

1973

Studies on inclusion body disease (herpesvirus infection) of falcons

David Lee Graham
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STUDIES ON INCLUSION BODY DISEASE (HERPESVIRUS
INFECTION) OF FALCONS.

Iowa State University, Ph.D., 1973
Veterinary Science

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Studies on inclusion body disease
(herpesvirus infection) of falcons

by

David Lee Graham

A Dissertation submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1973

TABLE OF CONTENTS

| | Page |
|---|------|
| INTRODUCTION | 1 |
| REVIEW OF THE LITERATURE | 3 |
| Prairie Falcon | 3 |
| Owls | 5 |
| Parrots | 8 |
| Pigeons | 9 |
| Geese | 11 |
| Chickens | 12 |
| Ducks | 13 |
| MATERIALS AND METHODS | 16 |
| Field Cases | 16 |
| Acquisition of field cases of inclusion body disease of falcons (IBDF) | 16 |
| Experimental Birds | 16 |
| Sources of Viruses | 16 |
| Collection, Preparation, and Examination of Specimens | 19 |
| Necropsy | 19 |
| Histopathology | 19 |
| Electron microscopy | 20 |
| Hematology | 20 |
| Virological methods | 20 |
| Inoculations | 21 |
| Experiments | 22 |
| Experiment I. Examination of field cases of IBDF | 22 |

| | |
|---|----|
| Experiment II. Pathogenicity of FHV strain S-18 for kestrels | 22 |
| Experiment III. Viremia in experimental IBDF in kestrels | 22 |
| Experiment IV. Progression of lesions in experimental IBDF in kestrels | 23 |
| Experiment V. Experimental reproduction of IBDF in a prairie falcon | 23 |
| Experiment VI. Pathogenicity of FHV strain S-18 in other raptor species | 24 |
| Experiment VII. Pathogenicity of FHV strain S-18 in non-raptorial birds | 24 |
| Experiment VIII. Comparative pathogenicity of OHV and PHV | 25 |
| RESULTS | 27 |
| Experiment I. Examination of Field Cases of IBDF | 27 |
| Field case 1. Prairie falcon (PF-I1) | 27 |
| Field case 2. Red-headed falcon (RHF-I1) | 30 |
| Field case 3. Prairie falcon (PF-I2) | 31 |
| Field case 4. Peregrine falcon (PER-I1) | 35 |
| Field case 5. Prairie falcon (PF-I3) | 39 |
| Field case 6. Gyrfalcon (GF-I1) | 40 |
| Field case 7. Prairie falcon (PF-I4) | 42 |
| Experiment II. Pathogenicity of FHV strain S-18 for Kestrels | 46 |
| Clinical signs | 46 |
| Hematology | 48 |
| Post-mortem examination | 48 |
| Virological examination | 62 |

| | |
|---|-----|
| Experiment III. Viremia in IBDF in Kestrels | 62 |
| Experiment IV. Progression of Lesions in IBDF in Kestrels | 64 |
| KIV1 | 64 |
| KIV2 | 64 |
| KIV3 | 64 |
| KIV4 | 72 |
| KIV5 | 72 |
| KIV6 | 81 |
| Experiment V. Experimental Production of IBDF in a Prairie Falcon (PFV1) | 81 |
| Experiment VI. Pathogenicity of FHV strain S-18 in Other Raptor Species | 90 |
| Experiment VII. Pathogenicity of FHV strain S-18 in Non-raptorial Birds | 99 |
| Experiment VIII. Comparative Pathogenicity of OHV and PHV | 100 |
| DISCUSSION | 102 |
| Proof of the Etiology of IBDF | 102 |
| Field Cases of IBDF | 102 |
| Experimental IBDF in Falcons | 103 |
| Comparison of the Lesions in Field and Experimental Cases of IBDF | 106 |
| Proposed Pathogenesis of Field and Experimental Cases of IBDF | 107 |
| Species Susceptibility Spectrum | 109 |
| SUMMARY | 114 |
| LITERATURE CITED | 117 |
| ACKNOWLEDGEMENTS | 121 |

INTRODUCTION

Among domestic and wild avian species there occur certain diseases which bear several points of mutual resemblance. The common denominators of owl hepatosplenitis, Pacheco's parrot disease, inclusion body disease of pigeons, duck plague, goose hepatitis, and inclusion body hepatitis of chickens are hepatitis and the development of hepatic intranuclear inclusion bodies. Herpesviruses are either strongly suspected of being or are known to be the causative agents of several of these diseases. Other clinical and pathological features are inconsistently shared.

In 1971 a previously unrecognized disease was reported to occur in the prairie falcon (Falco mexicanus), a native North American raptor (bird of prey). The clinical course was short, approximately 40 hours, and was characterized by the relatively non-specific signs of anorexia and listlessness. Post mortem findings were multiple necrotic foci in the liver, spleen, bone marrow and intestinal wall. Intranuclear inclusion bodies were found in cells adjacent to the necrotic areas. The name "inclusion body hepatitis" was proposed for the disease and a herpesvirus was suggested as the etiological agent. Liver and spleen material from a naturally occurring case was found to be infectious for embryonated chicken eggs and for American kestrels (F. sparverius) in which the lesions described above were reproduced. This falcon disease would thus deserve a place in the list of

"inclusion body hepatitis" diseases listed above.

The status of this falcon disease as a potential or real threat to the poultry industry and to other wildlife species has not been assessed.

Pesticide-related mortality and reproductive failure in wildfowl in general and raptors in particular have received much study. Less well understood, however, are the naturally occurring infectious diseases.

Because of the decline in wild breeding populations of several raptor species a number of federal agencies and private institutions have embarked on captive breeding programs. The effects of an epizootic of this disease in these breeding establishments could be devastating to the programs and to the species they were designed to preserve.

This research was undertaken to characterize the lesions of the falcon disease, study its pathogenesis, investigate the spectrum of species susceptible to its causative agent and to study the comparative pathogenicity of the agents of the falcon disease and others in the avian "inclusion body hepatitis" group of diseases.

REVIEW OF THE LITERATURE

Hepatic necrosis, in some instances accompanied by the development of intranuclear inclusion bodies, has been recognized as a feature of certain diseases occurring in several avian species. "Inclusion body hepatitis of falcons" (Ward et al., 1971), inclusion body disease of chickens (Pettit and Carlson, 1972), and a disease of muscovy ducks described by Kaschula (1950) are presumed to be of viral etiology. A viral etiology is accepted for owl hepatosplenitis (Burtscher, 1968), Pacheco's parrot disease (Rivers and Schwentker, 1932), herpesvirus infection of pigeons (Cornwell, Weir, and Follett, 1967), goose hepatitis (Schettler, 1971a), and duck virus enteritis (Leibovitz, 1972).

Prairie Falcon

The clinical and pathological aspects of a disease observed in a young captive male prairie falcon (Falco mexicanus) were described by Ward and co-workers (1971).

The falcon had been captured at an eyrie in South Dakota at about 10 days of age and was taken to Pennsylvania when about 5 weeks old. Anorexia and listlessness were observed at 7 weeks of age.

Physical examination was performed approximately 36 hours after the onset of clinical illness. The bird was depressed and shivered occasionally. Parasitological examinations of the feces and of oral swabs proved negative for helminth eggs and trichomonads. Hepatomegaly was observed radiographically.

The falcon was given an intramuscular (IM) injection of chloramphenicol (50 mg/kg) but died a few hours later.

Necropsy revealed focal necrosis in the liver, spleen, bone marrow, and small intestine lamina propria. Many intranuclear inclusion bodies were seen in parenchymal cells adjacent to the necrotic foci.

A suspension of liver from the falcon was inoculated on the chorioallantoic membrane (CAM) of 12-day old chicken embryos and white, thickened lesions of the CAM were present when examined 5 days post inoculation (PI).

Two kestrels (F. sparverius) were inoculated with liver suspension, 1 intramuscularly, the other orally. A third kestrel was inoculated IM with a suspension of CAM from infected eggs. All 3 birds died 4 to 6 days post inoculation and had hepatic necrosis and hepatic intranuclear inclusion bodies.

Another kestrel, uninoculated, was allowed to inhabit the same room with 2 of the fatally infected birds and remained healthy.

Similar lesions to those described by Ward et al. were observed by Bigland et al. (1964) in a prairie falcon which had been trapped as a juvenile in southern Alberta, Canada, in 1957. The bird was anorectic for 24 hours ante mortem. Necropsy revealed a large number of nematodes (Serratospiculum amaculata) in the air sacs, as well as multiple foci of necrosis in the liver and spleen. Inclusion bodies were not

noted. The hepatic and splenic lesions were believed to be causally related to the parasite infection (Bigland, University of Saskatchewan, personal communication, 1971).

Owls

Errington (1932) found a fresh cadaver of a nesting great horned owl (Bubo virginianus) in Wisconsin in February of 1932. The decomposed remains of its presumed mate were found about 6 weeks later.

The first owl was necropsied (Green, 1935; Green and Shillinger, 1936). It was a well-fleshed adult of unstated sex. Because of the large amount of food found in the upper digestive tract extreme illness was presumed to have been of short duration.

Gross lesions were limited to the liver and spleen. Both organs contained many small necrotic foci and there was splenomegaly. Histological examination of the liver revealed many discrete necrotic foci with central concentration of nuclear debris. The nuclei of adjacent degenerating hepatocytes contained eosinophilic inclusion bodies.

Burtscher (1965a, 1965b) reviewed necropsy protocols and histological preparations from 55 owls which had been necropsied at the veterinary school in Vienna, Austria, from 1915 to 1965. Thirty-seven or 67.3% of those cases had lesions identical to those described by Green in Wisconsin. Similar lesions were less consistently found in the bone marrow, alimentary tract, kidney, lung, and upper respiratory

passages. Catarrhal enteritis, hemorrhagic enteritis, and fibrinous serositis also were seen in some cases. On the basis of the consistent liver and spleen lesions Burtscher named the disease "hepato-splenitis infectiosa strigorum" (HSiS).

A disease described as "virus hepatitis" by Borg and Rockborn (1971) has been diagnosed in 15 of about 50 eagle owls (Bubo bubo) necropsied in Sweden between 1948 and 1971. Miliary necrotic foci in the liver and other organs were the typical lesions. No mention of intranuclear inclusion bodies was made by these workers.

The finding of intranuclear inclusion bodies led Green (1935) and Green and Shillinger (1936) to the opinion that the causative agent was a "filterable virus."

Burtscher and Schumacher (1966) performed ultrastructural studies on the virus in lesions from experimental HSiS infections of owls and embryonated chicken eggs. Replicative stages of the virus occurred in the nucleus of infected cells. The nucleocapsids were about 80 nanometers (nm) in diameter and gained an envelope upon egress through the inner nuclear membrane. The complete enveloped virion was about 122 nm in diameter. Recognizing that these morphological criteria alone are insufficient for definitive classification, they nonetheless suggest that the virus is a member of the herpesvirus group.

Schettler (1969, 1970) accomplished in vitro cultivation

of the owl hepatitis agent in various avian cells and reported greatest success with goose kidney cells. In vitro studies revealed that the agent is a DNA virus which is sensitive to ether, chloroform, heat (56°C), pH 3.0, and 1 molar MgCl₂. Detection of cell-free virus in the cell culture supernate led Schettler to classify the agent as a member of subgroup A of the herpesviruses.

A virus isolated from one of Borg and Rockborn's (1971) Swedish eagle owls with lesions of "virus hepatitis" was classified as a herpesvirus, but the criteria used for classification were not reported.

Green and Shillinger (1936) did not attempt to culture their agent in embryonated chicken eggs but did experimentally transmit the disease to another great horned owl and to a screech owl (Otus asio) by injection of a suspension of lesion material. Attempts to reproduce the disease in a barred owl (Strix varia), 2 pigeons, and 2 guinea pigs failed.

Burtscher (1968) succeeded in culturing the HSiS virus in the yolk sac, allantoic sac, and on the CAM of embryonated chicken eggs. He also attempted transmission of the disease to adult birds and to mammals. These attempts failed in 120 domestic fowl (Gallus domesticus), 10 mallard ducks (Anas platyrhynchos), 15 pigeons (Columba livia), 1 budgerigar (Melopsittacus undulatus), 1 swift (Apus apus), 1 blackbird (Turdus merula), 1 carrion crow (Corvus corone), 1 house sparrow (Passer domesticus), 20 adult and 14 neonatal

white mice (Mus musculus), and 4 guinea pigs (Cavia cobaya). Successful transmission of the disease to 1 little owl (Athene noctua), 1 boreal owl (Aegolius funereus) and 3 long eared owls (Asio otus) was accomplished. On the basis of these results the conclusion was drawn by Burtscher "... das es sich bei HSiS vermutlich um eine Eulen-spezifische Infektionskrankheit handelt" that with HSiS (one) presumably deals with an infectious disease specific for owls.

Attempts were made by Borg and Rockborn (1971) to transmit the Swedish owl virus to an eagle owl (Bubo bubo), a tawny owl (Strix aluco), and a buzzard (Buteo buteo). The eagle owl proved susceptible; neither the tawney owl nor the buzzard became clinically ill nor were lesions found at necropsy 4 to 6 weeks after inoculation.

Parrots

Rivers and Schwentker (1932) reviewed the early literature on a disease of Brazilian parrots subsequently termed "Pacheco's Parrot Disease" by Findlay (1933) and performed pathological and transmission studies with the viral agent.

The disease was produced experimentally in budgerigars (Melopsittacus undulatus) by intramuscular or intracerebral inoculation of a 10% emulsion of parrot liver lesion material in Locke's solution. Three to 4 days post inoculation the birds began to "... lose a certain spontaneity of action." The feathers became ruffled, progressive depression and weakness ensued, and death occurred 6 to 10 days after inoculation.

At necropsy there was focal necrosis of the liver and splenic necrosis which obliterated the normal architecture of the organ. Acidophilic intranuclear inclusion bodies were found in cells of the liver and spleen and occasionally in cells of the lungs, kidneys, and bone marrow. Depending upon the site of inoculation inclusions were also present in the brain, meninges, ependymal cells and choroid plexus (intra-cerebral route) or in the nuclei surrounding muscle fibers (intra-muscular route).

Attempts to transmit the disease to mice, guinea pigs, rabbits, and canaries failed. Rhode Island Red and White Leghorn chickens 1 to 29 days old were also inoculated. A few 1- and 2-day old chicks inoculated intracerebrally died; meningitis and meningeal intranuclear inclusion bodies were present.

Inoculation of the CAM of embryonated chicken eggs resulted in death of most of the embryos by the 5th post-inoculation day. Whitish opaque lesions of the CAM contained eosinophilic intranuclear inclusion bodies.

Andrews and Pereira (1967, p. 304) tentatively include this virus in the herpesvirus group.

Pigeons

In 1945 Smadel, Jackson, and Harman reported on a disease which occurred in United States Army carrier pigeons.

The lesions were focal necrosis of the liver, spleen, and pancreas with formation of eosinophilic intranuclear inclusion

bodies.

Identical or similar diseases have been reported in Denmark by Marthedal and Jylling (1966) and Jylling (1967), again in the United States by Lehner, Bullock, and Clarkson (1967) and in Scotland by Cornwell, Weir, and Follett (1967). The agent isolated by Cornwell and his coworkers was grown in cell culture (1970b) and in embryonated chicken eggs and was characterized morphologically and chemically as a herpesvirus (1970a).

The disease may be clinically inapparent or inactivity and anorexia may occur (Lehner et al., 1967). Cornwell and Wright (1970) describe a range of signs from slight malaise with mild serous rhinitis or conjunctivitis to marked dyspnea and gasping. Marthedal and Jylling (1966) observed weakness, dyspnea, diarrhea, and emaciation.

The disease observed in Scotland by Cornwell and Wright (1970) recurred annually for several years and affected birds 5 to 6 weeks old; Lehner et al. (1967) reported an age range of affected birds from 6 weeks to 4 months in the single outbreak they observed. The disease observed by Marthedal and Jylling (1966) occurred mainly in birds 1 to 6 months of age.

Cornwell, Wright, and McCusker (1970) demonstrated the pathogenicity of 2 pigeon herpesvirus isolates from Scotland for young pigeons but not for chicks using the intraperitoneal and intralaryngeal routes of inoculation.

Geese

Schettler (1971a, 1971b) briefly reviewed the literature on goose hepatitis and reported the isolation of a small DNA virus tentatively classified as a parvovirus from an outbreak of the disease in a flock of 2000 goslings.

In goslings up to 6 weeks of age mortality was 40 to 50%. The principal gross lesions were hepatomegaly with small hemorrhages on the surface of the liver, and severe ascites (Schettler, 1971b).

Lesions in goslings which died in the acute stage of the experimental disease following IM inoculation with the virus (Schettler, 1971b) were "severe non-fatty vacuolating degeneration of hepatocytes and focal coagulative necrosis around the liver capillaries, with invasion of heterophils." Some hepatocytes and Kupffer cells contained intranuclear inclusion bodies.

Attempts to propagate the virus on the CAM of embryonated chicken and duck eggs were not successful.

Schettler (1971c) has diagnosed goose virus hepatitis in 7 Canada goose goslings (Branta canadensis canadensis) and 1 snow goose gosling (Chen hyperborea atlantica). These birds died at a zoo 1 week after having been hatched in incubators at Schettler's laboratory where work on goose virus hepatitis was in progress.

Chickens

Helmboldt and Frazier (1963) classified as a "medical curiosity" a hepatitis characterized by intranuclear inclusion body formation which they recognized in 2 broiler flocks in Connecticut. Infectious laryngo-tracheitis and presumably Newcastle disease occurred concurrently with the hepatitis in the 1st and 2nd flock respectively. Mortality was less than 0.1%.

The liver lesion was that of "... an acute hepatic catastrophe" in which marked fatty change was seen in more than 90% of the parenchymal cells. There were widespread focal hemorrhages as well as diffuse and occasionally focal infiltration by leukocytes. Intranuclear inclusion bodies were numerous.

Seneviratna (1969, p. 45) reports that "an inclusion-body hepatitis causing moderate mortality, depression, and diarrhea has been described from Italy. The exact cause is not known; however, it may be due to a virus."

Twelve outbreaks of "inclusion body hepatitis" in broiler flocks were diagnosed in Alberta, Canada, by Howell et al. (1970) and 86 outbreaks were identified in Ontario by Pettit and Carlson (1972).

The lesions, summarized by Pettit and Carlson (1972), were markedly fatty livers with stellate to punctiform hemorrhages, enlarged pale kidneys, hemorrhages in fat and muscles, pale bone marrow, and extremely small, firm bursae

of Fabricius. Histologically there was massive hepatic necrosis with intranuclear inclusion bodies in hepatocytes, regression of bursal follicles, aplasia of the bone marrow, and moderate to severe nephrosis.

Pettit and Carlson (1972) obtained a "virus-like agent" which was cytopathogenic on chick and duck embryo cultures. They could not, however, isolate the agent on chick embryos or demonstrate distinct virus particles in lesions by electron microscopy.

Ducks

Duck plague (duck virus enteritis), an acute virus infection of ducks, geese, and swans, occurs in Europe, Asia, the Indian subcontinent, and North America, and its history has been reviewed by Leibovitz (1972).

The pathological changes of duck plague have been described by Leibovitz (1971) as "... those of specific, progressively developed, enanthematous lesions of the digestive tract, hemorrhage and necrosis of visceral organs, serosal hemorrhages, and collections of free blood within body cavities and hollow organs." Rupture of capillaries and venules with associated hemorrhage were observed histologically in many tissues. Cellular degeneration characterized by swelling, altered staining, division of the cytoplasm into subunits which are subsequently discharged from the ruptured cell, and the formation of intranuclear inclusion bodies occurs in epithelial cells of the esophagus, cloaca, liver, and

pancreas. These changes are, however, most severe and consistent in reticuloendothelial tissues.

Electron microscopic characterization of duck plague virus isolated from an outbreak on Long Island was performed by Breese and Dardiri (1968). The nucleic acid of virions embedded in methacrylate was susceptible to digestion by deoxyribonuclease. The diameter of the nucleocapsid was about 75 nm; that of the enveloped virion about 181 nm. The Long Island isolate was morphologically identical to an attenuated strain of duck plague virus from the Netherlands and both were classified as herpesviruses.

Kaschula (1950) recognized, in the area of Durban, South Africa, an acute disease apparently specific for the muscovy duck. Mortality ranged from 67 to 94% in flocks of muscovies. Chickens, turkeys, geese, and other breeds of ducks on the same premises were unaffected. Muscovies of all ages were susceptible but losses were heaviest in ducks 2 to 3 months old.

The clinical signs were anorexia, fever, thirst, pallor of the beak and legs, diarrhea, and listlessness. The incubation period was 2 to 4 days and the course of the clinical illness was 2 to 5 days.

At necropsy the livers were enlarged, soft, and friable; numerous yellowish-grey foci were scattered throughout the organ. The spleens were inconsistently enlarged but most did contain necrotic foci. Pulmonary hyperemia and edema and

catarrhal enteritis were also observed.

The disease could be reproduced in susceptible muscovies by inoculation with a Seitz filtrate of blood from freshly dead ducks.

Kaschula failed in attempts to transmit the disease to pigeons, chickens, Pekin ducks and guinea pigs and cited personal communication from J. D. W. A. Coles at Onderstepoort who reported no success in transmitting the disease to the Stanley crane, pea hen, budgerigar, canary, white mouse, white rat, and ferret.

Seneviratna (1969, p. 174), in his brief list of duck diseases, ascribed a description of "spleen necrosis of ducks" to Trager but did not reference the citation. The disease, fatal to young ducklings in 3 to 5 days, was characterized by severe anemia, necrotic lesions in the spleen, and hepatitis. The "filter-passing agent" was stated as being different from that causing duck virus hepatitis but the criteria of differentiation were not mentioned.

MATERIALS AND METHODS

Field Cases

Acquisition of field cases of inclusion body disease of falcons (IBDF)

An appeal for information regarding, or specimens from suspected cases of IBDF was made to members of the Pathology Committee of the Raptor Research Foundation, members of the North American Falconers Association, and veterinarians at zoos with extensive raptor collections.

Experimental Birds

The species and sources of the experimental birds are shown in table 1. All raptorial birds were fed white mice and day-old cockerels. The non-raptorial birds were fed appropriate commercial or prepared diets.

Sources of Viruses

The falcon herpesvirus (FHV) used in the studies on lesions and pathogenesis was isolated from the liver of a dead prairie falcon, one of the submitted field cases of IBDF, and has been designated "FHV strain S-18."

The pigeon herpesvirus (PHV) and owl herpesvirus (OHV) used in the comparative pathogenicity studies were obtained from Dr. C. John Maré, Department of Veterinary Microbiology and Preventive Medicine, Iowa State University. The PHV was the strain isolated by Cornwell et al. (1967) in Scotland. The OHV was originally isolated by Schettler (1970) in Germany.

Table 1. Sources of Experimental Birds

| Common Name ^a | Scientific Name |
|---|---------------------------------|
| American kestrels (K) | <u>Falco sparverius</u> |
| prairie falcon (PF) | <u>F. mexicanus</u> |
| merlin (M) | <u>F. columbarius</u> |
| Harris' hawks (H) | <u>Parabuteo unicinctus</u> |
| bald eagle (BE) | <u>Haliaeetus leucocephalus</u> |
| golden eagle (GE) | <u>Aquila chrysaetos</u> |
| Cooper's hawk (CH) | <u>Accipiter cooperii</u> |
| red-tailed hawks (RT) | <u>Buteo jamaicensis</u> |
| great horned owls (GHO) | <u>Bubo virginianus</u> |
| screech owl (SO) | <u>Otus asio</u> |
| coot ^f (CT) | <u>Fulica americana</u> |
| green heron ^f (GR) | <u>Butoroides virescens</u> |
| Baltimore oriole ^f (BO) | <u>Icterus galbula</u> |
| rose-breasted grosbeak ^f (RBB) | <u>Pheucticus ludovicianus</u> |
| budgerigars (B) | <u>Melopsittacus undulatus</u> |
| Amazon parrot (AP) | <u>Amazona ochrocephala</u> |
| muscovy ducks (MD) | <u>Cairina moschata</u> |
| pigeons (P) | <u>Columba livia</u> |
| ring-necked doves (RD) | <u>Streptopelia rhizoria</u> |
| turkeys (T) | <u>Meleagris gallopavo</u> |
| chickens (C) | <u>Gallus domesticus</u> |

^a Initials in parentheses are the species code.

^b Iowa Scientific Collecting Permits Nos. 19 (1971) and 14(1972).

^c South Dakota Scientific Collecting Permit No. 129 (1972).

^d From Mr. Peter Cragg, Bryan, Texas; Texas Scientific Collecting Permit No. 119 (1973).

^e Bureau of Sport Fisheries and Wildlife, Migratory Bird Permit No. 6-SP-3.

^f Injured bird not suitable for rehabilitation and release.

Source

trapped in Iowa^b

trapped in South Dakota^c

trapped in South Dakota^c

trapped in Texas^d

U. S. Department of the Interior^e

U. S. Department of the Interior^e

Iowa State Conservation Commission

Iowa State Conservation Commission

Iowa State Conservation Commission

Iowa State Conservation Commission

Iowa State Conservation Commission

Iowa State Conservation Commission

Iowa State Conservation Commission

Iowa State Conservation Commission

Taylor Pet and Hobby Center, Ames, Iowa

Des Moines Zoo

Department of Genetics, Iowa State University

Department of Genetics, Iowa State University

Department of Genetics, Iowa State University

Veterinary Medical Research Institute

Veterinary Medical Research Institute .

Collection, Preparation, and Examination of Specimens

Necropsy

All field cases of IBDF and all experimental birds which died or exhibited clinical signs of disease were necropsied by a standard procedure described by Stubbs (1954, p. 63-71) and selected gross lesions were photographed.

Tissue specimens routinely saved were liver, spleen, bone marrow, intestine, lung, and kidney. In selected cases specimens were also collected from the esophagus, proventriculus, ventriculus, ceca, bursa of Fabricius, heart, gonad, adrenal gland, thyroid gland, parathyroid gland, uropygial gland, peripheral nerves, eye, and brain.

Histopathology

Representative blocks of tissue were fixed in 10% buffered formalin for a minimum of 24 hours before being trimmed to an appropriate size for embedding. The trimmed blocks were dehydrated, cleared, and infiltrated with paraffin in an automatic tissue processing machine. After embedment, sections were cut at 6 to 8 microns (μm) on a rotary microtome, affixed to clean glass slides, stained with hematoxylin and eosin (H and E) stain, and coverslipped.

The histological slides were examined with a binocular microscope and photomicrographs of selected lesions were made with a Zeiss photomicroscope.

Electron microscopy

Specimens of liver, spleen, and bone marrow from selected cases were prepared for ultrastructural study. Thin (0.5 to 1.0 mm) slices of tissue were placed in 2% glutaraldehyde for 15 to 30 minutes. The hardened slices were finely diced with a razor blade and returned to fresh glutaraldehyde for 30 to 45 minutes. They were rinsed for 1 to 2 hours in cacodylate buffer and were stored in fresh buffer in a refrigerator. The tissue blocks were post-fixed in 1% osmium tetroxide, dehydrated, and were individually embedded in Epon 812 resin in gelatin capsules. The blocks were trimmed and ultrathin sections were cut on an LKB ultramicrotome. The sections were picked up on uncoated 300-mesh copper grids, stained with uranyl acetate and lead citrate, and were examined with an Hitachi model 11A electron microscope.

Hematology

Blood samples were collected by jugular venipuncture using disposable syringes and disposable half-inch 23 gauge needles.

Air-dried smears were stained with Wright's stain for differential leukocyte counts and total leukocyte counts were made using the method of Rees-Ecker as described by Lucas and Jamroz (1961).

Virological methods

Virus isolation, characterization, and identification were done by Dr. Maré. Isolations were made by inoculation

of the CAM of embryonated chicken eggs, chicken embryo fibroblast (CEF) cell cultures, or duck embryo fibroblast (DEF) cell cultures with antibiotic-containing suspensions of liver, spleen, bone marrow, or lysed peripheral blood.

The egg membranes were examined for lesions 5 days post-inoculation (PI) and cell cultures were examined daily for viral cytopathogenic effects (CPE).

The original isolate (FHV strain S-18) was cloned 3 times by plaque selection before being chemically and physically characterized as a herpesvirus. All other FHV isolates were compared with strain S-18 by one-way serum virus neutralization (SVN) using rabbit serum prepared against FHV strain S-18.

Inoculations

Unless otherwise specified all birds were inoculated with cell-culture-propagated virus. The dose of FHV ranged from $10^{3.0}$ tissue culture infective doses₅₀ (TCID₅₀) to $10^{4.5}$ TCID₅₀ per inoculum except for a dose of 100 TCID₅₀ per inoculum used in the muscovy ducks.

The dose of PHV was $10^{4.0}$ TCID₅₀ per inoculum and that of the OHV was $10^{3.5}$ TCID₅₀.

All birds were inoculated by the intramuscular (IM) route in the pectoral muscle mass except for one which was inoculated by intranasal aerosol.

Experiments

Experiment I. Examination of field cases of IBDF

Materials received included case histories, frozen tissues, frozen cadavers, formalin-fixed tissue, and stained histological slides. In those cases in which a cadaver was submitted a complete necropsy was performed. Tissues suitable for microbiological examination were submitted to Dr. Maré.

Experiment II. Pathogenicity of FHV strain S-18 for kestrels

One kestrel, KIII1, was inoculated with 0.25 cc of a 10% suspension of liver and spleen tissue from the prairie falcon from which FHV strain S-18 was isolated. Two other kestrels, KIII2 and KIII3, were inoculated with FHV strain S-18 cell culture inoculum.

All three birds were observed for signs of illness. Two blood samples were taken from kestrel KIII1, 1 on PI day 4, the other on PI day 6.

Experiment III. Viremia in experimental IBDF in kestrels

Two kestrels were inoculated with FHV strain S-18. One of the birds, KIIII1, was inoculated by the IM route and the other, KIIII2, by means of inhalation of an aerosol sprayed at the external nares.

Four drops of peripheral blood were collected at 8 hour intervals from each of the birds by clipping the ends of the talons. The blood was collected in 0.5 cc of sterile distilled water. The laked blood samples were submitted for

virus reisolation attempts. Both birds were necropsied.

Experiment IV. Progression of lesions in experimental IBDF in kestrels

Six kestrels (KIV1 through KIV6) were inoculated with FHV strain S-18. At 26 hours PI, and at 24 hour intervals thereafter, a bird was bled by jugular venipuncture and killed by decapitation. Some birds died before they were scheduled to be killed.

All birds were necropsied using aseptic technique. Specimens were collected from the liver, spleen, small intestine, kidney, lung, and tibial bone marrow in such a manner that cross-contamination between the organs did not occur and they were submitted, along with a specimen of blood, for virus reisolation attempts. Routine histopathological examinations were performed on all birds and hematological examinations were performed on the birds that were killed.

Experiment V. Experimental reproduction of IBDF in a prairie falcon

A male prairie falcon (PFV1) was inoculated with FHV strain S-18 and a blood sample was collected for SVN titer. The bird was observed for clinical signs of disease and hematological studies were performed on PI days 3, 4, 5, 6, and 7. Necropsy was performed upon death of the bird; liver tissue was collected for attempted virus reisolation and histological and ultrastructural examinations were performed.

Experiment VI. Pathogenicity of FHV strain S-18 in other raptor species

One merlin (MVI1), 3 great horned owls (GHOVI1 through GHOVI3), 1 screech owl (SOVI1), 4 red-tailed hawks (RTVI1 through RTVI4), 1 Harris' hawk (HVI1), 1 Cooper's hawk (CHVI1), 1 golden eagle (GEVI1), and 1 bald eagle BEVI1) were inoculated with FHV strain S-18. Blood samples were taken prior to inoculation from all but the merlin and screech owl, and the sera were submitted for determination of the presence of neutralizing antibodies.

All birds were observed for the development of clinical signs of disease. Those birds which died were necropsied and attempts were made to reisolate the virus from the livers.

Experiment VII. Pathogenicity of FHV strain S-18 in non-raptorial birds

One coot (CTVII1), 1 green heron (GRHVII1), 1 rose-breasted grosbeak (RBBVII1), 1 Baltimore oriole (BOVII1), 3 ring-necked doves (RDVII1, RDVII2, RDVII3), 5 budgerigars (BVII1 through BVII5), 1 Amazon parrot (APVII1), 10 18-day old turkey poults (TVII1 through TVII10), 10 3- to 4-day old muscovy ducklings (MDVII1 through MDVII10), 8 10-day old muscovy ducklings (MDVII11 through MDVII18), 5 7-week old muscovy ducklings (MDVII19 through MDVII23), 1 adult muscovy duck (MDVII24), 5 day-old chicks (CVII1 through CVII5), and 3 pigeons (PVII1 through PVII3) were inoculated with FHV.

Pre-inoculation pooled serum samples from the 3- to 4-day old ducklings, 10-day old ducklings, and day-old chicks, and individual serum samples from the turkey poults, 7-week old ducklings, adult duck, and pigeons were submitted for determination of the presence of neutralizing antibodies.

The birds were observed for signs of disease. All birds which died were necropsied and virus reisolation was attempted.

Experiment VIII. Comparative pathogenicity of OHV and PHV

In order to compare the spectrum of species susceptible to OHV and PHV with that of species of known susceptibility or non-susceptibility to FHV the former viruses were inoculated into kestrels, doves, pigeons, great horned owls, budgerigars, and red-tailed hawks (table 2). Preinoculation serum samples were collected from the owls and red-tailed hawks for determination of the presence of neutralizing antibodies. All birds were observed for signs of illness. Necropsies, including virus reisolation attempts, were performed on all birds which died.

Table 2. Birds Inoculated with OHV and PHV

| Species | OHV | PHV |
|------------------|-------------------------------|--|
| kestrel | KVIII1 | KVIII2 KVIII3 KVIII4 |
| ring-necked dove | RDVIII1 RDVIII2 RDVIII3 | RDVIII4 RDVIII5 RDVIII6 |
| pigeon | PVIII1 PVIII2 PVIII3 | PVIII4 PVIII5 PVIII6 |
| great horned owl | GHOVIII1 GHOVIII2 | GHOVIII3 GHOVIII4 |
| red-tailed hawk | RTVIII1 RTVIII2 | RTVIII3 RTVIII4 |
| budgerigar | BVIII1 BVIII2 BVIII3 | BVIII4 BVIII5 BVIII6 BVIII7 BVIII8 BVIII9 |

RESULTS

Experiment I. Examination of Field Cases of IBDF

A wide variety of specimens was received in response to the author's request for case material from possible or suspected cases of IBDF and 7 previously unreported or unrecognized cases were found (Table 3).

Field case 1. Prairie falcon (PF-I1)

Clinical history A fledgling tiercel (male) prairie falcon was captured from an eyrie in western South Dakota in May, 1971. It was raised and trained by a falconer in eastern South Dakota. Its diet consisted of whole dead birds, including pigeons and day-old chicks, and beef and horsemeat supplemented with vitamins and minerals. At about 4 months of age the tiercel became anorectic, depressed, and weak and experienced a moderate (2 ounce) weight loss over a 3- to 4-day period of time. Force-feeding by gavage was attempted but the bird died on the 4th day of illness. The cadaver was frozen several hours after death and was inadvertently thawed and refrozen before being submitted for necropsy.

Post-mortem examination The frozen cadaver was quickly thawed in tepid water to minimize further autolysis.

Gross lesions The cadaver was thin but not emaciated. All internal organs were reddish-tan to reddish-brown because of autolysis and blood pigment imbibition. The abdominal air sacs contained 5 adult nematodes (Serrato-
spiculum amaculata).

Table 3. Summary of the Clinical, Pathological, and Virological Aspects of Field Cases of IBDF

| Case | Clinical Signs | | | | | | duration of illness (days) | Lesion Distribution | | | | | | FHV isolated (strain) |
|-----------|----------------|------------|----------|-------------|----------|----------------|----------------------------|---------------------|--------|-----------------|------------------|----------------|--------------------------------|-----------------------|
| | anorexia | depression | weakness | weight loss | diarrhea | other | | liver | spleen | b. marrow | alimentary tract | other | intracellular inclusion bodies | |
| 1(PF-I1) | + | + | + | + | - | - | 4 | + | + | A ^a | + | - | A ^a | S-18 |
| 2(RHF-I1) | - | + | + | - | - | - | 3 | + | + | + | + | + ^b | A ^a | H-4 |
| 3(PF-I2) | + | + | + | + | + | - | 2 | + | + | + | + | - | + | H-5 |
| 4(PER-I1) | + | + | + | + | - | + ^c | ? ^d | + | + | - | - | + ^c | + | 2-A |
| 5(PF-I3) | + | + | + | - | + | + ^e | 1 | + | + | NE ^f | NE | + ^g | + | none ^h |
| 6(GF-I1) | + | + | + | - | - | - | 3 | + | + | + | - | - | + | none ^h |
| 7(PF-I4) | + | + | + | + | + | - | 4 | + | + | + | + | - | + | H-48 |

^a Autolysis precluded evaluation.

^b Focal necrosis in ovarian stroma.

^c Radiographic, pathological, and cultural confirmation of concurrent aspergillosis.

^d Concurrent disease precludes determination.

^e Black casting.

^f Not examined.

^g Concurrent Serratospiculiasis.

^h Not attempted.

The liver was enlarged. Its edges were rounded and there was a slight convexity of its normally concave posterior-dorsal surfaces. Numerous reddish-tan foci, pinpoint-size to 1.0 mm in diameter, were randomly scattered throughout the dark brown liver. They were easily visible through the capsule but rarely caused capsular elevations (Fig. 1).

The spleen was slightly enlarged (1.1 x 0.8 x 0.8 cm) and many pale reddish-tan to orange pinpoint foci were scattered throughout the organ (Fig. 1).

The bone marrow had a semi-fluid consistency.

The walls of the jejunum and ileum were turgid and many fine light yellowish-tan transverse streaks were faintly visible through the serosa.

There were no other gross lesions.

Microscopic lesions The normal lobular architecture and cell cord arrangement of the liver was obliterated by autolysis and ice crystal clefts (freezing artifacts). There were multiple foci of parenchymal necrosis which were well circumscribed and occasionally coalesced with adjacent foci. There was no apparent zonal distribution of the lesions. The necrotic foci were characterized by karyolysis and by a marked, more homogeneous eosinophilia not found in the surrounding normal, albeit autolysed, parenchyma.

There was focal to diffuse necrosis of the spleen and focal necrosis of the small intestinal lamina propria.

Virological examination The entire spleen and a portion of the liver were submitted for virus isolation attempts. A virus was isolated and was characterized both physically and chemically as a herpesvirus. This virus was designated "FHV strain S-18."

Field case 2. Red-headed falcon (RHF-II)

Clinical history Two red-headed falcons (*F. chiquera*), a male and a female, were imported from India in 1969 by a falconer in California. One year later they were shipped to a falconer in Iowa who, after 4 to 5 months, donated them to the Raptor Research Foundation captivity breeding project directed by Mr. Donald V. Hunter, Jr., Centerville, South Dakota. After 4 months in South Dakota, during which time the diet consisted of day-old chicks and occasional pigeons, the female died. Her death followed 3 days of listless behavior. During the illness there was no anorexia or weight loss, but on one occasion the bird regurgitated recently-ingested food. She was treated orally with Emtryl[®] and Terramycin[®]. The cadaver was frozen within 8 hours of death and was stored in a home freezer for 13 months before being submitted for necropsy.

Post-mortem examination

Gross lesions The cadaver was well fleshed and contained normal internal fat depots. The crop contained 4 grams of recently ingested white muscle meat.

Hepatomegaly was evidenced by slight rounding of the liver edges. There were many small (pinpoint to 0.5 mm) light tan foci scattered throughout the dark reddish-brown liver.

The dark purple-brown spleen contained several small white to tan foci.

The small intestine was slightly reddened and within the intestinal wall there were numerous pale yellowish-tan punctiform lesions visible through the serosa (Fig. 2).

There were no other gross lesions.

Microscopic lesions There were freezing artifacts in all tissues. The liver contained numerous irregularly shaped, non-zonally distributed foci of coagulation necrosis which were well delineated from the adjacent parenchyma.

Multiple foci of necrosis also occurred in the spleen, bone marrow, intestinal lamina propria and ovarian stroma.

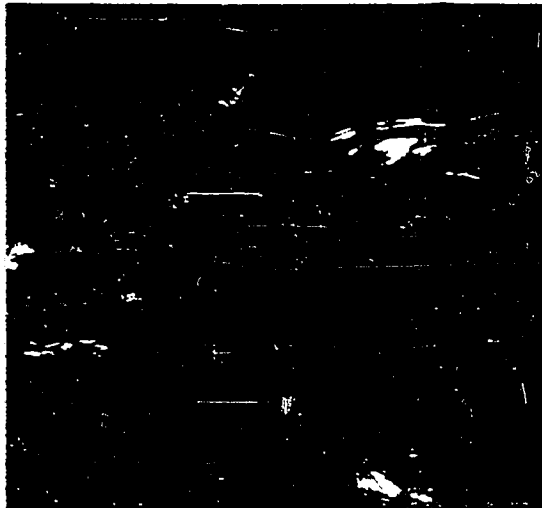
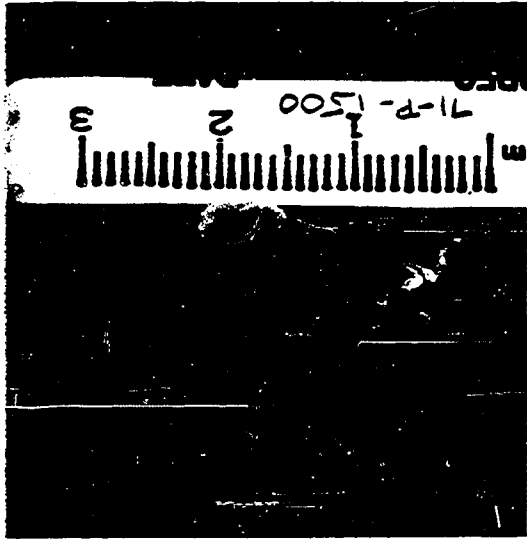
Virological examination Portions of the liver and spleen were submitted for attempted virus isolation. A virus was isolated which produced a CPE typical of that produced by herpesviruses in cell cultures. The virus was neutralized by rabbit anti-FHV strain S-18 serum and was designated FHV strain H-4.

Field case 3. Prairie falcon (PF-I2)

Clinical history A female prairie falcon was captured as a nestling from a Wyoming eyrie in 1971 and was raised by

Figure 1. The liver of PF-I1 contains randomly distributed foci of necrosis. The spleen (bottom center) is heavily studded with necrotic foci.

Figure 2. Pale tan foci are visible through the serosa of the small intestine of RHF-I1. This segment has been opened and laid mucosa-side down.



a falconer in New York State. By the age of 5.5 months the bird often pursued feral pigeons during training flights. On November 13th the falcon refused to chase other birds. On November 14th its feces were uniformly green and that evening the bird refused to fly and was lethargic and anorectic. She was force-fed a small amount of pigeon breast meat dipped in egg yolk and was treated with Emtryl[®] orally. On the morning of November 15th the bird was somnolent in sternal recumbency and the food fed the previous night remained in the crop. The bird died that evening and was necropsied by Dr. Malcom Peckham, Cornell University.

Post-mortem examination

Gross lesions The lesions observed and reported by Dr. Peckham were liver necrosis, spleen enlargement and hemorrhage, and gastroenteritis.

The cadaver was frozen following necropsy and was sent, along with stained histological slides, to the author.

Examination of the tibial bone marrow of the thawed specimen revealed many pale tan to light reddish-tan foci randomly distributed through the red-brown to purple-brown tissue.

Microscopic lesions No significant post-mortem autolytic change was found in the slides submitted by Dr. Peckham.

There were many discrete, non-zonal foci of necrosis in the liver. They were markedly eosinophilic and thus delimited

sharply from the surrounding more basophilic intact parenchyma. Pycnotic nuclei and basophilic nuclear fragments were sparsely scattered through the necrotic areas (Fig. 3) but in the centers of occasional foci there was a more dense accumulation of basophilic nuclear debris.

Many intact hepatocytes immediately adjacent to the necrotic areas contained eosinophilic (red to reddish-magenta) intranuclear inclusion bodies. In most instances the inclusion bodies were solitary, but occasional nuclei contained 2 inclusions. The inclusions were, in the great majority of cases, centrally disposed within the nucleus and were separated from the peripherally margined and condensed chromatin by a relatively clear, chromophobic halo (Fig. 4).

There was 1 poorly delimited focus of degeneration and necrosis in the lamina propria of the small intestine.

There were no microscopic lesions of the lung, myocardium, pancreas, and kidney.

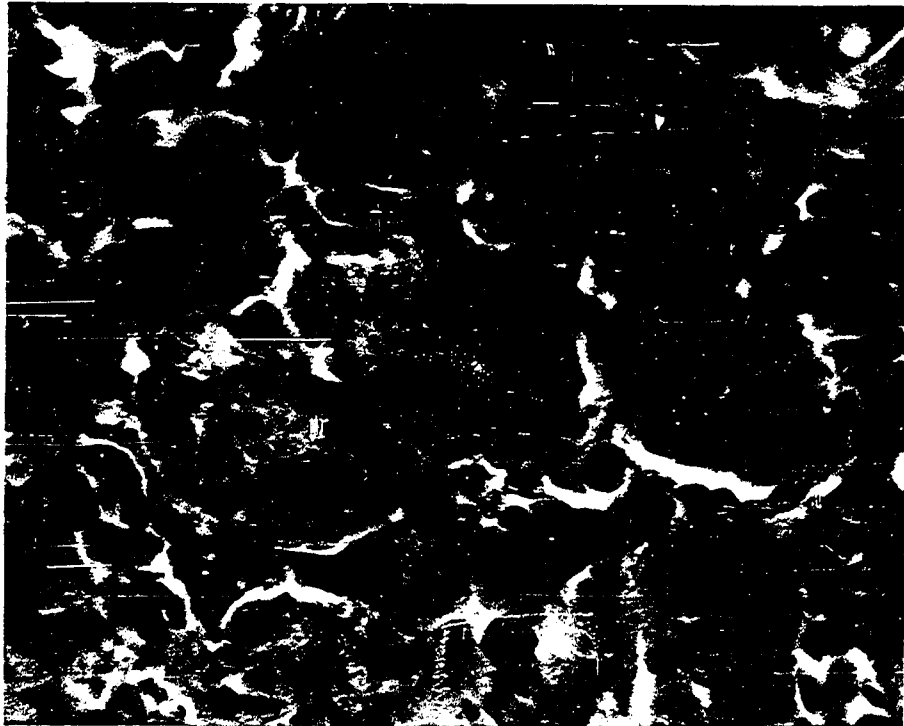
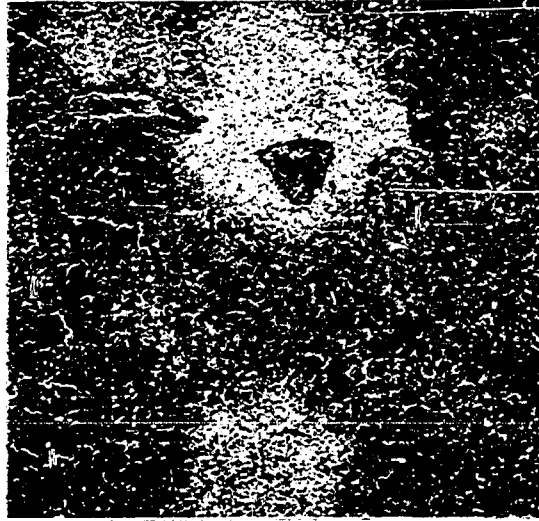
Virological examination A portion of the bone marrow was submitted for virus isolation attempts. A virus was isolated which produced CPE on DEF and CEF cell cultures and was inactivated by rabbit anti-FHV strain S-18 serum. This isolate was designated "FHV strain H-5."

Field case 4. Peregrine falcon (PER-I1)

The history and the gross and microscopic post-mortem findings were submitted by Dr. F. Prescott Ward, Edgewood Arsenal, Maryland.

Figure 3. Focal necrosis in the liver of PF-I2. H and E. x77.

Figure 4. Hepatocytes containing intranuclear inclusion bodies are found adjacent to the necrotic foci in PF-I2. H and E. x750.



Clinical history An immature female peregrine falcon (F. peregrinus tundrius) was captured and banded on Assateague Island, Maryland, during the fall migration in 1970. The bird was released in apparent good health. Sixteen days later the bird was trapped again 250 miles south of the original capture site. At recapture the bird was noticeably thin, appeared to be ill and was therefore retained in captivity for recuperation. The bird's appetite was normal for 3 days, then began to decrease and a slow weight loss began. On the 7th post-recapture day a fecal exam was negative for parasites or their eggs. Examination of a mouth swab revealed no trichomonads but there were many yeast-like organisms.

Intrathoracic nodules suggestive of a mycotic infection were found on radiographic examination. On the basis of a presumptive diagnosis of pulmonary aspergillosis the bird was treated intravenously with Fungizone[®]. Death occurred 2 hours later and the falcon was necropsied.

Post-mortem examination

Gross lesions The cadaver was emaciated. The air sacs and lungs contained plaque-like and focal caseous lesions, respectively, considered characteristic of aspergillosis. Many yellow foci 0.5 to 1.0 mm were scattered throughout the liver which did not appear to be enlarged. There was splenomegaly and the spleen was pale yellowish-brown and friable.

Microscopic lesions The lung and air sac lesions were granulomas containing branching septate mycelia and

occasional fruiting bodies characteristic of Aspergillus species.

The spleen and liver contained foci of coagulation necrosis; intact cells adjacent to the necrotic sites contained intranuclear inclusion bodies.

Virological examination Ward prepared a suspension of liver tissue which was used to inoculate the CAM of chicken embryos. Lesions of the CAM were present 5 days PI and were used to inoculate a kestrel by the IM route. The kestrel was found dead on PI day 6 and its liver contained "countless tiny white foci." A portion of this kestrel's liver was sent to the author who submitted it for attempted virus isolation.

A viral agent was isolated which produced cell "rounding" and giant cell formation typical of the CPE of herpesviruses in cell culture. The virus was neutralized by rabbit anti-FHV strain S-18 serum and was designated "FHV strain 2A."

Field case 5. Prairie falcon (PF-I3)

Because of the similarity between the lesions described and illustrated by Bigland et al. (1964) in a prairie falcon with air sac worms and those of falcon inclusion body disease the author solicited more information on this case. Copies of the summarized case history and gross necropsy findings and the original stained slides of liver and spleen were sent by Dr. C. H. Bigland, Department of Veterinary Microbiology, University of Saskatchewan.

Clinical history The immature prairie falcon had been trapped in southern Alberta, Canada, in February of 1957 and was being trained by a falconer. The owner observed the sudden onset of anorexia, depression, and weakness; the feces were watery and green and the bird's casting (regurgitated indigestible material) was black. The falcon was treated with Enheptin[®] and Nystatin[®] per os but died within 24 hours of the onset of illness.

Post-mortem examination

Gross lesions There were numerous air sac worms and the liver and spleen contained "multiple yellow necrotic foci."

Microscopic lesions In both the liver and spleen there were foci of coagulative necrosis, and eosinophilic intranuclear inclusion bodies were present in many of the intact hepatocytes contiguous to the edges of the focal lesions.

Virological examination No tissues were collected for bacterial or virus isolation attempts.

Field case 6. Gyrfalcon (GF-I1)

Mr. Donald V. Hunter, Jr., Centerville, S. D., the last owner of Field Case 2 (the red-headed falcon) has been an active falconer for more than 30 years and has kept a relatively complete file of reports of post-mortem examinations performed on his birds. This file yielded a report of a necropsy performed on a gyrfalcon by Morgan Berthrong, M.D., Penrose Hospital, Colorado Springs, Colorado, who sent the

author a set of histological slides from the case.

Clinical history A tiercel gyrfalcon (F. rusticolis) was captured as a nestling in the Canadian arctic in 1962 and trained for falconry. The bird suddenly became listless and easily fatigued in November of 1963. It was anorectic for 24 to 48 hours ante mortem but there was no significant weight loss. The bird died 2 to 3 days after illness was first noted.

Post-mortem examination

Gross lesions The necropsy was performed by Morgan Berthrong, M.D. who reported that the liver and spleen were both enlarged and "... diffusely infiltrated by tiny gray-yellow abscess-like lesions."

Microscopic lesions No significant post-mortem autolysis except of the epithelial cells of the intestinal mucosa was noted on the histological slides sent by Dr. Berthrong.

The liver contained many non-zonal foci of coagulation necrosis which were separated from normal parenchyma by an incomplete narrow margin of hepatocytes undergoing degeneration. The nuclei of the degenerating cells, as well as those of adjacent, otherwise normal cells, contained central red to magenta inclusion bodies and had margined chromatin.

There were accumulations of lymphoid cells in perivascular and portal regions.

In the spleen there were numerous well circumscribed individual and coalesced foci of coagulation necrosis. Many of the foci contained relatively dense accumulations of basophilic particulate nuclear debris (Fig. 5 and 6). Intranuclear inclusion bodies occurred infrequently in cells of this spleen.

There was necrosis of the bone marrow in the medullary spaces of the ossified tracheal rings.

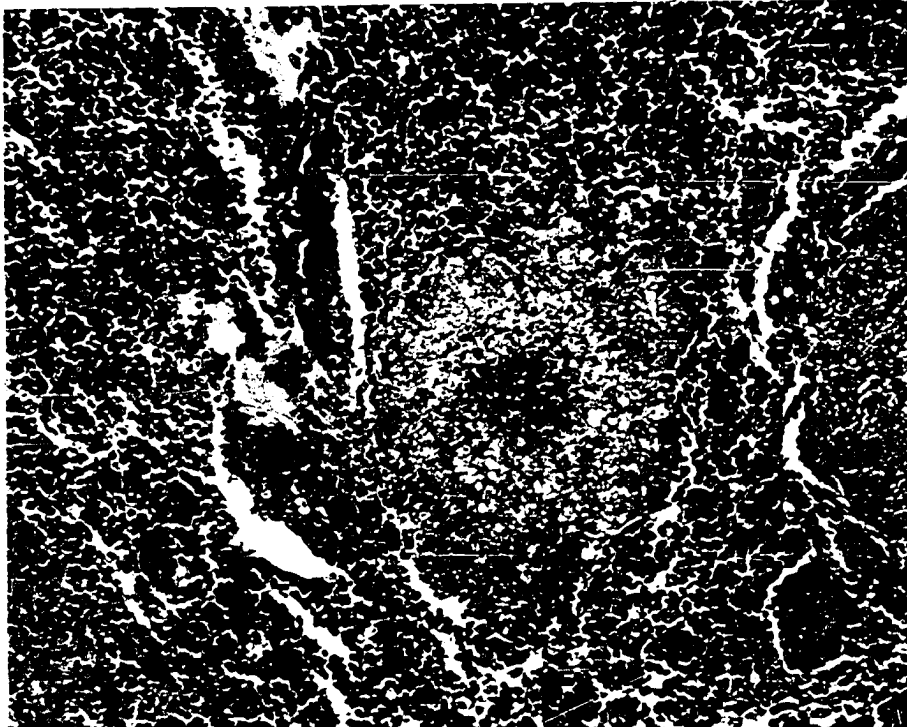
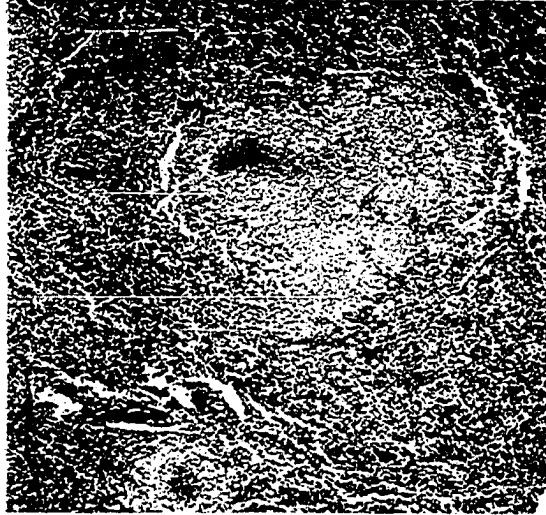
Virological examination No tissues were saved for virus isolation attempts.

Field case 7. Prairie falcon (PF-I4)

Clinical history Three 28-day old prairie falcons, 2 males and a female, were taken from an eyrie in Bonneville County, Idaho in June, 1972. The eyrie was endangered by nearby construction and the birds were removed for hand rearing and subsequent release. They were housed in a nest box open to the outdoors and were fed twice daily on a ledge outside the nest box. When fully fledged, they were not confined and flew free but returned to the ledge twice a day to eat. They were fed dead sparrows, ground squirrels, pigeons, meadow larks, magpies and other birds.

Figure 5. Focal splenic necrosis in GF-I1. H and E. x50.

Figure 6. The foci of splenic necrosis contained central accumulations of nuclear debris. (Higher power view of Figure 5.) H and E. x 220.



When the birds were 41 days old, one of the tiercels failed to return to the feeding ledge and was obviously ill when recaptured 2 days later. The bird was anorectic, weak, and finally was unable to fly. It sat with ruffled feathers and its eyes were dull. The feces were watery and green. The bird was fed a blended mixture of non-carbonated soft drink and ground liver by gavage and was treated daily with an oral antibiotic. The tiercel died 3 days after recapture.

Post-mortem examination A cursory necropsy was performed within a few hours of death by a local veterinarian with the owner in attendance. Tan spots were noted on the liver and spleen. Pieces of the liver and spleen were fixed in 10% formalin and the cadaver was immediately frozen. The fixed specimens and the cadaver were shipped to the author.

Gross lesions The carcass was quickly thawed in warm water. It was thin but not emaciated; most of the internal fat deposits were depleted. There were many small (pinpoint to 1.5 mm) white to tan foci scattered throughout the liver (Fig. 7), spleen, and bone marrow.

The pancreas and most of the small intestine had been removed from the cadaver; there were no gross lesions of the ileum and large intestine.

Microscopic examination Many non-zonal foci of coagulation necrosis were scattered throughout the section of liver. In several cases the necrotic foci were oriented around or adjacent to large venous channels. Occasional

foci were coalesced into large, irregularly shaped regions of massive necrosis. Central eosinophilic intranuclear inclusions were present in many hepatocytes.

The spleen contained focal to massive areas of necrosis. Intranuclear inclusions, though present, were not numerous.

Accumulations of nuclear debris were absent from the hepatic and splenic lesions.

Tissues from the frozen cadaver contained freezing artifacts.

There were multiple foci of necrosis in the bone marrow and in the lamina propria of the vestigial ceca. No microscopic lesions were noted in the heart, brain, proventriculus, kidney, adrenal gland, and lungs.

Virological examination A virus which was isolated from the liver caused typical herpesvirus CPE on cell culture and was neutralized by rabbit anti-FHV strain S-18 serum. This isolate was designated "FHV strain H-48."

Experiment II.

Pathogenicity of FHV strain S-18 for Kestrels

Two birds became ill and died with lesions of IBDF within 5 days of inoculation. Bird KII1 was killed in extremis on PI day 6. The findings are summarized in Table 4.

Clinical signs

Clinical evidence of illness was observed as early as the 3rd PI day in kestrel KII2; there was anorexia but no

Table 4. Summary of the clinical, hematological, and pathological findings in KII1, KII2, and KII3

| Bird | Clinical signs | | | leucocytopenia | days PI to death | FHV reisolated | Lesion Distribution | | | | |
|------|----------------|----------|------------|-----------------|------------------|----------------|---------------------|--------|-------------|------------------|----------------|
| | onset (PI day) | anorexia | depression | | | | liver | spleen | bone marrow | alimentary tract | other |
| KII1 | 5 | + | + | + | 6 | + | + | + | + | + | + ^a |
| KII2 | 3 | + | - | NE ^b | 5 | + | + | + | + | + | + ^c |
| KII3 | 0 | - | - | NE | 4 | + | + | + | + | + | + ^d |

^a Kidney.

^b Not examined.

^c Pancreas.

^d Adrenal gland.

obvious lethargy or depression was noted. Death occurred on PI day 5.

Anorexia and depression were noted in bird KII1 on PI day 5. He was comatose and hypothermic exactly 6 days PI, was considered to be in extremis and was killed by exsanguination.

No clinical illness or premonitory signs of impending death were seen in KII3. This bird was observed vigorously eating a mouse on PI day 4 and was found dead 4 hours later.

Hematology

The results of the 2 hematological examinations on KII1 are shown in Table 5.

Post-mortem examination

Gross lesions Many extremely small, barely visible white to pale tan foci were scattered throughout the liver (Fig. 8) and spleen of all 3 birds. Gross lesions of the alimentary tract and bone marrow were not seen.

Microscopic lesions All 3 birds had microscopic lesions in the liver, spleen, bone marrow and alimentary tract and a single description of each organ will suffice for all the birds.

Liver The livers contained innumerable microfoci of degeneration and coagulative necrosis. These foci were proportionately smaller and more numerous than those observed in the livers of the field cases. The lesions were of a completely non-ordered, non-zonal distribution being found with equal ease near or around central veins, in portal

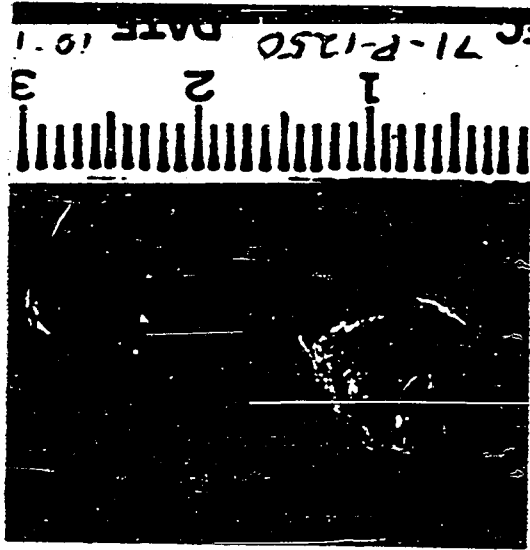
Table 5. Hematological studies on KIII1

| | Sample Collected on | |
|---------------------|---------------------|-----------------------|
| | PI day 4 | PI day 6 ^a |
| WBC/mm ³ | 22,500 | 350 |
| Differential count: | | |
| heterophils | 84 | 1 |
| lymphocytes | 10 | 10 |
| monocytes | 0 | 0 |
| eosinophils | 6 | 0 |
| basophils | 0 | 1 |

^a There were too few leucocytes in the peripheral blood smears to complete a differential count.

Figure 7. The focal liver lesions of PF-I⁴ varied markedly in size and produced a granular to finely nodular roughening of the capsule.

Figure 8. The hepatic necroses in KIII¹ are typical of the pinpoint focal lesions found in experimental I.B.D.F.



regions and in mid-zonal loci. Intranuclear inclusion bodies were common throughout the liver parenchyma for no point or locus within the organ was more than 10 to 15 cells away from a necrotic focus. Inclusion bodies of 2 obviously different types occurred. One was eosinophilic, irregularly spherical, centrally situated, and surrounded by a clear, or at least hypochromatic halo within a nucleus with peripherally condensed or margined chromatin. The other common inclusion was either darkly basophilic or deep bluish-magenta and filled the somewhat enlarged nucleus rarely permitting visualization of the thin zone of margined chromatin. Although these inclusions were, as described above, clearly separable it must be emphasized that they were the extremes of a continuum, the intermediate members of which comprised the greater proportion of the inclusion population.

Spleen The splenic parenchyma, and the basic stromal and vascular scaffold of the organ were almost entirely obliterated by foci of necrosis of such size and numbers that each one had several peripheral points of coalescence with its several neighbors. The larger arteriolar structures remained; there was hemorrhage in the interstices between the regions of dead tissue. Inclusion bodies, although present, were found with difficulty.

Bone marrow There was either diffuse necrosis of the marrow as in case KII2, in which only erythrocytes and occasional primitive reticular cells remained, or there

was multifocal necrosis as exemplified in the tibial marrow of KII1 (Fig. 9). As in the spleen, inclusion bodies were found with difficulty.

Alimentary tract Focal areas of degeneration and necrosis were present in the esophagus, proventriculus, ventriculus, small intestine, and large intestine but no one bird had lesions in all these sites. The smaller, more discrete lesions were limited to the lamina propria in most instances (Figs. 10 and 11). Occasionally the integrity of the basement membrane of the overlying epithelium was violated (Fig. 12). Lesions, even the smaller, more subtle ones, of the ventriculus were accompanied by some degree of thinning and fibrillar fragmentation of the normally dense, homogeneously eosinophilic submucosal connective tissue layer (Fig. 13).

Pancreas There was a focus of coagulation necrosis in the pancreas of KII2 (Fig. 14) and there are eosinophilic intranuclear inclusions in the adjoining intact acinar cells.

Kidney A single, well circumscribed focus of renal necrosis (Fig. 15) was found in KII1. The necrosis surrounded 1 degenerating glomerulus and encroached upon the surrounding tubules. Magenta to blue intranuclear inclusion bodies were found in nuclei of adjacent tubule lining cells.

Adrenal gland None of the adrenal glands contained necrotic lesions; however there were pale red to light magenta inclusions in occasional cortical cell nuclei

Figure 9. Focal necrosis (N) of the bone marrow in KIII1.
H and E. x90.

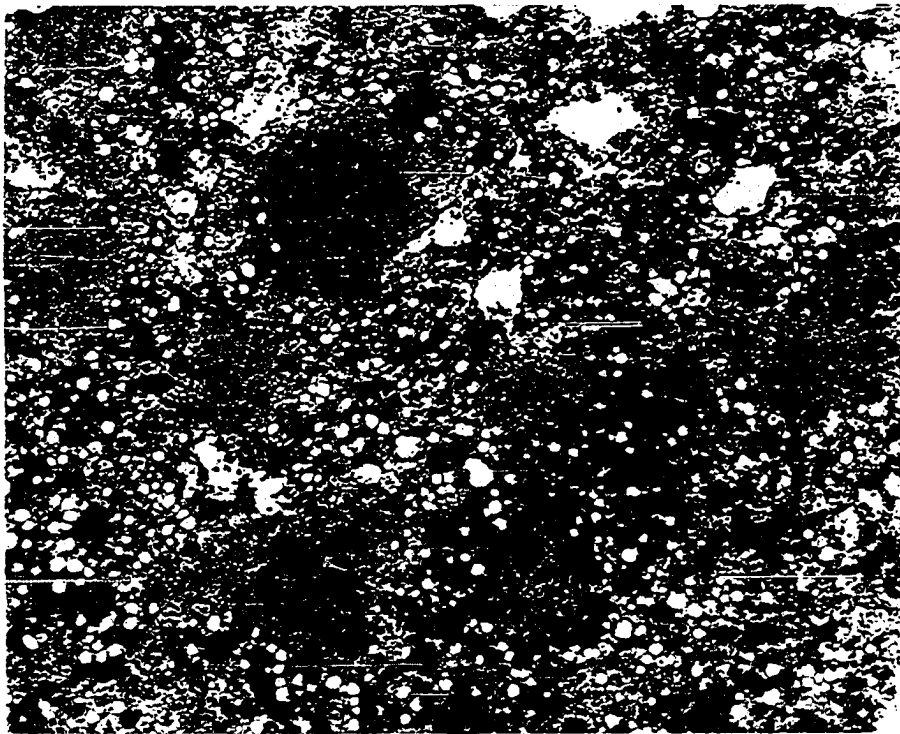


Figure 10. Wall of small intestine of KIII1 with 2 foci of degeneration and necrosis (arrows) in the lamina propria. H and E. x50.

Figure 11. Higher power view of Figure 10. Compare the distinct eosinophilia and pycnotic nuclei in the lesion (arrow) with the basophilia of the unaffected lamina propria. H and E. x420.

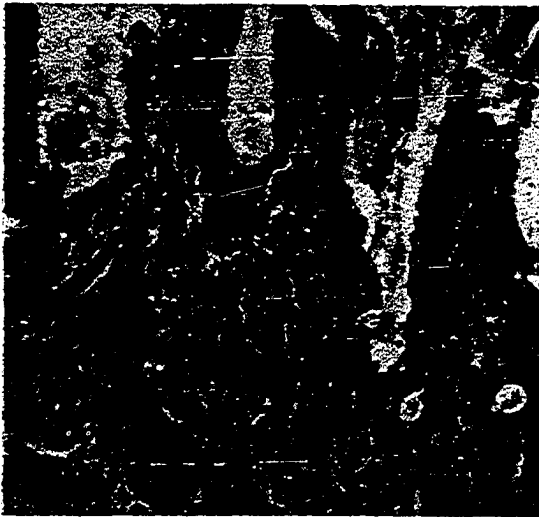


Figure 12. Focal necrosis in the esophagus of KII1 involving the basement membrane and deep epithelial layers. H and E. x420.

Figure 13. Necrosis in the lamina propria of the ventriculus in KII1. H and E. x600.

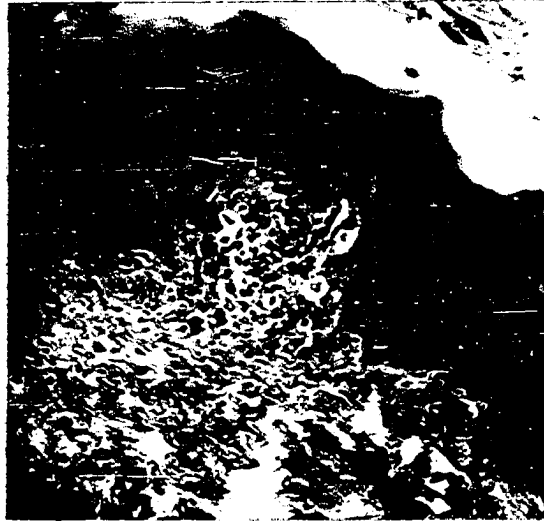
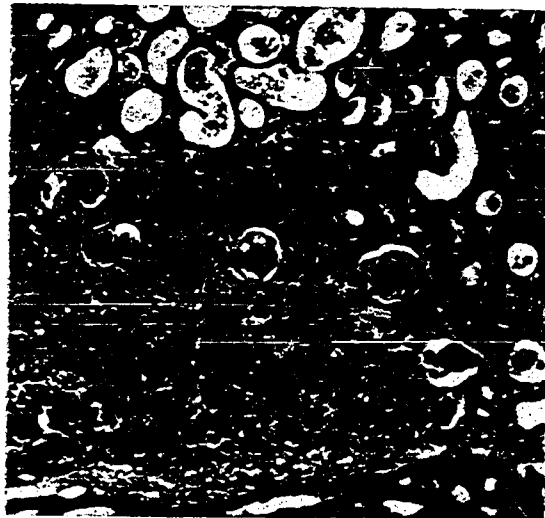
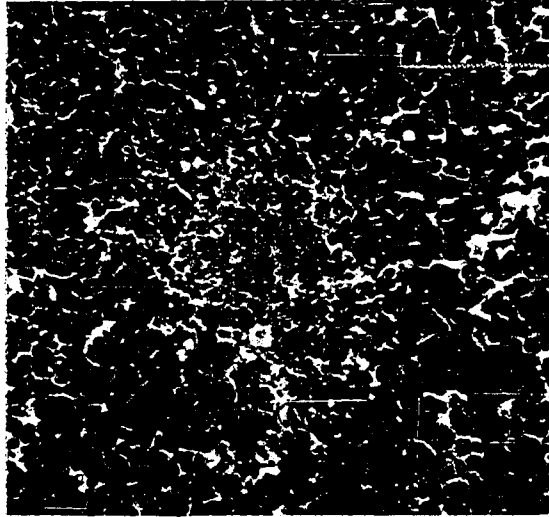


Figure 14. Focal necrosis of the pancreas in KII1. H and E.
x500.

Figure 15. Renal necrosis in KIII1 surrounding a degenerating
glomerulus (arrow) and encroaching upon tubules
and another glomerulus. H and E. x300.



in KII3.

Other organs No microscopic lesions were noted in the hearts, brains, eyes, lungs, and gonads.

Virological examination

FHV was reisolated from the livers of all 3 birds.

Experiment III. Viremia in IBDF in Kestrels

Virus was first detected in the blood of KIII1 collected at 64 hours PI and was found in each sample of blood taken at 8 hour intervals thereafter until it died. The bird had been anorectic and lethargic at 96 hours PI and died at 115 hours PI.

Virus was first reisolated from the blood of KIII2 collected at 72 hours PI and was found at 8 hour intervals thereafter until death. No clinical signs of illness were noted and the bird ate a full meal 5 hours before its death at 100 hours PI.

The results of the serial virus reisolation attempts are presented in Table 6.

Both birds were necropsied. Focal (pinpoint) light tan lesions were found in the liver, spleen, and bone marrow of both birds. These lesions were also seen on microscopic examination and there were hepatic intranuclear inclusion bodies. Except for multiple foci of necrosis in the wall of the bursae of Fabricius, both birds were devoid of lesions in the alimentary tract and other organ systems.

Table 6. Results of serial virus reisolation attempts from the blood of kestrels inoculated with FHV strain S-18

| Hours PI | KIII1 | KIII2 |
|----------|----------------|----------------|
| 8 | - | - |
| 16 | - | - |
| 24 | - | - |
| 32 | - | - |
| 40 | - | - |
| 48 | - | - |
| 56 | - | - |
| 64 | + | - |
| 72 | + | + |
| 80 | + | + |
| 88 | + | + |
| 96 | + | + ^a |
| 104 | + | |
| 112 | + ^b | |

^a Death at 100 to 101 hours PI.

^b Death at 115 hours PI.

Experiment IV.

Progression of Lesions in IBDF in Kestrels

Four birds, KIV1, KIV2, KIV3, and KIV6, were killed at 24, 48, 72, and 96 hours PI respectively. Birds KIV4 and KIV5 died spontaneously of the disease.

The distribution of lesions is summarized in Table 7.

The hematological findings are shown in Table 8.

The results of virus reisolation attempts from different tissues are shown in Table 9.

KIV1

This bird was killed 24 hours PI. Post-mortem examination revealed no gross or microscopic lesions.

KIV2

This bird was killed 48 hours PI and no gross lesions were noted at necropsy.

Microscopic examination of the liver revealed several minute, non-zonally distributed foci of degeneration and necrosis (Fig. 16). Intranuclear inclusion bodies were present. There were similar microfocal lesions in the spleen and bone marrow (Fig. 17). There was heterophil infiltration in a poorly delimited focus of degeneration and necrosis in the lamina propria of the duodenum (Fig. 19).

KIV3

This bird was killed 72 hours PI. Gross lesions were seen only in the liver and spleen.

Microscopic examination revealed focal lesions in the

Table 7. Distribution of lesions in kestrels in experiment IV.

| Bird | Hours PI to death | Lesion Distribution ^a | | | | | | | | | | | | |
|------|-------------------|----------------------------------|--------------------------------|-----------|-------|--------|-------------|--------|------|--------|-------|-------|---------------|---------------|
| | | esophagus | esoph.-proventric. junction | intestine | liver | spleen | bone marrow | thymus | lung | kidney | gonad | brain | adrenal gland | thyroid gland |
| KIV1 | 24 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| KIV2 | 48 | - | - | + | + | + | + | - | - | - | - | - | - | - |
| KIV3 | 72 | - | + | + | + | + | + | + | + | - | - | - | - | - |
| KIV4 | 95 | + | + | + | + | + | + | + | + | - | + | - | + | + |
| KIV5 | 93 | - | + | - | + | + | + | - | - | - | + | + | + | - |
| KIV6 | 96 | - | + | + | + | + | + | + | + | + | - | - | + | - |

^a Organs which were examined but in which no lesions were found were the pancreas, proventriculus, ventriculus, parathyroid gland, skeletal muscle, heart, eye, and uropygial gland.

Table 8. Hematological findings in kestrels in experiment IV.

| | KIV1 | KIV2 | KIV3 ^a | KIV5 ^a |
|---------------------|------------------------|------------------------|------------------------|------------------------|
| Hours PI | 24 | 48 | 72 | 96 |
| RBC/mm ³ | 2.66 x 10 ⁶ | 2.57 x 10 ⁶ | 2.73 x 10 ⁶ | 2.51 x 10 ⁶ |
| WBC/mm ³ | 6.8 x 10 ³ | 10.2 x 10 ³ | 350 | 600 |
| Differential Count: | | | | |
| heterophils | 85 | 94 | 12 | 49 |
| lymphocytes | 14 | 5 | 27 | 0 |
| monocytes | 0 | 0 | 0 | 0 |
| eosinophils | 0 | 0 | 0 | 0 |
| basophils | 1 | 1 | 11 | 1 |

^a There were too few leukocytes in the peripheral blood smears to complete a differential count.

Table 9. Results of virus reisolation attempts from tissues of kestrels in experiment IV.

| Bird | Hours PI to death | Tissues Examined | | | | | | |
|------|-------------------|------------------|-------|--------|-------------|------|--------|-----------------|
| | | intestine | liver | spleen | bone marrow | lung | kidney | blood |
| KIV1 | 24 | - | - | + | - | - | - | - |
| KIV2 | 48 | - | + | - | + | - | - | - |
| KIV3 | 72 | - | + | + | + | - | + | + |
| KIV4 | 95 | - | + | + | + | + | + | NE ^a |
| KIV5 | 93 | NE | + | NE | NE | NE | NE | NE |
| KIV6 | 96 | + | + | + | + | + | + | + |

^a Not examined.

Figure 16. An early microfocus of degeneration and necrosis (arrow) in the liver of KIV2. H and E. x170.

Figure 17. Early microfocal lesion (arrows) in the bone marrow of KIV2. H and E. x170.

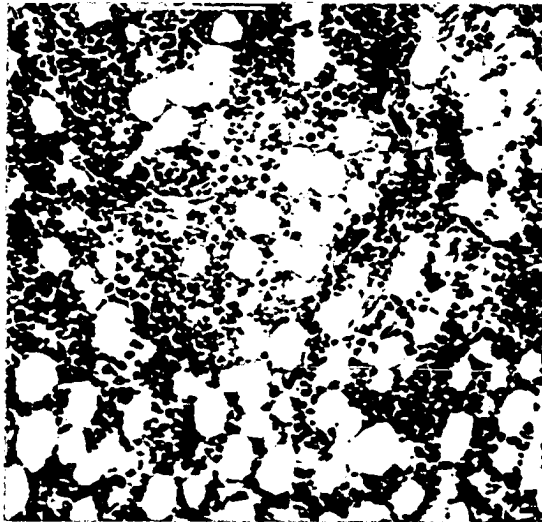
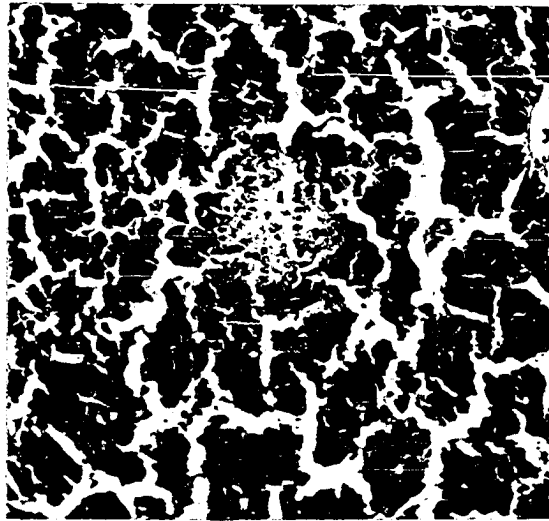


Figure 18. Nests of lymphoid cells in the lamina propria of the intestinal tract (KIV1). H and E. x200.

Figure 19. Lamina propria of the small intestine of KIV2 containing a focus of degeneration and necrosis accompanied by heterophil infiltration. H and E. x200.



liver, spleen, bone marrow, thymus, small intestine lamina propria, esophageal-proventricular junction, adrenal gland, and bronchiolar submucosa.

KIV4

This bird appeared clinically normal 2 hours before death at 95 hours PI. Blood was not collected.

At necropsy focal lesions in the liver and spleen were noted.

Histological examination revealed the typical necrotic lesions of this disease in the liver, spleen, bone marrow, thymus, esophagus, lamina propria of the intestine and esophageal-proventricular junction (Fig. 20), thyroid gland (Fig. 21), ovary (Fig. 22), and mucosa and submucosa of a bronchiole (Fig. 23). Although no necrosis was noted in the adrenal gland there were intranuclear inclusion bodies in the cortical epithelial cells.

KIV5

This bird was found dead in rigor mortis 93 hours PI. Blood was not collected.

Pale tan pinpoint foci were noted at necropsy in the liver and spleen.

Histological examination revealed the typical lesions of this disease in the liver, spleen, bone marrow, thymus, esophageal-proventricular junction, adrenal gland and in the sub-ependymal stroma of the choroid plexus of the 4th ventricle (Fig. 24).

Figure 20. Focal necrosis in the lamina propria and sub-
mucosa at the esophageal-proventricular junction
of KIV4. H and E. x50.

Figure 21. Multiple foci of interstitial necrosis (arrows)
in the thyroid gland of KIV4. H and E. x80.

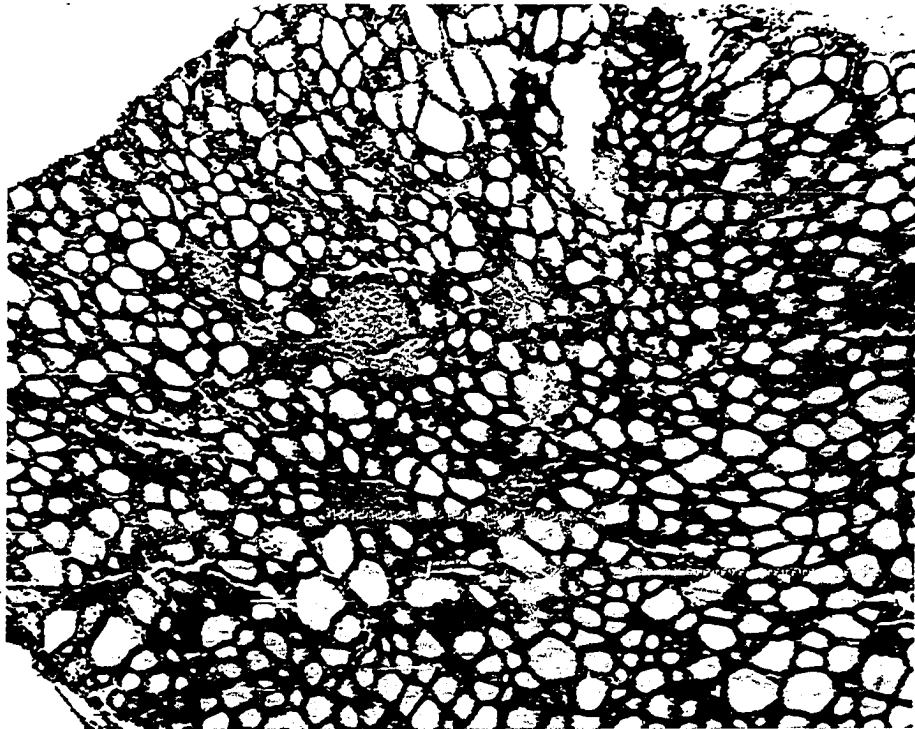
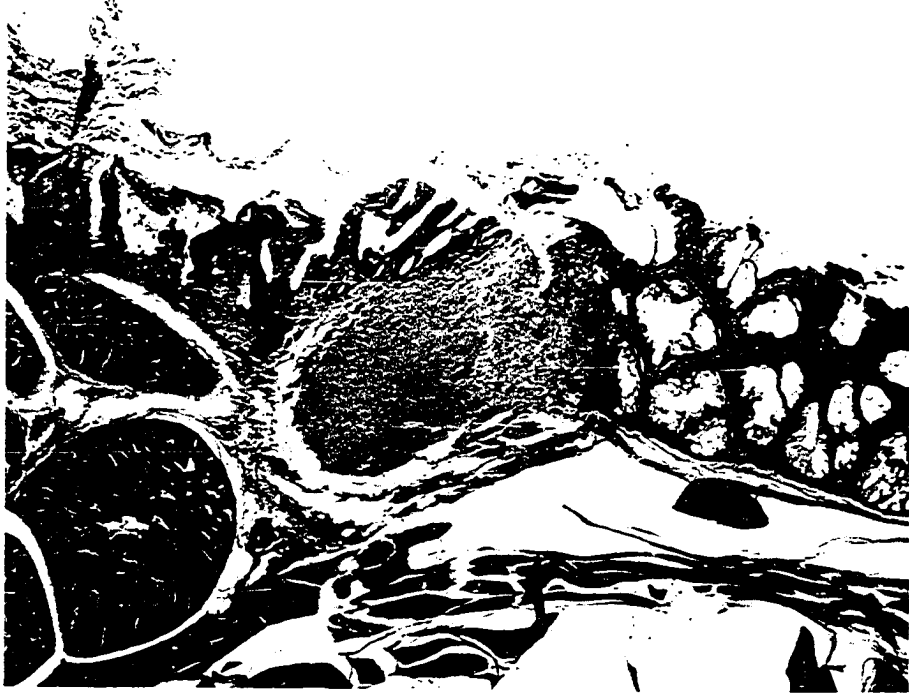


Figure 22. Necrosis in the stroma of the wall of a graffian follicle in the ovary of KIV4. H and E. x210.

Figure 23. Necrosis in the mucosa and submucosa of a bronchiole in KIV4. H and E. x49.

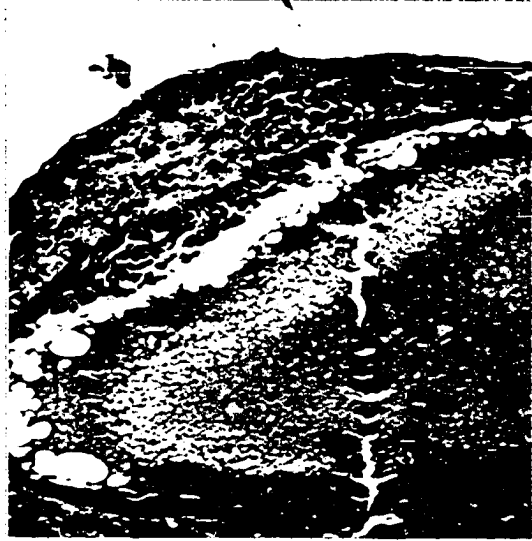


Figure 24. Necrosis in the subependymal stroma in the brain of KIV5. H and E. x200.

Figure 25. The hepatic lesions at 96 hours PI in KIV6 with dense central accumulations of basophilic nuclear debris. H and E. x80.

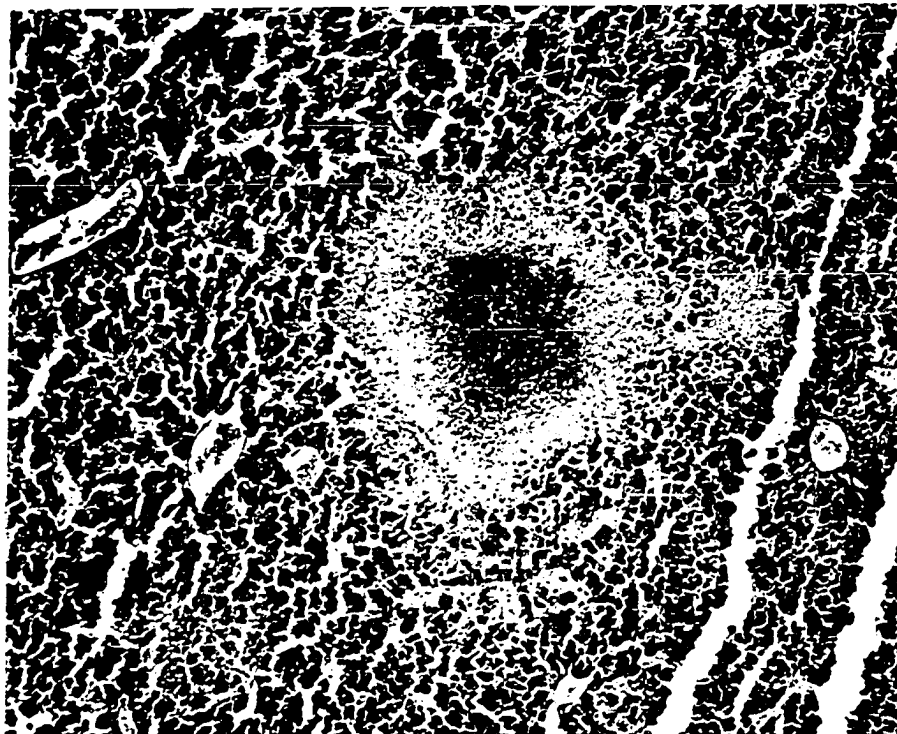
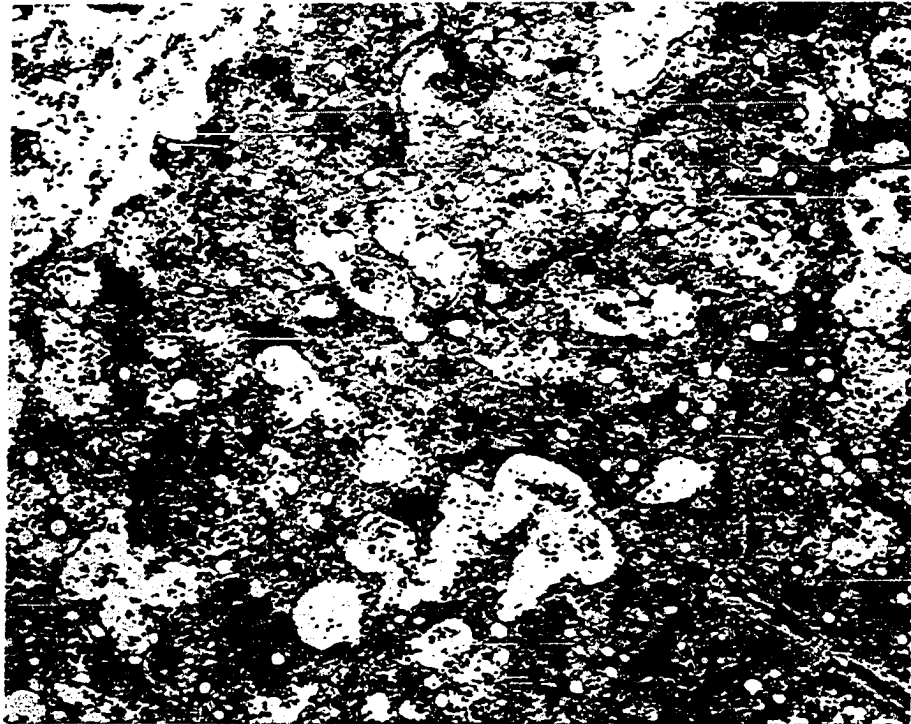


Figure 26. Irregular coalescence of necrotic foci in the spleen of KIV6. H and E. x35.

Figure 27. Diffuse necrosis of the bone marrow in KIV6 at 96 hours PI. H and E. x90.



KIV6

This kestrel was killed 96 hours PI.

Typical gross lesions were noted in the liver and spleen and microscopic lesions similar to those previously described were found in the liver (Fig. 25), spleen (Fig. 26), bone marrow (Fig. 27), thymus, lung, kidney, small intestine, esophageal-proventriculus junction, and adrenal gland (Fig. 28).

Experiment V.

Experimental Production of IBDF in a Prairie Falcon (PFV1)

Falcon PFV1 remained clinically normal until PI day 5 when his appetite was reduced. Anorexia was complete on PI days 6 and 7. At 154 hours PI (PI day 7) he was alert and active but was recumbent, though easily aroused, at 155 hours PI. Death occurred during PI hour 161.

Blood samples for virus reisolation attempts and hematological examination were collected at 72, 96, 110, and 134 hours PI, and FHV was reisolated from all samples.

The results of the hematological examinations are shown in Table 10.

Twelve adult air sac worms were found in the left abdominal air sac.

Gross lesions similar to those described in the field cases were noted in the spleen and bone marrow. The liver lesions were, for the most part, miniscule puncta far smaller

Table 10. Hematological findings in a prairie falcon (PFV1) with IBDF

| | Samples taken at PI hour: | | | | |
|--|---------------------------|-------|------|------|-------|
| | 0 | 72 | 96 | 110 | 134 |
| RBC/mm ³ (x 10 ⁶) | 2.19 | 2.13 | 2.28 | 2.10 | 2.73 |
| WBC/mm ³ (x 10 ³) | 11.70 | 11.55 | 5.60 | 4.75 | 0.800 |
| Differential Count: | | | | | |
| heterophils | 52 | 80 | 86 | 91 | 88 |
| lymphocytes | 46 | 18 | 12 | 9 | 10 |
| monocytes | 0 | 0 | 0 | 0 | 0 |
| eosinophils | 0 | 0 | 0 | 0 | 0 |
| basophils | 2 | 2 | 2 | 0 | 2 |

than those observed in the previously described field cases of IBDF. There were also petechial to fine stellate hemorrhages on the liver (Fig. 29).

Microscopic lesions similar to those heretofore described were found in the following organs:

muscularis at the level of the proventricular-ventricular junction

small intestine lamina propria

wall of cecal protuberances

liver

pancreas

spleen

bone marrow

thymus

thyroid gland

parathyroid gland

adrenal gland

testis.

Virus was reisolated from the liver.

Ultrastructural study of the liver lesions revealed numerous virus particles within the nuclei of degenerating hepatocytes (Fig. 30). Most particles had a dark-staining core region and capsids with an average diameter of 95.7 nm and a range of 83 nm to 105 nm (Fig. 31). Occasional empty capsids were seen.

Some nucleocapsids were in immediate contact with, and

Figure 28. Focal degeneration of the adrenal gland in KIV6 characterized by hypochromasia of the cytoplasm and formation of intranuclear inclusion bodies (right side of figure). H and E. x210.

Figure 29. Multiple pinpoint foci of necrosis (black arrows) and petechial hemorrhages (white arrow) in experimental IBDF (PV1).



Figure 30. Non-enveloped nucleocapsids (white arrow) are numerous in the central region of the nucleus of an hepatocyte. Note the peripherally clumped chromatin (C) and the chromatin-free zone partially surrounding the somewhat eccentric dark-staining inclusion body. There are occasional extranuclear non-enveloped nucleocapsids (black arrows) (PFV1). Uranyl acetate and lead citrate. x29,000.



Figure 31. The dark-staining core of nucleic acid (NA) and the surrounding protein coat or capsid (C) comprise the nucleocapsid of the virus particles as they typically appear in the nucleus of an infected cell. A virus particle in the perinuclear space (PNS) (outside the inner nuclear membrane) has an envelope. Uranyl acetate and lead citrate. x64,500.



produced a bulging of the inner nuclear membrane which was thickened at the point of contact (Fig. 32). Virus particles situated in the perinuclear space were contained within an envelope (Fig. 31). Occasionally an enveloped virion was found within the nucleus but invariably was situated within a membrane-bound vacuole (Fig. 33). Many complete virus particles were found in large dilatations of the cisternae of the endoplasmic reticulum (Fig. 34) or in smaller cytoplasmic vacuoles (Fig. 35). Degenerative changes in the cytoplasm of infected cells were nonspecific and included mitochondrial swelling with fragmentation of the cristae, and dilatation of the endoplasmic reticulum. In the necrotic cells there was fragmentation and disintegration of most membrane structures.

Experiment VI.

Pathogenicity of FHV strain S-18 in Other Raptor Species

Falcon herpesvirus was reisolated from all birds which died with lesions similar to those previously described.

Mortality due to FHV infection in the following species is expressed within parentheses as the number of birds which died of herpesvirus infection (numerator) over the number inoculated with FHV (denominator):

| | |
|-----------------|-------|
| merlin | (1/1) |
| red-tailed hawk | (0/4) |
| Harris' hawk | (0/1) |

Figure 32. Some intranuclear virus particles were in contact with the inner nuclear membrane (arrows). At the area of contact there was bulging and thickening of the membrane. Uranyl acetate and lead citrate. x35,500.



Figure 33. An occasional enveloped virus particle (arrow) was found within a membrane-bound vacuole, or pseudoinclusion, within the nucleus. Uranyl acetate and lead citrate. x31,000.



Figure 34. Many enveloped virus particles were present in dilatations of the endoplasmic reticulum of degenerating hepatocytes (PFV1). Uranyl acetate and lead citrate. x31,000.

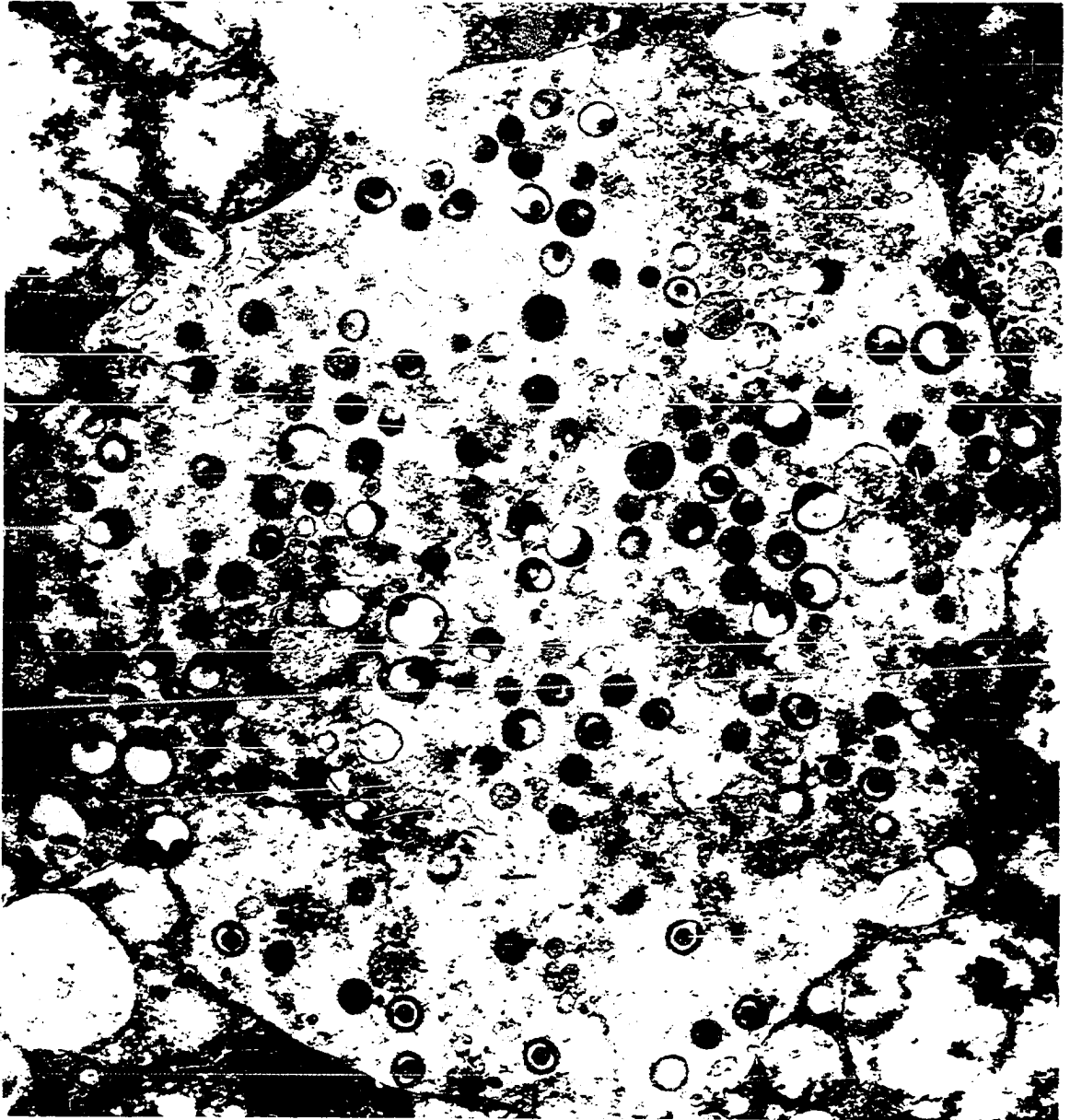


Figure 35. Small cytoplasmic vacuoles occasionally contained enveloped virus particles. Note the polyhedral nucleocapsids (PFV1). Uranyl acetate and lead citrate. x29,000.



| | |
|------------------|-------|
| Cooper's hawk | (1/1) |
| golden eagle | (0/1) |
| bald eagle | (0/1) |
| great horned owl | (3/3) |
| screech owl | (1/1) |

Preinoculation sera from the red-tailed hawks, eagle, and great horned owls proved negative for neutralizing antibodies against FHV strain S-18.

Experiment VII.

Pathogenicity of FHV strain S-18 in Non-raptorial Birds

Virus was reisolated from all birds which died with lesions similar to those described in susceptible raptors.

Mortality due to FHV infection in the following species is expressed within parentheses as the number of birds which died of herpesvirus infection (numerator) over the number inoculated (denominator):

| | |
|-------------------------------|--------|
| green heron | (1/1) |
| coot | (0/1) |
| rose-breasted grosbeak | (0/1) |
| Baltimore oriole | (0/1) |
| ring-necked doves | (3/3) |
| budgerigars | (5/5) |
| Amazon parrot | (1/1) |
| turkey poults | (0/10) |
| 3-4 day old muscovy ducklings | (9/10) |

| | |
|------------------------------|-------|
| 10 day old muscovy ducklings | (0/8) |
| 7 week old muscovy ducklings | (0/5) |
| adult muscovy ducklings | (0/1) |
| day-old chicks | (0/5) |
| pigeons | (0/6) |

All pre-inoculation sera proved negative for neutralizing antibodies against FHV strain S-18.

Experiment VIII. Comparative Pathogenicity of OHV and PHV

A summary of the mortality caused in each species by OHV and PHV is shown in Table 11.

Virus was reisolated from all birds in which lesions were found at necropsy.

The lesions which resulted from infection with either OHV or PHV were very similar. They were also similar in type and distribution to the lesions produced in the same species by FHV in Experiments II, III, IV, VI, and VII.

Table 11. Comparative pathogenicity^a of OHV and PHV

| Species | Virus | |
|------------------|-------|-----|
| | OHV | PHV |
| kestrel | 1/1 | 2/3 |
| great horned owl | 2/2 | 0/2 |
| red-tailed hawk | 0/2 | 0/2 |
| ring-necked dove | 3/3 | 3/3 |
| pigeon | 0/6 | 0/6 |
| budgerigar | 3/3 | 4/6 |

^a Mortality caused by OHV and PHV is expressed in the appropriate column by a fraction; the number of birds which died of herpesvirus infection (numerator) over the number inoculated with virus (denominator).

DISCUSSION

Proof of the Etiology of IBDF

The agent isolated from the liver and spleen of Field Case 1 (PFI1) was propagated in cell culture, repeatedly cloned to exclude extraneous infectious agents and was characterized as a herpesvirus. This herpesvirus, FHV strain S-18, was inoculated into kestrels and produced a disease with lesions which were identical in their significant aspects with those observed in the field case from which it was originally isolated. The virus was reisolated from the kestrels and was inoculated into a healthy prairie falcon (PFV1) in which the lesions of IBDF were reproduced.

The classical criteria for proving the identity of the etiological agent of a disease were thus fulfilled for the herpesvirus of IBDF and the validity of using FHV strain S-18 to produce experimental IBDF was confirmed.

Field Cases of IBDF

The clinical histories of the 7 field cases of IBDF noted above, as well as that of the case described by Ward et al. (1971), indicate that the disease has a short clinical course of 1 to 4 days. Depression and weakness were observed in all cases and most birds were anorectic. Weight loss and diarrhea were not consistently noted.

Focal necrosis of the liver and spleen was found in all cases. Less consistently seen were lesions in the bone

marrow, alimentary tract, and other tissues. Intranuclear inclusion bodies were demonstrated in 5 of the 7 field cases; freezing artifacts and autolysis precluded determination of the presence or absence of inclusion bodies in 2 cases.

Herpesviruses were isolated from all but 2 of the field cases. In both of those cases only histological preparations were available for study and no virus isolation attempts had been made by the original prosectors.

Experimental IBDF in Falcons

Thirteen birds of the genus Falco were inoculated with FHV strain S-18 (experiments II, III, IV, V, and VI). Lesions of I.B.D.F. were found in 12 of the 13 birds. The bird in which lesions were not found (KIV1) had been killed 24 hours PI. Falcon herpesvirus was reisolated from the spleen of KIV1 as well as from all the birds in which lesions were demonstrable.

Nine of the 13 inoculated falcons either died of the disease or were killed in extremis and their lesions are representative of terminal IBDF. The remaining 4 birds (KIV1, KIV2, KIV3, and KIV6) were killed according to a predetermined schedule and their lesions thus represent sequential stages of development and distribution.

Initial lesions were first noted at 48 hours PI (KIV2). These pathologic alterations occurred in the liver and reticuloendothelial tissues (spleen, bone marrow, and intestinal

lamina propria) and were recognized as miniscule foci of necrosis with heterophil infiltration.

At 72 hours PI (KIV3) the lesions in the parenchymatous organs were uniformly larger than those seen at 48 hours indicating that a radial spread of virus occurs from necrotic cells at the limits of the foci to adjacent, immediately peripheral hepatocytes. Electron micrographs of the liver lesions in the experimentally infected prairie falcon (PFV1) illustrate the large numbers of virus particles found in the cytoplasm of the degenerating and necrotic cells (Fig. 34) and light microscopic demonstration of intranuclear inclusion bodies in the hepatocytes adjacent to necrotic tissue (Fig. 4) indicates relatively recent infection of these cells. Thus the concept of direct, peripherally sequential infection of radially adjacent cells is supported.

Further enlargement of the lesions was evident by 96 hours PI in KIV6. Coalescence of the heretofore discrete foci was pronounced in the spleen and resulted in the formation of large irregularly shaped regions of necrosis. Lesion coalescence in the bone marrow was complete to the point of diffuse necrosis.

The prominence and density of the central accumulations of basophilic nuclear debris in the necrotic foci could not be attributed solely to remnants of the nuclei of the hepatocytes which originally occupied the area. The additional basophilic debris was probably provided by the heterophils

found in and about the lesions when they were in an early stage of development.

Results of the repeated hematological studies on KIII1 (Table 4), PFV1 (Table 10) and the findings on the single blood samples from KIV1, KIV2, KIV3, and KIV6 (Table 8) indicate that leukocytopenia occurs during the terminal 24 to 72 hours of the disease. The degenerative and necrotic lesions of the bone marrow and other reticuloendothelial tissues probably account for the leukocytopenia. This marked depression of circulating leukocytes late in the disease explains the absence of inflammatory cellular response to the more peripheral, most recently formed zones of necrosis.

No remarkable alterations in the total red blood cell counts occurred in either the prairie falcon (PFV1) or in the kestrels of Experiment IV.

Ultrastructural examination of liver lesions in the prairie falcon (PFV1) revealed that the nucleocapsid of the virus formed in the nucleus of the infected cell. Some nucleocapsids were seen in contact with the inner nuclear membrane which bulged and was thickened and more densely staining at the point of contact than in other regions. Enveloped virus particles were present outside the inner nuclear membrane or in membrane bound vacuoles in the nucleus interpreted as invaginations of the nuclear membrane. This indicates that the nucleocapsid gained its envelope from, and upon passage through, the inner nuclear membrane. The

presence of occasional non-enveloped particles indicated that the inner nuclear membrane was incomplete. Most of the extranuclear virus particles were found in small to large vacuoles which were interpreted as dilatations of the endoplasmic reticulum. These observations and interpretations are compatible with the generally accepted concepts of herpesvirus maturation and envelopment as reviewed by Darlington and Moss (1969).

Comparison of the Lesions in Field and Experimental Cases of IBDF

In field and experimental cases a definite affinity of FHV for liver and reticuloendothelial tissues was evident. Although the lesions in the liver and reticuloendothelial tissues of both groups of birds were qualitatively identical, they differed on a quantitative basis. The necrotic foci in the livers of the field cases (Fig. 1 and 7) were larger and had a much greater size range than did those in the experimental cases (Fig. 8 and 29). Lesions of the intestinal lamina propria were visible on gross examination only in some of the field cases (Fig. 2).

The difference in lesion size between the field and experimental cases suggests a difference in pathogenesis of the disease between the 2 groups.

Proposed Pathogenesis of
Field and Experimental Cases of IBDF

The birds in which IBDF was produced experimentally undoubtedly received a far higher infective dose of FHV than did the field cases and the use of intramuscular injection accomplished direct and immediate internal deposition of the virus. Gross lesions indicating the site of IM inoculation were not found in the falcons.

Virus was reisolated from only the spleen of a kestrel 24 hours PI but was isolated from an increasing number of other organs (Table 9) as the PI interval increased. This implies that a viremia was present at least 48 hours before it was detected by reisolation of virus from the blood on cell culture. It is hypothesized that free virus particles and particles in circulating macrophages were present in the blood at levels sufficient to produce a detectable concentration in the spleen at 24 hours PI and to establish foci of infection with lesion development in the spleen, liver, bone marrow, and intestine by 48 hours PI. It was not until 72 hours PI, however, that the concentration of virus in the blood reached a sufficient level to be detected by CPE production in tissue culture cells.

Thus, the high dose of virus administered by IM inoculation provided for the rapid establishment of viremia with subsequent initiation of large numbers of simultaneously developing lesions in the spleen, liver, bone marrow, and

intestine. Small lesions in other tissues were noted with greater frequency as the PI interval increased and are interpreted as being foci of infection secondary to dissemination of progeny virus particles from the lesions in the spleen, marrow, liver, and intestine. The occasional larger foci, however, may also have been primary lesions of the initial viremia of inoculation.

The natural route of infection in the field cases of IBDF is not known. Well developed lesions in the intestinal lamina propria of PFI1 and RHFI1 suggest that, in those cases at least, infection may have occurred by the oral route and that the primary lesions developed in the focal accumulations of reticulendothelial cells in the lamina propria of the intestine (Fig. 18). The simultaneous presence in the liver of large, well developed lesions and smaller, more recently initiated lesions denotes that primary foci elsewhere served as a continuing source of virus for the production of these secondary foci.

The lower infecting dose of naturally occurring IBDF would result in the establishment of fewer primary lesions than obtained in the experimental cases. This would permit a longer PI survival period and, consequently, the opportunity for initiation of more numerous secondary focal lesions of different ages and stages of development than were observed in the experimentally infected birds.

The source of infection for the field cases is not known.

A corollary of the proposed oral route of infection is that the virus is acquired from one of the falcon's prey species in which it probably exists as a mild, subclinical, or latent infection. Other herpesviruses are known to cause mild disease in a reservoir host yet produce severe or fatal disease in another species. A similar phenomenon may obtain in the case of IBDF.

Species Susceptibility Spectrum

The field cases of IBDF as well as the species experimentally inoculated with FHV strain S-18 are listed according to their taxonomic order and family in Table 12 and their susceptibility to FHV infection is indicated.

Twenty-four species representing 12 families and 9 orders were studied. Fourteen species representing 6 families and 6 orders were susceptible to FHV.

Among the species susceptible to infection with FHV were 2 species of owls, 2 psittacine species, 1 member of the pigeon family and 1 species of duck. It was not possible to definitively differentiate the lesions produced by FHV infection in the owls, psittacine birds, and ring-necked doves from those of owl hepatosplenitis (Burtscher, 1965a, 1965b), Pacheco's parrot disease (Rivers and Schwentker, 1932), and pigeon herpesvirus infection (Cornwell et al., 1967) respectively. The lesions in the liver and reticuloendothelial tissues of the 3 to 4-day old muscovy ducklings in Experiment

Table 12. Summary of species susceptible or not susceptible to the pathogenic effects of FHV.

| Order | Family | Genus and species | Susceptibility |
|----------------|---------------|--|----------------|
| Falconiformes | Falconidae | <u>Falco mexicanus</u> | + |
| | | <u>Falco chiquera</u> ^a | + |
| | | <u>Falco perigrinus</u> ^a | + |
| | | <u>Falco rusticolis</u> ^{a,b} | + |
| | | <u>Falco sparverius</u> | + |
| | | <u>Falco columbarius</u> | + |
| | Accipitridae | <u>Buteo jamaicensis</u> | - |
| | | <u>Parabuteo unicinctus</u> | - |
| | | <u>Accipiter cooperii</u> | + |
| | | <u>Haliaeetus leucocephalus</u> | - |
| | | <u>Aquila chrysaetos</u> | - |
| Anseriformes | Anatidae | <u>Cairina moschata</u> | ± ^c |
| Gruiformes | Rallidae | <u>Fulica americana</u> | - |
| Ciconiiformes | Ardeidae | <u>Butoroides virescens</u> | + |
| Strigiformes | Strigidae | <u>Bubo virginianus</u> | + |
| | | <u>Otus asio</u> | + |
| Passeriformes | Icteridae | <u>Icterus galbula</u> | - |
| | Fringillidae | <u>Pheucticus ludovicianus</u> | - |
| Galliformes | Phasianidae | <u>Gallus domesticus</u> | - |
| | Meleagrididae | <u>Meleagris gallopavo</u> | - |
| Psittaciformes | Psittacidae | <u>Melopsittacus undulatus</u> | + |
| | | <u>Amazona ochrocephala</u> | + |
| Columbiformes | Columbidae | <u>Columba livia</u> | - |
| | | <u>Streptopelia rhizoria</u> | + |

^a Field case.

^b Diagnosis based on gross and microscopic lesions, no attempt to isolate causative agent.

^c 3- to 4-day old ducklings +; 10 days old and older -.

VII were qualitatively comparable to those described in duck plague (Leibovitz, 1971). Table 13 summarizes the comparative pathogenicity of FHV, OHV, and PHV in 6 avian species.

The PHV was not pathogenic for the great horned owls and thus was differentiated from FHV and OHV, both of which killed owls. All 3 viruses were pathogenic in doves and none were pathogenic in pigeons.

It was the opinion of Burtscher (1968) that owl hepatosplenitis was "... a disease specific for owls" however the OHV used in the present work, originally isolated by Schettler (1970), was pathogenic not only for owls but for 3 other species as well. The spectrum of species susceptible to infection with OHV was, in fact, identical to that of species susceptible to FHV infection.

On the basis of the spectrum of susceptibility of, and lesions produced in mutually tested species a marked similarity between FHV, OHV, and PHV is indicated. The falcon and owl viruses appear to be indistinguishable on the basis of the studies described above. These observed similarities in biological activity are reinforced by the serological work of Purchase et al. (1973) who showed that "... two strains of PHV, the OHV, and the FHV were indistinguishable serologically." The OHV, FHV, and one of the PHV strains studied by Purchase were the same strains used in the present study.

These findings suggest that further investigation of FHV, OHV, and PHV, as well as the viruses of Pacheco's parrot

Table 13. Comparative pathogenicity^a of FHV, OHV, and PHV

| Species | FHV | virus OHV | PHV |
|------------------|-------|--------------|-----|
| kestrel | 13/13 | 1/1 | 2/3 |
| great horned owl | 3/3 | 2/2 | 0/2 |
| red-tailed hawk | 0/4 | 0/2 | 0/2 |
| ring necked dove | 3/3 | 3/3 | 3/3 |
| pigeon | 0/6 | 0/6 | 0/6 |
| budgerigar | 5/5 | 3/3 | 4/6 |

^a Mortality caused by each virus is expressed in the appropriate column by a fraction; the number of birds which died of herpesvirus infection (numerator) over the number inoculated with virus (denominator).

disease and duck plague must be carried out before a comprehensive understanding of their significant interrelationships or dissimilarities can be gained.

SUMMARY

A disease of falcons has been characterized clinically and pathologically and named "inclusion body disease (herpesvirus infection) of falcons" (IBDF). Various aspects of its pathogenetic mechanisms have been elucidated in 13 cases of the disease produced experimentally in falcons.

The pathogenicity of the causative agent has been demonstrated in 14 of 24 avian species and compared with that of 2 other closely related avian herpesviruses.

Inclusion body disease of falcons has a short clinical course of 1 to 4 days characterized by mild to severe depression and weakness sometimes accompanied by anorexia. Weight loss and diarrhea occur inconsistently.

Gross lesions occur in the liver, spleen, bone marrow, and intestinal wall and are light tan foci ranging from pinpoint size to 1.5 mm in diameter. Histologically the lesions are foci of degeneration and necrosis; there is a conspicuous absence of inflammatory cell reaction to the peripheral zones of necrosis. Intranuclear inclusion bodies are most numerous in the liver and less so in other organs.

The disease was diagnosed in 4 prairie falcons (Falco mexicanus), 1 peregrine falcon (F. peregrinus), 1 gyrfalcon (F. rusticolis) and 1 red-headed falcon (F. chiquera).

A herpesvirus isolated from one of the field cases was designated "falcon herpesvirus (FHV) strain S-18." This isolate, grown in avian cell cultures, was used to experi-

mentally reproduce the disease in other falcons and to assess the pathogenicity of FHV in other avian species.

Experimental IBDF was produced in 11 kestrels (F. sparverius), 1 merlin (F. columbarius), and 1 prairie falcon. As in the field cases, a definite affinity of FHV for the liver and reticuloendothelial system was evident. Ultrastructural studies of liver lesions confirmed the presence of typical herpesvirus particles in the nuclei and cytoplasm of degenerating and necrotic cells. Lesions of the experimentally produced disease were considerably smaller, more numerous, and of more uniform size than those of the field cases indicating more simultaneity of nidation of infectious foci than occurred in the latter. This disparity in lesion size is accounted for by all experimental birds having received a higher infective dose of virus and, in most cases, having received it by a more direct route (IM) than the field cases were likely to have experienced.

The prominence of the intestinal lesions in some of the field cases suggests that natural infection in those falcons may have occurred by the oral route. Thus the primary foci of infection were established in the reticuloendothelial cells of the lamina propria of the alimentary tract from which continued dissemination of virus occurred to establish secondary foci of infection in the liver, spleen, and bone marrow.

Leukocytopenia occurred during the later stages of the

experimental disease in falcons as a likely consequence of necrosis of the bone marrow and other reticuloendothelial tissues.

The lesions in all susceptible species were essentially similar and in the owls, doves, psittacine birds, and muscovy ducklings were not able to be definitively differentiated from the lesions of known herpesvirus infections occurring in those species or closely related species.

The comparative pathogenicity of FHV, owl herpesvirus (OHV), and pigeon herpesvirus (PHV), 3 serologically indistinguishable viruses, was studied in 6 species of birds and found to be strikingly similar.

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ACKNOWLEDGEMENTS

The encouragement and patient counsel of Dr. F. K. Ramsey, my major professor during the course of the research and preparation of this manuscript, are gratefully acknowledged.

A note of appreciation is extended to Dr. R. A. Packer, Dr. J. H. Greve, Dr. W. R. Richter, and Dr. S. L. Balloun, the other members of my graduate committee, for their time and advice.

Special thanks is due Dr. C. J. Maré whose continuing enthusiasm, encouragement, council, friendship, and supporting virological expertise are reflected throughout this manuscript.

The technical assistance of Mrs. Alvina Owenson, Mrs. Kathy Brown, Mrs. Larson, Mrs. Jolene Olah, Mrs. Linda Knell, Mrs. Karen Weiss, Mrs. Lois Dille, Mrs. Maureen Rohret, and Mr. James Heminover was greatly appreciated as were the services rendered by Mr. Ron Moses and Mr. Fred Porter in the care of the experimental birds.

Finally, I am grateful to Mrs. Diana Madsen for her patient cooperation in the typing of this manuscript.