The samples from the control birds were collected with an identical postmortem interval and fixed in the same manner. Ultrathin sections were mounted on grids, stained with uranyl acetate and lead citrate, and examined with a Tesla BS 500 transmission electron microscope at 60 kV. In order to exclude concurrent fungal or bacterial infections, samples of liver, spleen, kidney, and intestine were examined by standard microbiological methods.

RESULTS

Clinical signs of infection such as apathy, diarrhea, shaggy plumage, and inappetence were noticed 7 days postinfection. Grossly, there was marked hepatic congestion in all infected birds, distension of the gall bladder, and fluid feces. No macroscopic lesions were present in the intestine.

Six of the 10 infected animals had renal congestion and enlargement, whereas four had only congestion. Histologically, only hyperemia was present. Grossly and histologically, the control birds showed no lesions.

Scattered ultrastructural alterations were present in the liver and kidney of all 10 infected chickens. In the liver, there was widespread mitochondrial swelling, reduction or loss of the mitochondrial cristae (cristolysis), and rarefaction of mitochondrial matrix. The mitochondria contained coiled myelin figures (Fig. 1). In a small number of hepatocytes, there was nuclear deformation and pyknosis and dilatation of rough endoplasmic reticulum. Glycogen granules were observed only in the least sick bird and in the control birds. There was also marked cytoplasmic swelling of sinusoidal endothelial cells in all infected birds (Fig. 2). Phagolysosomes were found in several Kupffer cells in infected birds only.

In the kidneys, ultrastructural findings in the tubular epithelial cells were similar to those in

Fig. 2. Liver, chicken. Endothelial cell cytoplasmic swelling (★). g = granulocyte, er = erythrocyte, m = mitochondria of adjacent hepatocytes. Uranyl acetate, lead citrate. Bar = 1 μm.

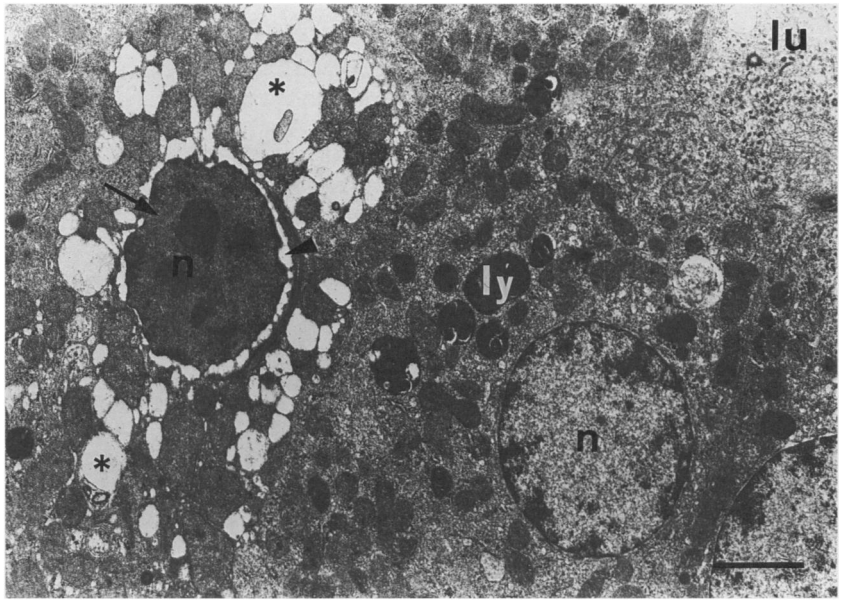

Fig. 3. Renal proximal tubule, chicken. Note multiple vesicles (*) and the pyknotic nucleus (arrow) with dilated perinuclear space (arrowhead). n = nucleus, ly = lysosomes, lu = lumen. Uranyl acetate, lead citrate. Bar = 2 μm.

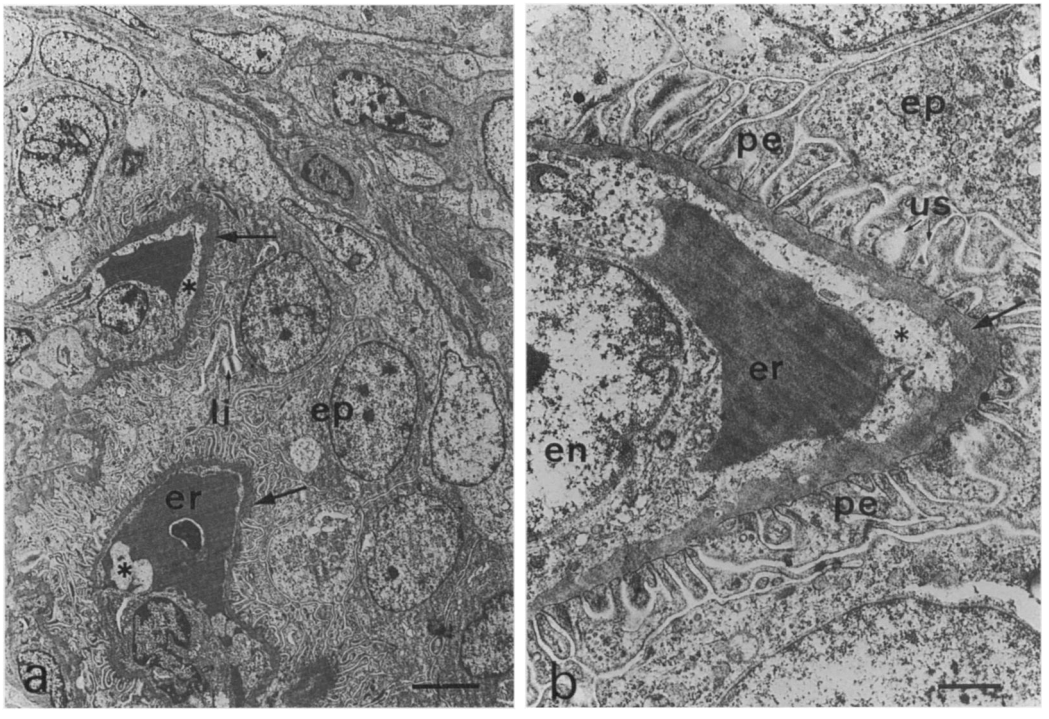

Fig. 4. Glomerulus, chicken. a) Endothelial cell cytoplasmic swelling (*) and thickening of the lamina basalis of glomerular basement membrane (arrow). Bar = 3 μm; b) Higher magnification of a glomerular capillary. Bar = 1 μm. en = endothelial cell nucleus, er = erythrocyte in capillary lumen, ep = epithelial cell, us = urinary space, pe = pedicel, li = lipid droplet (a rare finding). Uranyl acetate, lead citrate.

the liver: there was marked mitochondrial swelling, cristolysis, and matrix rarefaction (especially in the proximal tubules); occasional hydropic swelling of the proximal tubular epithelial cells; and the appearance of multiple perinuclear vesicles caused by dilatation of the endoplasmic reticulum. Nuclear degeneration (Fig. 3) was uncommon. There was marked cytoplasmic swelling of glomerular and intertubular capillary endothelial cells (Fig. 4a) and thickening of the glomerular basal membrane (Fig. 4b). No lesions were found in the control birds.

Clostridium perfringens was reisolated only from the intestines. There were no signs of underlying infection.

DISCUSSION

The observation that *Clostridium perfringens* Type A occurs in the intestine of healthy chickens (4,13,15) infers that *Clostridium perfringens* enterotoxemia requires additional factors such as a high animal concentration (15–30 birds/m²) (7,10), a protein-rich diet (6,16), and the presence of birds older than 2 weeks (17).

Previous reports on spontaneous (4,8,9,11) and experimental (1,3,5,7,12,14,16,18) *Clostridium perfringens* enterotoxemia in chicken have focused on the intestine. The observations described here demonstrate that toxins of *Clostridium perfringens* Type A not only damage enterocytes, the primary target organ (18), but also hepatic parenchymal and endothelial cells in liver and kidney. The main pathological effects were mitochondrial lesions in hepatocytes and renal tubular epithelial cells and cytoplasmic swelling of capillary endothelial cells in both organs. Thickening of glomerular basement membrane was also observed. The mitochondrial lesions described in the present investigation were also observed in cardiomyocytes (Vissiennon, unpublished observations). The alterations described in the present investigation presumably were caused by the *Clostridium perfringens* infection because they were

not found in the control birds. Therefore, it can be hypothesized that 1) toxins, mainly α-toxin, produced by *Clostridium perfringens* in the intestine enter the circulatory system via the intestinal mucosa (=enterotoxemia) and reach other organs. In this case, the findings suggest that α-toxin of *Clostridium perfringens* (after enteric absorption) in chickens is endothelio-, hepato-, and nephrotoxic; and 2) toxins produced by other intestinal germs, whose resorption was favored by ultrastructural alterations in the intestine (18) caused by the *Clostridium perfringens* infection, triggered the observed hepatic and renal alterations. These hypotheses remain unproven, and the mechanisms require further studies.

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