

# INCLUSION BODY HEPATITIS IN CHICKENS

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INCLUSION BODIES are not a common feature of disease in the avian species. The virus of infectious laryngotracheitis will produce intranuclear bodies in the respiratory system of domestic fowl (10) and in chick embryo kidney cells (9). A turkey strain of Ornithosis agent when inoculated into White Leghorn chickens has produced intracellular inclusion bodies in the trachea, lung, air sac, cerebrum and heart (2). In viral inclusion body hepatitis and splenitis of owls, intranuclear inclusions of Cowdry's Type A were observed in liver and reticular cells of the spleen and bone marrow (4). Racing pigeons affected with a Herpes virus suffered from hepatic and renal necrosis. Basophilic intranuclear inclusions were observed in the affected livers and in the ectodermal cells of the chorioallantoic membranes of ten day embryonated hen eggs which had been inoculated with material from infected pigeons (5). Hepatic intranuclear inclusion bodies have been described in two chicken broiler flocks by Helmboldt *et al* (6). A similar condition is described in this paper.

## Description of Affected Flocks

During 1968 and 1969, a syndrome characterized by sudden death and massive liver necrosis was observed in chicken broiler flocks on twelve premises. Outbreaks were not confined to a single strain of bird, hatchery or feed source. On two premises only, two separate outbreaks occurred several months apart in different groups of birds. On all other premises the disease occurred only once.

Birds were usually found dead but were occasionally seen in an extremely depressed condition shortly before death. Death occurred within a few hours following initial observation of signs. It was only on rare occasions that sick birds were still alive on arrival at the laboratory. Outbreaks occurred most frequently at the age of five weeks, but were observed as early as three weeks and as late as three months of age. Total losses were as high as 8% and outbreaks lasted for up to three weeks. Daily losses sometimes approached 1% per day. Mass medication with various broad spectrum antibiotics and multiple vitamin preparations did not alter the course of the disease. The condi-

tion was observed in broiler flocks only. Marek's disease and enteritis were also present in some of the affected flocks and coliform septicemia was diagnosed concurrently in one flock.

## Gross Pathology

The most consistent findings in this condition were encountered in the liver and kidney. On gross examination, the appearance of the livers was very similar in all outbreaks. Characteristically, the livers were swollen, mottled, and had a reticular pattern of fine linear and stellate hemorrhages beneath the capsule (Figure 1). The parenchyma of the liver was soft in consistency. The kidneys were pale, swollen and had a mottled appearance. Hemorrhage into the renal cortex was noted in some of the birds. All other internal organs appeared to be normal.

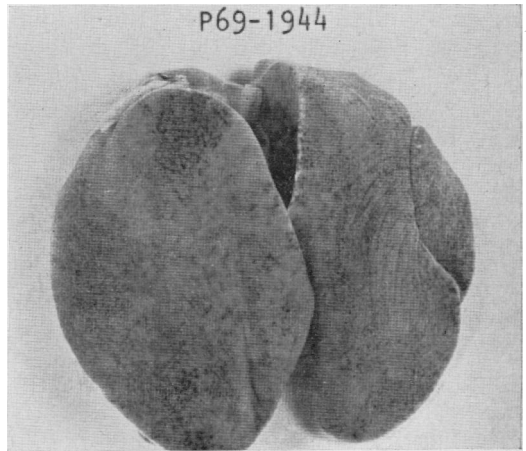


FIGURE 1. Liver from an affected bird. Note the reticular pattern of fine linear stellate hemorrhages beneath the capsule.

## Histopathology

Histopathological findings encountered in the kidney were congestion, small focal hemorrhages and nephrosis. Helmboldt and Frazier's term "hepatic catastrophe" aptly describes the severity of the changes observed in the liver (6). In the majority of cases, liver destruction was almost complete. The hepatic cords were dissociated and marked vacuolation of the hepatic cells was apparent (Figure 2). Cryostat sections were prepared on one case and stained with Oil Red O. Examination of the

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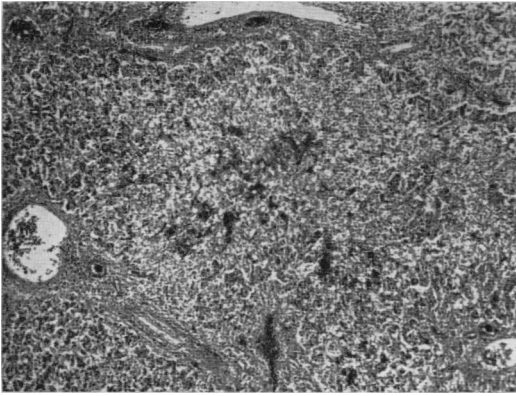


FIGURE 2. Photomicrograph showing dissociation and marked vacuolation of hepatic cells. X60.

stained slides revealed that the marked vacuolation noted in the hepatic cells was attributable to fatty metamorphosis. Focal areas of degeneration and necrosis were scattered at random throughout the liver substance. Focal collections of round cell leucocytes were noted in some areas. In many of the hepatic cells, the nuclei had disappeared entirely, leaving a ghost cell consisting of one or more large vacuoles. Varying degrees of pyknosis, karyorrhexis and karyolysis were observed in the majority of the hepatic cells. In some hepatic cells, swelling of the nuclei and margination of the chromatin were apparent. Large Cowdry Type A intranuclear inclusion bodies were noted in many of the degenerating liver cells (Figure 3). The inclusion bodies were mauve colored using hematoxylin and eosin stain. They were surrounded by a clear halo and were irregular in outline. In some areas of the liver, inclusion bodies were very numerous.

#### *Electron Microscopic (E.M.) Studies*

E.M. studies were attempted on three separate outbreaks. Specimens were fixed in buf-

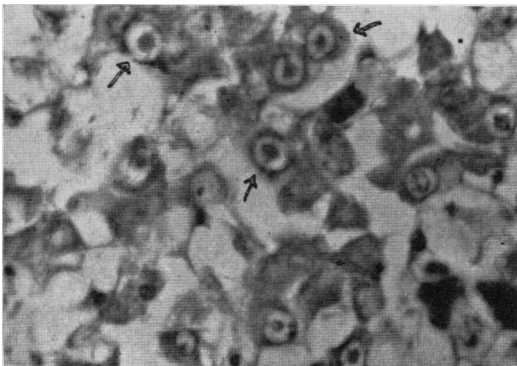


FIGURE 3. Photomicrograph showing several large Cowdry Type A inclusion bodies. X570.

fered osmic acid, dehydrated and embedded in Epox 812. Sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. The prepared specimens were examined with a Philips E.M. 200 electron microscope.

No intranuclear virus particles were observed in hepatic cells. In two cases, structures resembling virus particles were observed in the cytoplasm of hepatic cells. These particles appeared to be "budding" from the plasma membrane (Figure 4) and were similar to virus particles seen in Rous sarcoma (3).

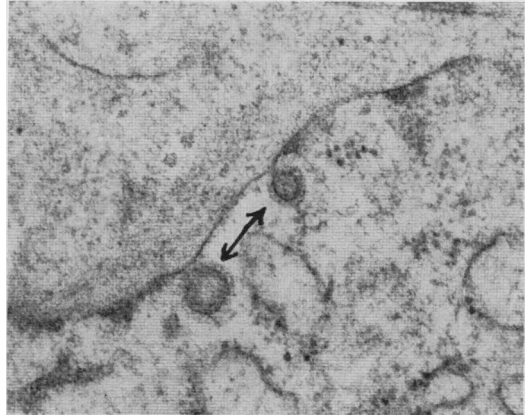


FIGURE 4. Electron micrograph showing structures resembling virus particles "budding" from the plasma membrane. X47,000.

#### *Bacteriology*

Tissues were cultured aerobically and anaerobically on bovine blood agar and McConkey's plates. No pathogenic organisms were demonstrated in any of the numerous cultures made on these cases.

#### *Virology*

Several attempts were made to demonstrate pathogenic viruses using primary chick embryo fibroblast tissue culture, mice, and embryonated hen eggs. Whole chick embryo fibroblast tissue cultures made from 10-day-old chick embryos were inoculated with bacteria-free buffered saline suspensions of liver and kidney. Suckling mice were inoculated intracranially. Nine-day-old chick embryos were inoculated with an extract of kidney and liver in nutrient broth using triple inoculation technique.

Newcastle disease virus was isolated from the liver of one submission using the chick embryo technique. Since Newcastle disease vaccination had been conducted on a routine basis on the premises it was considered probable that the isolated virus was a vaccine strain. No other viruses were demonstrated.

*Biochemistry*

Crude fat determination was carried out on the liver of an affected bird and on the liver of a normal control according to an A.O.A.C. method (1). The liver of the affected bird contained 9.21% fat and the liver of the control bird contained 5.08% fat on a wet weight basis. The lipid portions were subsequently submitted for a fatty acid analysis. No significant difference was observed between the two lipid extracts with respect to their fatty acid composition.

*Discussion*

The etiology and pathogenesis of this condition has not been established. The consistent findings of Cowdry Type A inclusion bodies suggest a viral etiology. This possibility has not been supported by the results of many attempts at viral isolation. The particles noted on electron microscopy were located in the cytoplasm and therefore were not considered to be related to the intranuclear inclusions seen on light microscopy.

Inclusion bodies are generally associated with a viral etiology. However, inclusion bodies have been demonstrated in Mallard ducks which had been fed lead shot (8) and in dogs and rats which had been given endotoxin (7). For these reasons, it is considered possible that some factor other than a virus infection may be associated with the formation of the inclusion bodies observed in this condition.

A similar condition in chickens has been categorized as a "medical curiosity" (6). In most Alberta outbreaks, the losses were economically significant.

*Summary*

A syndrome characterized by sudden death, massive liver necrosis, vacuolation of hepatic cells and formation of Cowdry Type A intranuclear hepatic inclusion bodies has been observed in broiler chickens on twelve premises in Alberta over a two-year period. Losses which commonly started at five weeks of age continued in spite of various treatments until the eighth week. Mortality usually varied from 5 to 8% but, in a few cases, was less than 1%.

*Résumé*

On a observé chez des poulets appartenant à douze élevages Albertains, au cours d'une période de deux ans, un syndrome caractérisé

par une mort subite accompagnée de nécrose étendue du foie, de vacuolisation des cellules hépatiques et de formation de corps d'inclusion intra-nucléaires hépatiques Cowdry de type A. Les pertes commençaient habituellement à l'âge de cinq semaines et se poursuivaient, en dépit des différents traitements, jusqu'à la huitième semaine. Habituellement, la mortalité variait entre 5 et 8% mais, dans quelques cas, elle était inférieure à 1%.

*Acknowledgments*

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