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EXPERIMENTAL FELINE INFECTIOUS ENTERITIS
IN THE GERMFREE CAT

DISSERTATION

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the Degree Doctor of Philosophy in the Graduate
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By

Michael William Rohovsky, D.V.M., M.Sc.

The Ohio State University

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Approved by

Richard Griesemer
Adviser
Department of Veterinary
Pathology

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VITA

February 26, 1937 . Born--Youngstown, Ohio

1960 D.V.M., The Ohio State University, Columbus, Ohio

1960-1962 Captain, USAF (VC), NASA Veterinarian for Project Mercury in Europe; Base Veterinarian, 20th Tactical Fighter Wing, RAF Wethersfield, England

1962-1966 Research Associate, Morris Animal Foundation Fellow and NIH Trainee in Comparative Pathology, Department of Veterinary Pathology, The Ohio State University, Columbus, Ohio

1965 M.Sc., The Ohio State University, Columbus, Ohio

PUBLICATIONS

"The Germfree Cat." Lab. Animal Care, Vol. 16, pp. 52-59, 1966.

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FIELDS OF STUDY

Major Field: Veterinary Pathology

Studies in Gnotobiology. Professor Richard A. Griesemer

Studies in Pathology of Viral Diseases. Professor Richard A. Griesemer

Studies in Pathology of Diseases of the Gastrointestinal Tract. Professor Richard A. Griesemer

Studies in Comparative Neuropathology. Professor Adalbert Koestner

Studies in Animal Oncology. Professor Adalbert Koestner
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CHAPTER I

FELINE INFECTIOUS ENTERITIS, A LITERATURE REVIEW

Historical

Feline infectious enteritis has been described under a variety of synonyms since Verge and Cristoforoni (75) experimentally transmitted "cat gastro-enteritis" with a bacteria-free filtrate in 1928. Hindle and Findlay (29) used the name "feline distemper" in 1932, a name which is still used frequently on commercial vaccines. Leasure et al. (45) designated the disease in cats as feline infectious enteritis. Lawrence and Syverton (44) reported their studies in 1938 on the feline disease "spontaneous agranulocytosis." Hammon and Enders (24) used the term "feline malignant panleucopenia" in 1939. All of these diseases are apparently the same, and are caused by a virus which will be referred to in this review as the feline infectious enteritis virus. This designation reflects the most striking histologic lesion of the disease.

Properties of the Virus

The size of the feline infectious enteritis virus has been estimated by filtration to be from 80-100 millimicrons (7). Filtration studies have shown that the virus readily traverses Berkefeld candles of "V", "N", and "W" grades, and Seitz E-K discs. Viability is retained in 50% glycerol suspensions for 138 days at -80 C. The

virus remains viable in decomposing fecal matter for six days. Following freeze-drying the virus retains its infectivity for an indefinite period. Although immunity can be readily conferred after lyophilization, the infectivity is lost. The virus is resistant to heating at 37 C for 48 hours but is destroyed at 56 C for 30 minutes, or at 100 C for one minute (7).

In 1957, Bolin (6) reported the cultivation of feline infectious enteritis virus in trypsinized cat lung, spleen and kidney cell cultures. Cohen et al. (10) in 1961 established that this virus was not feline infectious enteritis virus. They designated it as Bolin's feline virus isolate (FVI).

Pranato (59) using an Indonesian isolate of the virus demonstrated lesions on the chorio-allantoic membranes of eight 10-day-old chicken embryos. At 37-38 C the lesions were fully developed in three days. They were described as white, opaque areas with central necrosis, mesodermal cell-hyperplasia, edema and vascular dilatation. This work has not been confirmed.

Lawrence et al. (42) determined by inoculation of susceptible cats that the feline infectious enteritis virus was not related to the viruses of hog cholera, lymphocytic choriomeningitis, fox encephalitis, vesicular stomatitis, western equine encephalomyelitis, herpes or B virus infection. These viruses did not alter the hemogram and did not cause clinical signs in the inoculated cats.

Burger (8) attempted to demonstrate viral antigen serologically by routine laboratory methods. The antibody could not be detected by complement-fixation, hemagglutination or hemabsorption.

No evidence of cytonecrosis or specific cellular changes were observed after attempts were made to propagate the virus in primary cell cultures of feline kidney, feline bone marrow, feline spleen, canine kidney, raccoon kidney, ferret kidney, and mink kidney. It was not determined whether viral multiplication occurred in these tissue cultures. Viral multiplication did not occur after cultivation in serially propagated cell lines of canine kidney, human synovia (McCoy) and human carcinoma (HeLa).

In 1964, Johnson isolated a herpes-type virus from the spleen of a 9-month-old leopard cub from the Bristol Zoo in England (33). The cub had a disease that resembled feline infectious enteritis both clinically and pathologically. This agent was between 100 and 180 mu in size as determined by gradocol filtration. Exposure to ethyl ether overnight at 4 C reduced the titer in exposed fluids from $10^{5.2}$ to 10^3 tissue culture infective doses. No visible evidence of multiplication occurred in bovine, canine, monkey, human epidermoid cancer, or Hela cell cultures. The agent did not affect chicken embryos, guinea pigs, neonatal and adult mice, or rabbits by either corneal scarification or parenteral inoculation. Hemagglutination and hemadsorption tests at 4 C and room temperature using washed bovine, equine, ovine, avian, guinea pig, and murine erythrocytes were all negative. The agent did not grow on a wide variety of culture media.

A later report by Johnson in 1966 (37) compared properties of the leopard-agent to the herpes virus of feline rhinotracheitis. Treatment of the leopard-agent at room temperature with chloroform

resulted in an increase in titer from $10^{6.2}$ to $10^{6.6}$ tissue culture infective dose 50/ml. A corresponding increase in cytopathogenicity in feline kidney tissue cultures was also noted after this treatment. Overnight treatment with ether at 4 C produced a decrease in titer of 0.2 log from $10^{5.8}$ TCID 50/ml. This drop in titer was of less magnitude than Johnson's earlier report in 1964. The virus retained its titer of 10^6 TCID 50/ml after acidification to pH 3 with 1 N HCl for three hours. At pH 9, the titer was not significantly altered. No loss in infectivity was observed at 50 C for one hour although the infectivity was completely lost between 80-85 C for 30 minutes. A potentiation of titer from 10^5 to $10^{5.5}$ TCID 50/ml occurred after incubation for one hour at 37 C with 0.1% crystalline trypsin. After three weeks of exposure to 0.5% phenol in media 199, the infectivity of the virus was not lost. The virus resisted 0.1% formalin for 2 days and 0.05% formalin for 3 days but was completely inactivated after 24 hours in 0.2% formalin. The infectivity of the suspension was lost in the range of 100 mu to 80 mu average pore diameter with graded collodion membranes. In the presence of 5-bromodeoxy-uridine, there was a retardation of the cytopathic effect which suggests that the agent is a DNA virus. This observation was further strengthened by positive feulgen and acridine orange staining for DNA. Johnson concluded that the virus was not in the Herpes group. He postulated that it had properties suggestive of either the adenovirus or papova virus group.

In 1965, King and Croghan (41) reported the isolation of feline panleukopenia virus in cell culture and the identification of the virus by immunofluorescent techniques. This procedure provided the first in vitro system for propagation and quantitation of this virus. Primary feline kidney cell cultures were used with a direct immunofluorescence method for identification of the virus. This tissue culture system was subsequently used in a serum neutralization test. Tissue culture cells were classified as being negative for neutralization if they had typical cellular fluorescence. On the other hand, neutralization occurred if the tissue culture cells did not exhibit fluorescence. The virus which was used in this system also produced a cytopathic effect in the feline kidney culture cells although this CPE was inconsistent.

A cytopathic effect on feline kidney culture cells had been mentioned by Johnson in his original report of 1964 (33). The cytopathic changes in the monolayers were slight and disappeared rapidly. They could be detected in material that had been stained with hematoxylin and eosin. Nuclear changes included condensation of chromatin and the presence of a halo around the nucleolus. These changes became evident in occasional cells within 24 to 72 hours after inoculation of the cultures. The inclusion masses became deeply basophilic and disappeared within three days. Occasionally the inclusions would take an eosinophilic stain but only in the later stages of incubation. The numbers of affected cells varied considerably with the type of media, the serum used, and the age of the primary monolayer.

Johnson published a second report in 1965 concerning the cytopathic effects of his virus isolate in feline kidney monolayers (36). The CPE involved less than 5% of the cells at one time. The results essentially confirmed his earlier published report.

Lust et al. in 1965 reported the occurrence of intranuclear inclusions in feline kidney cell cultures inoculated with the same virus strain (Philips Roxane) which had been used by King (47). These intranuclear inclusions were similar to those reported for the Johnson isolate. The first specific evidence of cellular degeneration was seen on post-inoculation day (PID) 3. The nuclei became swollen and the chromatin became darker and more granular. Early inclusion bodies were diffuse and granular but they subsequently became larger and more dense. A surrounding halo was a feature of this change within the nucleus. As cellular degeneration progressed, the cytoplasm of these cells retracted and became stringy. In the final stages of degeneration, the cells became pyknotic and terminally detached from the coverslips. DNA content of the inclusion bodies was suggested by a positive feulgen and acridine orange reaction. The number of inclusion bodies was directly proportional to the titer of the virus. These workers confirmed the fluorescence which King et al. had reported.

In a later publication in 1966, Gorham et al. (22) reported their ability to recognize CPE with the Philips Roxane strain in unstained culture preparations.

There are no unequivocal reports in the literature concerning the ultrastructure of the virus with electron microscopy. Johnson in

1965 reported spherical structures resembling virus particles in bone marrow cells, hepatic cells, lymphocytic cells, and epithelial cells from the crypts of the small intestine (32). These particles were limited by a single membrane and measured 150 mu to 280 mu in diameter. They contained an inner spherical core which was 60 to 160 mu in diameter. These structures, however, were never demonstrated in the nucleus but only in the cytoplasm. He demonstrated intranuclear filamentous aggregates but they did not contain any virus-like particles. These inclusion bodies contained material which could not be distinguished from strands of chromatin in the halo area surrounding the inclusion. The histochemical properties of the inclusion bodies in the liver differed from those in the other tissues. This evidence suggests that more than one virus could have been present.

Species Susceptibility

Torres (74) reported that a disease, similar to feline infectious enteritis, affected wild felines in the Zoological Garden of Recife City in Brazil. Cockburn (9) described the disease in the wild cats of the Zoological London Regent's Park. Goss (23) has stated that all species of the family Felidae are susceptible as well as the raccoon from the family Procyonidae. Infectious gastroenteritis in raccoons had been previously documented by Waller (76). Sick raccoons recovered after administration of anti-feline infectious enteritis serum. Active immunity can be demonstrated in the raccoon following injection of commercial feline infectious enteritis vaccine.

Another member of the raccoon family, the coati-mundi, has also been reported to be affected by the disease. Miller's report concerning "distemper in the coati-mundi" appeared in 1961 (53).

Cross immunization between feline infectious enteritis and mink virus enteritis has been accomplished in cats, minks, and raccoons (7, 8). The clinical signs and pathologic changes in these two diseases are similar in their natural host species. Minks can be immunized against mink virus enteritis by subcutaneous or oral inoculation with feline infectious enteritis virus or commercial inactivated vaccine (21, 60). Cats can be immunized against feline infectious enteritis with either mink enteritis virus or commercial inactivated mink virus enteritis vaccine. The feline infectious enteritis virus does not acquire pathogenicity for mink, even after serial passage in mink virus enteritis-susceptible animals. Although the two viruses have a close immunologic relationship, they currently are regarded as two distinct viral entities, since neither can produce the corresponding disease in the other species (7).

Hammon et al. (24) failed to produce any clinical signs of disease after inoculating rabbits, guinea pigs, ferrets, and mice. Hindle et al. (29) tried to adapt the virus to dogs, rats, mice, rabbits, guinea pigs, ferrets, and mongooses without success. Syverton (73) used serial and alternate passage techniques in rabbits, guinea pigs, ferrets, suckling mice, and ground squirrels. Although he used intracerebral, intranasal, and intraperitoneal inoculations, no viral multiplication was demonstrated.

Transmission

Feline infectious enteritis can be transmitted by many routes of inoculation. Hindle and Findlay (29) experimentally infected cats by the intranasal spraying of an aerosol, and by intracerebral, subcutaneous, and intraperitoneal inoculation. They also proved that cats are readily infected by contact and by fomites. Syverton et al. (73) confirmed these results and also demonstrated that infection occurred after ingestion of the virus. They determined that blood, most tissues and organs, nasal secretions, feces, and urine were infective. The virus could be recovered 48 hours after inoculation during the incubation period and also during the course of clinical illness. At the British National Institute for Medical Research, isolation techniques which had proved to be sufficient to prevent the spread of canine distemper in dogs and ferrets, failed to prevent the spread of feline infectious enteritis (19). Hammon and Enders (24) suggested the possibility of an arthropod vector. Torres (74) collected fleas from a diseased wild cat, washed them in 5% cresyl solution for 10 minutes, and saline twice. He prepared an emulsion, passed it through a Seitz EK-A filter, and inoculated young, healthy kittens with the filtrate. The kittens died from a disease compatible with feline infectious enteritis. At Mill Hill in London, the idea was pursued that the virus could be carried in Ascaris sp. eggs, but the results were inconclusive (19).

Active and Passive Immunity

Enders and Hammon (14) suggested that the resistance of naturally immune female cats is transferred to the offspring. Kittens suckling immune mothers were exposed to heavy doses of the virus but did not develop signs of the disease. The explanation was considered to be that sufficient maternal antibody was acquired in utero and through the colostrum to enable kittens to resist natural infections.

Little is known about passive maternal immunity in kittens. Harding et al. (28) immunized queens against Salmonella montevideo in the last third of pregnancy. They determined that the queen's titer reached a peak of 1:3000 before parturition and kept this level during the first week postpartum. Prior to nursing, the newborn kittens had titers against S. montevideo of 1:200. Following nursing this titer reached 1:1000 where it was maintained for the first week of life. Harding concluded therefore, that the in utero transfer of antibodies equaled about 20% of the total concentration of antibodies in a colostrum-fed kitten. By age one month, the serum titer of the kittens had dropped to 1:50.

Miller-Ben Shaul (70) studied the immune response of neonatal cats in 1965. He found that the immunological response of the neonatal kitten to soluble antigen varies with the antigen. Even though antibodies were produced against bovine fibrinogen and human fibrinogen, immunological tolerance followed injection of bovine serum albumin, human gamma globulin, and egg albumin. In addition,

antibodies were produced to the flagella of Salmonella typhi. Two of six kittens tested developed antibodies against rat collagen.

No antibodies were demonstrated in the neonatal kittens following oral administration of human and bovine fibrinogen. These antigens are apparently not absorbed from the gastrointestinal tract. Twenty-five to 30 days appears to be the maximum period during which immunological tolerance can be induced in the neonatal kitten. The significance of this information in relationship to either active or passive immunity to feline infectious enteritis in the suckling kitten remains to be determined. Gorham feels that young kittens may retain some degree of passive immunity to the virus until 16 weeks of age (personal communication, 1966).

Dalling (13) stated that kittens, regardless of age, were frequently not susceptible to feline infectious enteritis virus when obtained through ordinary sources. He claimed that cats could be immunized with formalin-inactivated virus. Enders and Hammon (14) indicated that formalized virus conferred active immunity. They also passively immunized cats with serum from convalescent animals. These results were confirmed by Lawrence et al. (42). Burger protected experimental cats against exposure to virulent virus by administration of inactivated mink virus enteritis vaccine containing an adjuvant (8).

Clinical Signs

The clinical signs associated with feline infectious enteritis are variable. Generally speaking, younger cats are

affected more frequently although the disease does occur occasionally in older animals. The morbidity rates of various age groups of cats are undoubtedly a reflection of the degree of passive immunity conferred upon the kitten. The possibility also exists that active immunization occurs during the period when the kitten has high levels of antibody from the queen.

Other important factors in the consideration of morbidity and mortality are the virulence of the virus strain and/or the co-existence of other feline viruses. In 1961 Bittle et al. reported a comparative study of feline cytopathogenic viruses and feline panleucopenia virus (4). They demonstrated an apparent synergistic effect in dual infections with Bolin's isolate and infectious enteritis virus. The mortality was increased from 10% to 83%. The role of concomitant virus infections in spontaneous cases of feline infectious enteritis has not been critically studied.

Jubb and Kennedy (39) agree with other authors that the disease has world wide distribution. The many synonyms of this disease reflect the inconsistency in use of the name for this condition.

In 1949 Bentinck-Smith reviewed 574 cases of feline infectious enteritis from the files of the Angell Memorial Hospital (3). This review covered the period from 1946-1948. A white blood count that was diagnostic of the disease was available in only 192 of these cases. The other cases were diagnosed on the basis of the clinicians opinion. It is the only report in the literature, however, which attempts to document the clinical signs of a large number

of spontaneous cases. Briefly, the most characteristic signs were reported as follows: anorexia 80.1%, emesis 67.9%, prostration 50.7%, diarrhea 24.0%, dehydration 7.8%, dyspnea 3.1%, and painful abdomen 1.2%. In the Boston area, the disease was enzootic in July, August, and early September. Riser reported an epizootic in Iowa in August (61). Schnelle's results showed two peaks in seasonal incidence; the first in the winter months of February and March and the second in the summer months of July and August (67).

Leasure et al. also attempted to classify the signs of feline infectious enteritis (45). Consistently observed clinical signs included pyrexia, anorexia, depression, unkempt hair coat, and emaciation. Clinical signs that were less frequently observed included emesis, diarrhea, conjunctival or nasal discharge, ataxia, painful abdomen, and recumbency.

There is a difference between the reported incubation periods of the experimentally induced and naturally occurring diseases, the former being shorter. A range of from 3-15 days for the naturally occurring disease is not uncommon although most authors agree on a range of 4-8 days. In 1935 Apple reported an incubation period of 12 hours in cats that were experimentally infected (1). This is questionable, however, since adequate measures had not been taken to insure that the cats had not already been in the incubation stage of the disease at the time of inoculation.

Frequently a diphasic rectal temperature curve is manifested with the initial rise to 103 F and the secondary rise reaching 105 F. Cats in the terminal stages of the disease have subnormal

temperatures. An abrupt onset of illness usually occurs with anorexia and weakness developing rapidly. Vomiting of bile-tinged mucus in small quantities often occurs. Diarrhea occurs only in cases of longer duration where secondary bacterial invaders may play an important role. Diarrhea is associated most often with the spontaneous disease. Severe loss of weight and dehydration are soon followed by profound depression. The animals maintain no interest in their surroundings and assume a prone position on their abdomen. Coryza and lacrimation are not commonly observed. Sudden death may occur in 2-3 days, preceded by fibrillary twitching and possibly terminating in clonic convulsions. The duration is usually 3-7 days with an average of 4 days. Animals that survive may undergo a long recovery period due to their debilitated condition.

The most striking clinico-pathologic manifestation occurs in the hemogram. Hammon et al. reported a fulminating panleucopenia within 1 or 2 days after the onset of clinical signs (25). From this evidence they proposed the name of malignant panleucopenia to designate the disease. Lawrence et al., on the other hand, noted the striking neutropenia which occurred with an associated relative lymphocytosis (43). Consequently they designated the disease, infectious agranulocytosis. Jubb and Kennedy agree that the neutrophil is affected first (39). Other reports are conflicting concerning the specific leukocyte which is most severely depleted. Most authors report, however, that the earliest significant decrease is usually in the lymphocytes. By the fourth day following inoculation, the leucocytes have almost completely disappeared from the peripheral

circulating blood. Both a precipitous drop in the white blood cell count and a more gradual decrease have been reported (25, 43). Riser believed that in experimental cases, the leucopenia was most severe at the height of the fever (63). White blood cell counts of less than $500/\text{mm}^3$ have been reported. Usually a range of 1,000 to $3,000/\text{mm}^3$ is present at the time of death.

Johnson's 25 experimental cases had white blood cell counts of less than $5,000/\text{mm}^3$ at the height of the disease (32). He also observed a typical "differential shift" in all of his cases. This shift consisted of a relative lymphopenia and neutropenia followed by a relative lymphocytosis and neutropenia at the height of the disease.

The reports of both Hammon et al. and Riser indicate that some degree of anemia coexists with the panleucopenia (25, 63). Hammon and Enders felt that the decline of erythrocytes occurred as a result of increased destruction of erythrocytes as well as decreased erythropoiesis. Riser showed in chart form that the decline in erythrocytes paralleled that of the leucocytes. Both of these reports vary from that of Lawrence and Syverton who demonstrated no decrease in the erythrocyte count (44). Johnson's results indicate a variable but slight decline in the total erythrocyte count (32).

Hammon and Enders reported a cat with panleucopenia in which there was thrombocytopenia (25). Lawrence and Syverton felt that there was no demonstrable change in the platelet count (44). Johnson indicated a variable decrease in platelets (32). Other authors have not made any definitive statements regarding the platelet count.

The mortality rates which are reported in the literature reflect a wide range of variation. Some of these reports include: Riser, 80% mortality; Lawrence, Syverton et al., 66%; Hagen, 65%; Arlein, 64%; and Angell Memorial Animal Hospital records 53% (3). In regarding the validity of these figures, one must consider the susceptibility of the population in question, the strain of virus, and the possible concomitant infection with other feline viruses.

Macroscopic Lesions

At necropsy, the animal may be emaciated and extremely dehydrated. Mucopurulent conjunctivitis has been reported but this lesion is probably a result of the effects of another feline virus (61). The hair coat may be rough, dry, and unkempt. Cats usually stop grooming themselves before the height of clinical disease and do not resume grooming until late into the recovery period. If the animal has had diarrhea, the tail and perineal region may be stained with feces.

The gastrointestinal tract usually has grossly observable lesions. The subserosal vessels are hyperemic. Diffuse congestion of the submucosal vessels may be striking. The areas of congestion are usually more extensive in the terminal portion of the ileum. In addition, the jejunum may also be involved. The stomach contains a small amount of bile-stained mucous material. Depending upon the duration of the anorexia, the remainder of the intestinal tract is empty. A pseudomembranous, fibrinous exudate, however, may be within the lumen of the small intestine. Hemorrhagic enteritis with or without grossly visible ulcerations has also been reported (62).

Many reports have been made concerning the gross changes in the mesenteric lymph nodes which are usually enlarged, edematous and hemorrhagic. The cut surface of these lymph nodes bulges from the capsule. Johnson felt that the gross lesions of the mesenteric lymph nodes were the most consistent gross lesions associated with the disease (32).

Gross lesions are inconsistently found in the bone marrow (32, 54). In some cases, it may be replaced by a greasy, semi-liquid material. The color may vary from dark red to pale pink. The marrow often has a fatty appearance. Jubb and Kennedy do not attribute any diagnostic significance to the color of the bone marrow (39).

Microscopic Lesions

Histological lesions in the intestinal tract are found in the majority of the cases. However, several authors have reported the absence of lesions in the intestine (32, 43, 54). In general, however, the intestinal lesion is the most striking one associated with the disease. The small intestine is characterized by lesions of various degrees of severity with the most severe lesions in the terminal ileum. Petechial hemorrhages and congestion are located within the lamina propria and occasionally the submucosa. Goblet cells are hyperplastic and distended with mucus. Various stages of degeneration, from cloudy swelling to coagulation necrosis occur in the epithelial cells lining the crypts. The number of mitotic figures is increased in the glandular epithelium. Necrotic epithelial cells are desquamated and cause distention of the crypts.

As the disease progresses, the number of goblet cells decreases. The erosion of the epithelium covering the villi results in shortened, thickened villi. The lamina propria and submucosa become edematous and are infiltrated by lymphocytes and macrophages. The lack of a severe cellular inflammatory response is striking, especially since extremely large numbers of bacteria are usually found in the lumen of the intestine adjacent to the altered intestinal villi. Strands of fibrin and other detritus may also be found within the lumen.

Intranuclear inclusion bodies are considered by some to be pathognomonic for feline infectious enteritis (30). The reported frequency of occurrence of these inclusion bodies is quite variable. Most authors agree that inclusion bodies occur in the gastrointestinal mucosa (13, 24, 30, 40, 44, 46). They have been described as granular, eosinophilic, intranuclear masses in cells deep in the crypts that have vesicular nuclei and a peripheral margination of chromatin. Indeed, Lucas and Riser classified these inclusions into two separate categories, granular and homogeneous (46). The granular inclusions were further subdivided into diffuse-granular and clustered-granular. The diffuse-granular inclusion was reported to be less common than the clustered-granular. Lucas considered the clustered-granular inclusions to be similar to Cowdry's type A and the same as the inclusions reported by Hammon and Enders, by Lawrence and Syverton, and by Kikuth et al. The homogeneous inclusions were classified with Cowdry's type B inclusions.

On the other hand, other authors have reported that inclusion bodies did not exist or were merely the result of enlarged, swollen nucleoli. In Newberne's experimental series of 25 cats, only two intestinal epithelial cells in a single cat had intranuclear masses which resembled type A inclusion bodies (54). He believed that the presence of inclusion bodies should not be considered a consistent part of the histopathologic criterion for identification of feline infectious enteritis. Jubb and Kennedy refer to the presence of intranuclear conglomerates of granules within intestinal epithelial cells but refrain from using the term inclusion body (39).

The lymphopoietic tissue also is uniformly affected during this disease. Microscopic lesions have been observed in the tonsils, spleen, lymph nodes, and Peyer's patches (25, 30, 43, 54, 62, 66). The lymphocytic tissue becomes enlarged due to an increase in reticulo-endothelial cells. The lymphoid follicles eventually undergo depletion and necrosis of lymphocytes. Large reactive centers containing reticulo-endothelial cells and cellular debris become prominent in the primary lymphoid follicles. The sinusoids of the lymph nodes become dilated and contain large macrophages filled with cellular debris. Erythrophagia may be evident. Congestion and edema are apparent in both the cortex and medulla. These lesions are the most consistent in the mesenteric lymph nodes. In the small intestine, the Peyers patches show similar changes but the Peyers patches within the colon and cecum are not so severely affected.

The spleen occasionally contains small, subcapsular hemorrhages. Hyperemia and edema are conspicuous throughout the parenchyma. The reticulo-endothelial cells are hyperplastic and prominent within the white pulp. The lymphoid follicles are similar to those described within the lymph nodes. The same controversy concerning eosinophilic intranuclear inclusion bodies within lymphocytes and reticulum cells of the lymphopoietic system occurs here as in the intestinal tract.

The bone marrow is the third area with the most consistently reported lesions. Hammon and Enders and Lawrence et al. reported changes in the bone marrow (24, 43). Bone marrow aplasia is severe with a decrease in the cellular elements, vacuolation, and replacement by adipose connective tissue. Most of the cells within the reticular framework are from the erythrocytic series with an occasional megakaryocyte. Areas of congestion or hemorrhage are present with erythrophagia by large macrophages. All cats do not show the same degree of aplasia. The aplastic appearance is primarily due to a decline or loss of all forms of cells comprising the myeloid system. Lawrence and Syverton reported that the erythroid elements persisted throughout the course of the disease (43). Hammon and Enders considered that hypoplasia of the erythroid system accompanied that of the myeloid system (24). Again, the presence and significance of inclusion bodies within cells of the bone marrow are controversial.

The liver has also been implicated as a site for microscopic lesions (62). Some degree of dissociation of hepatic cords has been

reported. Johnson reported inclusion bodies within hepatic cells which differed histochemically from those found in intestinal epithelium and bone marrow (32).

Electron Microscopy

Johnson's dissertation is the only study which concerns the electron microscopy of feline infectious enteritis (32). He used the tissues from 11 experimentally infected cats in an effort to resolve the question of the viral etiology of the inclusion bodies.

In the epithelium of the small intestine, there were morphologic alterations of mitochondria, increased numbers of lysosomes and multivesicular bodies. Myelin-like whorls often were found within the lysosomes. There was a smudgy appearance to the cristae of some of the mitochondria. These ultrastructural changes were very severe 5-10 days post-inoculation. In degenerating cells, the nuclear chromatin was clumped near the nuclear membranes leaving a relatively electron-translucent area in the center of the nucleus. Various degrees of flattening occurred in goblet cells, argentaffin cells, and columnar cells. In these cells the long axes were parallel to the basement membranes, whereas they were perpendicular in normal cells. Villi that were devoid of an epithelial covering were never visualized by the electron microscope. A severe reduction occurred in the number of microvilli per linear unit of the lumenar surface of the epithelial mucosa. Prominent, enlarged nucleoli were a feature of many crypt cells.

Many phagocytic cells with prominent lysosomes and digestive vacuoles were present in the lamina propria. The capillaries and arterioles were very prominent.

An attempt was made to find virus-like particles and intranuclear inclusions in osmium-fixed sections of bone marrow, ileocecal lymph node, and intestinal epithelial cells. Changes suggestive of inclusion body formation were found in only 2 of the 11 cats studied. The nucleus of only one epithelial cell in a crypt in each of 2 kittens had structures suggestive of inclusions. These nuclei contained filaments arranged into distinct aggregates. They were more osmiophilic than the surrounding chromatin except for dense clumps of chromatin which were just as osmiophilic as the filamentous aggregates. Nothing resembling virus particles was found in these filaments.

Virus-like particles were observed in the cytoplasm of occasional bone marrow cells, lymphatic cells, and crypt-epithelial cells. They were usually singular rather than in aggregates. These particles were spherical, measured 150-280 mu in diameter, and were enclosed by a single membrane. A relatively clear zone separated the outer membrane from an osmiophilic core measuring 60-160 mu in diameter. The particles were within the cytoplasm of the affected cells but they were not in association with any organelles or the cytoplasmic membrane.

Intranuclear inclusion bodies were also found in two hepatocytes but they were histochemically different from those observed elsewhere. These inclusions were probably composed of chromatin since they could not be differentiated from chromatin by the electron microscope.

CHAPTER II

FELINE INFECTIOUS ENTERITIS IN THE GERMFREE CAT

Introduction

The incidence and significance of coinfection with different feline viruses in the domestic cat have never been fully evaluated. Crandell et al. (4) isolated 31 cytopathic agents from the nasopharyngeal region of cats in feline kidney cell cultures. In most instances, the carriers of these feline respiratory isolates were not exhibiting signs of clinical illness. Bittle et al. (2) recovered an additional group of 17 feline viruses from cats with clinical signs of rhinitis and conjunctivitis. One agent was obtained from the spleen of a cat which was believed to have had feline enteritis. Neutralization tests of 12 of these viruses and 11 viruses supplied by other investigators showed that 8 shared common antigens. The 15 remaining viruses were different serologic types. A serologic field study revealed neutralizing antibody for several of the viruses in the sera of 40-50% of young cats which were tested. In adult cats, the incidence of significant antibody titers reached 70-90%.

Bittle et al. (3) studied 3 viruses (FPL, F-20, and KCD) which were isolated from the spleens of cats and were cytopathogenic in feline kidney cell cultures. They compared the disease-producing potential and antigenic relationship of these 3 viruses to each other

and to panleucopenia (feline infectious enteritis) virus. The mortality rate from panleucopenia virus alone was 10%. Panleucopenia virus inoculated simultaneously with FPL virus produced a serious disease which was comparable to that seen in some epizootics of panleucopenia. The mortality rate from the dual infection rose to 82%. From this evidence, there is a definite possibility that concomitant infection with one or more viruses may significantly modify the pathogenesis and course of both diseases.

With the development of methods to raise germfree cats by Rohovsky et al. (18), feline infectious enteritis could be studied without coexisting infections by viruses and other microorganisms. The objectives of this study were to: (1) determine the effects of feline infectious enteritis virus in germfree cats and (2) compare the lesions produced in germfree cats with those reported for the disease in conventional cats.

Materials and Methods

The methods of raising germfree cats have been described (18). Pregnant queens were obtained from the specific pathogen-free colony of cats which is maintained by the Department of Veterinary Pathology, The Ohio State University. The kittens were removed by hysterectomy and passed through a tank filled with a disinfectant into flexible plastic isolators. The diet consisted of an infant milk replacer which was supplemented with amino acids and multiple B vitamins. A sterile solid food was subsequently added to this diet after approximately 3 weeks of age. Sterility was determined

by culturing aliquots of the diet, fecal material, and urine at weekly intervals and at the times when supplies were taken into the isolator. The culture media consisted of thioglycollate broth and tryptose agar plus 5% defibrinated equine blood. Incubations were made at 22 C, 37 C, and 57 C in aerobic and anaerobic environments. Samples of lung, spleen, and colon were routinely cultured at the necropsy of each animal. Media for mycobacteria and mycoplasma were also used. Wet mounts of feces for protozoa, helminths, and motile bacteria were examined. The presence of viruses was investigated by microscopic examination of tissues and by the inoculation of feline kidney cultures with suspensions of spleen.

The feline infectious enteritis stock virus was obtained from Dr. John Gorham, College of Veterinary Medicine, Washington State University, Pullman, Washington. It had been originally isolated from pooled tissues from 4 cats with naturally occurring feline infectious enteritis and passaged 12 times through susceptible cats. The inoculum consisted of a mixture of ground mesenteric lymph node, ileum, colon, and spleen with 5% glycerin and antibiotics. Upon arrival the material was inoculated into feline kidney cell cultures. No cytopathic effect was seen. When injected into a conventional cat intraperitoneally, the cat developed feline infectious enteritis. The spleen was harvested aseptically and ground into a 10% suspension with Hank's solution and antibiotics. Aliquots of this splenic suspension were inoculated into feline kidney cell cultures and were tested for sterility. No cytopathic effect was seen in tissue culture and the aliquots were bacteriologically sterile. Aliquots were also inoculated intraperitoneally

into four conventional cats. All of them developed signs of feline infectious enteritis and died or were moribund by the seventh day after inoculation. Characteristic lesions of feline infectious enteritis were demonstrated microscopically.

A clinically healthy, 5-month-old, male cat taken from the specific pathogen-free breeding colony was used as a source of inoculum for the control animals. The spleen was harvested aseptically and ground into a 10% suspension with Hank's solution and antibiotics. Aliquots were inoculated into feline kidney cell cultures and no cytopathic effects occurred. When cultured the splenic suspension was bacteriologically sterile. Intraperitoneal injections of this suspension into conventional animals produced no signs or lesions.

Nineteen germfree kittens ranging in age from 19 to 24 weeks were used in this experiment. Of these, 14 kittens were injected with the feline infectious enteritis splenic suspension and 5 with the control splenic suspension.

During the baseline period and following inoculation intraperitoneally of the respective suspensions of spleen, daily physical examinations of all animals were performed. These included the recording of rectal temperatures five times daily, food and water consumption, appearance of feces, and the general appearance of the animals.

Blood samples were obtained by cardiac puncture during both the baseline and experimental periods and the following determinations were made: hemoglobin by the oxyhemoglobin method, packed

cell volume by the microhematocrit method, total erythrocyte and leucocyte counts, and differential leucocyte counts. Bone marrow smears were made from the proximal femur at the time of necropsy for differential counts.

Euthanasia was performed with pentobarbital sodium at intervals representing the various stages of disease (Table 1). Sections of the following tissues were fixed in 10% phosphate-buffered formalin and examined microscopically: all lobes of the lung, trachea, bronchial lymph node, tonsil, salivary gland, mandibular lymph node, tongue, esophagus, planum nasale, turbinate, conjunctiva, upper eyelid, membrana nictitans, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph node, pancreas, liver, spleen, kidney, ureter, urinary bladder, testis or ovary, uterus, thyroid, parathyroid, thymus, adrenal, pituitary, brain, rib at costochondral junction, psoas muscle, diaphragm, myocardium, aorta, and femur. The eyes were fixed in Zenkers fluid with acetic acid for one hour, diluted with an equal volume of distilled water for 2 more hours, washed overnight in running tap water, and placed in 70% alcohol for 24 hours. The tissues were embedded in paraffin and sectioned at 6 microns. Transverse sections of the brain were examined at the level of the basal ganglia, thalamus and optic chiasm, mesencephalon and hippocampus. Sections were also cut through the pons and cerebellum, medulla oblongata, anterior cervical cord, thoracic cord, and lumbar enlargement.

Schorr's triple stain as modified by Foot and Giemsa stain were used on selected sections in addition to hematoxylin and eosin.

Histochemical procedures used on lymphocytic tissues included the Feulgen reaction and the flurochrome acridine orange staining of frozen cryostat sections at a concentration of .01 for 12 minutes.

TABLE 1
THYMUS-BODY WEIGHT RATIO OF GERMFREE CATS WITH FELINE
INFECTIOUS ENTERITIS

Number	Sex	Age (weeks)	Euthanasia (PID)	Body Wgt. (gm)	Thymus Wgt. (gm)	Thymus/Body Wgt. %
<u>CONTROL</u>						
K1895	M	22	3	2142	6	0.28
K1896	Fe	22	4	2368	10	0.42
K1894	M	22	5	2868	9	0.31
J1624	M	20	7	3200	15	0.46
J1741	M	24	14	3680	20	0.54
<u>EXPERIMENTAL</u>						
K1836	M	22	1	3360	14	0.42
K1083	Fe	19	3	2202	8	0.36
K1837	M	22	3	3236	9	0.28
K1835	M	22	4	2476	6	0.24
K1085	Fe	19	5	1938	4	0.20
K1146	Fe	19	5	2326	6	0.25
K1840	M	21	5	2860	4	0.14
K 935	Fe	24	5	2354	4	0.17
K1147	Fe	19	6	1810	3	0.16
K1148	Fe	19	7	1800	1	0.06
K 934	Fe	24	7	2746	2	0.07
K1627	Fe	24	8	2480	2	0.08
K1839	M	21	10	2100	2	0.09
K1838	M	21	12	2290	2	0.08

Results

Clinical signs

All of the animals injected with the viral suspension had significant rises in temperature above that of the baseline period (Fig. 1). Two distinct fever peaks occurred during the course of the

Fig. 1. The rectal temperatures of 14 germfree cats inoculated with feline infectious enteritis virus. The heavy black bar and dotted line in Figures 1-4 represent the pre-inoculation standard deviation and mean values for all 19 germfree cats. The mean rectal temperatures of the five control germfree cats remained within one standard deviation of the pre-inoculation period throughout the course of the experiment. Note the peaks of fever which occurred on post-inoculation days 2 and 4.

Fig. 1

disease. The first peak was evident on post-inoculation day (PID) 2, followed by the second peak which occurred 32 hours later. The temperatures did not return to the baseline recording during the interval between the two peaks. The mean value of the temperature peaks was only 1.5 F above the mean value of the baseline period but four cats had individual temperature rises above 104 F. By PID 6, the temperatures of the experimental group had returned to the baseline values. No later rise in temperature occurred. The temperatures of the control group remained within the range of the baseline value throughout the course of the experiment.

Following inoculation of the virus, the appetites of the experimental group were not affected until the middle of PID 2. At this time the animals consumed only 50% of the expected food intake which was offered to them. By PID 4 the cats refused to eat completely. Water consumption was also decreased during this period but some water was always consumed at each feeding period. Coincidentally with the loss of appetite, the cats also stopped grooming themselves. Their hair coats were no longer sleek and shiny but rough and coarse. Hair was shed from their coats in strands and tufts. The legs and tail became matted with dried waste material and the cats made no attempt to clean themselves. Their attitude changed from alertness and playfulness to listlessness. From PID 3 to PID 5, the cats preferred to lie on the cage floor and remained inactive. By PID 5, weight loss was noticeable. The abdomens appeared tucked up and there was a decrease in the palpable subcutaneous fat. The skin was no longer supple. The ribs

became prominent and the eyes slightly sunken in their orbits. Starting on PID 6, however, the appetite gradually returned and the cats began grooming themselves again. By PID 8 they were again playful and alert and their appetites were back to the baseline level. They began to regain the weight lost. By PID 9, the remaining experimental cats could no longer be distinguished from the control cats so far as behavior was concerned. At no time during the observation period was diarrhea noted. Neither conjunctivitis nor nasal discharge occurred in any of the cats. None of the inoculated cats died.

The total leucocyte count decreased significantly on PID 1 (Fig. 2). The lowest mean value of 2250 per mm^3 was recorded on PID 4. The leucopenia remained at a significantly low level but the total leucocyte count began to rise again by PID 6. At PID 10, the leucocyte count was again within one standard deviation of the recorded baseline value. The leucopenia was due primarily to a severe absolute lymphopenia (Figs. 3 and 4). The total leucocyte count and total lymphocyte count almost paralleled each other. By PID 10, however, the lymphocyte count was still significantly lower than the value of the baseline period. An absolute neutropenia occurred later in the course of the disease than the absolute lymphopenia (Fig. 3). The total neutrophil count was not significantly decreased until PID 4. It remained low through PID 8 and was again within one standard deviation of the baseline value at PID 10.

Fig. 2. The total circulating leucocyte counts of 14 germfree cats inoculated with feline infectious enteritis virus. The mean total leucocyte count of the five germfree controls remained within one standard deviation of the pre-inoculation period throughout the course of the experiment. Note the severe leucopenia.

Fig. 3. Absolute neutrophil counts of 14 germfree cats inoculated with feline infectious enteritis virus. The mean total neutrophil count of the five control germfree cats remained within one standard deviation of the pre-inoculation period throughout the course of the experiment. Note the lag in the appearance of neutropenia compared with that of the lymphocytes in Figure 4.

Fig.2

Fig.3

Fig. 4. Severe lymphopenia in 14 germfree cats inoculated with feline infectious enteritis virus. The mean total lymphocyte count of the five germfree controls remained within one standard deviation of the pre-inoculation period throughout the course of the experiment. The leucopenia (Fig. 2) is due primarily to lymphopenia.

Fig. 4

It should be noted that the total number of lymphocytes far exceeds the total number of neutrophils in the germfree cat. The absolute eosinophil count of the experimental group did not significantly differ from the pre-inoculation value which consisted of a mean of 787 and a standard deviation of ± 314 . The mean erythrocyte and hemoglobin values of the two groups showed no significant differences.

Macroscopic lesions

Only one gross lesion was consistently present in the experimental group (Fig. 5). The thymus was appreciably reduced in size and weight after PID 4 (Table 1). Occasionally the interlobular spaces were filled with yellowish, gelatinous material. Tiny white foci could also be seen on the surface of the thymic lobules below the capsule. The decrease in weight became more severe as the course of the disease progressed. By PID 7, the thymus weighed only 2 grams or less.

Cats which were necropsied from PID 3 to 5 had mild to moderate congestion of the mesenteric vessels on the serosal surface of the intestine. This lesion was inconsistent, however, and was never spectacular. It did not occur in the control group of cats.

Intestinal parasites were not found in any of the germfree cats at necropsy. The lack of any gross evidence of enteritis in the experimental group was noteworthy (Figs. 6 and 7).

Fig. 5. Thymic atrophy (top) in a germfree cat (K 1627) eight days after inoculation of feline infectious enteritis virus, compared with the thymus (bottom) of a control germfree cat (J 1741) of the same age.

Fig. 5

Fig. 6. Photograph of the unfixed mucosal surface of the ileum of a germfree cat 12 days after inoculation with feline infectious enteritis virus (K 1838). Note the normal pattern of the intestinal villi. X 50.

Fig. 7. Photograph of the mucosal surface of the ileum of a conventional cat six days after inoculation with feline infectious enteritis virus (K 1476). The intestinal villi are completely eroded. Compare with Figure 6. X 50.

Fig. 6

Fig. 7

Microscopic lesions

The microscopic appearance of the intestinal mucosa of the experimental group was significant in that it did not differ from that of the control group. At all levels of the intestine the villi were completely covered by a layer of columnar epithelial cells and goblet cells. The villi were long and slender and projected well out into the intestinal lumen. There was no evidence of blunting of the tips of the villi. The epithelium of the intestinal crypts was not altered. There was no apparent increase in the mitotic rate of these cells. The crypts were not dilated or filled with necrotic cellular debris (Figs. 8 and 9). In contrast the intestinal mucosa of conventional cats inoculated with the same virus was nearly destroyed (Fig. 10).

Microscopic lesions were consistently present in the thymus and in the lymphocytic tissues. The lobules of the thymus were drastically reduced in size compared with those from control cats (Figs. 11 and 12). The interlobular space was increased in width and contained more adipose connective tissue. The interlobular connective tissue had a rather loose arrangement and contained scattered lymphocytes. After PID 5 the cortex was almost entirely depleted of lymphocytes. This caused the medulla to appear much more prominent in the experimental group. A few lymphocytes remained in the atrophic cortex along with several clusters of reticuloendothelial cells. There was marked hyperplasia of the reticuloendothelial cells of the medulla. Many of these cells were enlarged and contained phagocytized cellular debris within their

Fig. 8. Photomicrograph of the ileum of a control germ-free cat (K 1896). The Peyer's patches contain a zone of mature lymphocytes near the muscularis mucosae (arrows). Compare with Figure 9. H & E stain; X 60.

Fig. 9. Photomicrograph of the ileum of a germfree cat seven days after inoculation with feline infectious enteritis virus (K 934). The mucosal surface appears normal. The Peyer's patches have undergone depletion of lymphocytes. Compare with Figure 8. H & E stain; X 60.

Fig. 8

Fig. 9

Fig. 10. Photomicrograph of the ileum of a conventional cat five days after inoculation with feline infectious enteritis virus (K 658). The mucosal epithelial cells have desquamated and the villi are extremely blunted. Large groups of hyperplastic reticuloendothelial cells are located within the Peyer's patches. H & E stain; X 55.

Fig. 11. Photomicrograph of a normal thymus from a control germfree cat (J 1741). The cortex is well populated with thymic lymphocytes. H & E stain; X 50.

Fig.10

Fig.11

Fig. 12. Photomicrograph of severe lymphocytic atrophy and mineralization of Hassall's corpuscles in the thymus of a germfree cat six days after inoculation of feline infectious enteritis virus. Note the depletion of lymphocytes and hyperplasia of reticulo-endothelial cells in comparison to the control thymus in Figure 11 taken at the same magnification. H & E stain; X 50.

Fig. 13. Photomicrograph of the thymus of a conventional cat five days after inoculation with feline infectious enteritis virus (K 658). The lobules are decreased in size due to the loss of thymic lymphocytes. H & E stain; X 50.

Fig. 12

Fig. 13

cytoplasm. The nucleoli were enlarged and brightly eosinophilic. Indeed, some nucleoli were so prominent that they could be interpreted as inclusion bodies. However, they were connected to the nuclear membrane by fine strands of chromatin. Also, there were no other nucleoli in the nucleus. These intranuclear bodies stained red with the acridine orange fluorochrome staining reaction. This was comparable to the staining characteristics of the nucleoli in control sections. The intranuclear bodies did not stain magenta with the Feulgen reaction. The epithelial component and the Hassall's corpuscles were more conspicuous due to the loss of lymphocytes. Many of the Hassall's corpuscles were in various stages of degeneration. By PID 5, these degenerated cells began to undergo extensive mineralization. Some of the Hassall's corpuscles were greatly enlarged and contained a large mass composed of concentric layers of keratin. Mineralization or degeneration of Hassall's corpuscles did not occur in any of the control cats. By PID 12 there was still little evidence of regeneration occurring in the thymus. The lesions in the thymus of germfree cats were similar to those in conventional cats (Fig. 13).

The lymphocytic tissues of the control germfree cats resembled that of other germfree animals (Figs. 14 and 16) (14). The mesenteric lymph nodes, mandibular lymph nodes and tonsils infrequently contained poorly developed secondary reactive centers which varied slightly in number according to the age of the respective animal. The Peyer's patches usually contained only primary follicles. Plasma cells were rarely seen in any of the lymphocytic

Fig. 14. Photomicrograph of the mesenteric lymph node of a control germfree cat (J 1741). Note the lack of delineation of the primitive lymphocytic follicle (arrows) from the remainder of the cortex. H & E stain; X 125.

Fig. 15. Photomicrograph illustrating cortical lymphocytic depletion and reticuloendothelial cell hyperplasia in the mesenteric lymph node of a germfree cat seven days after inoculation with feline infectious enteritis virus (K 934). Compare with Figure 14. H & E stain; X 125.

Fig.14

Fig.15

Fig. 16. Photomicrograph. Higher magnification of the interfollicular tissue in Figure 14. Note the lack of reticuloendothelial cells in comparison with Figure 17. H & E stain; X 850.

Fig. 17. Photomicrograph. Higher magnification of the interfollicular tissue in Figure 15. Note the increased number of large reticuloendothelial cells and lymphoblasts. Some of the reticuloendothelial cells contain enlarged nucleoli (arrows). Compare with Figure 16. H & E stain; X 850.

Fig.16

Fig.17

tissue. They were occasionally found, however, in small numbers in the lamina propria of the intestinal mucosa in the region of the intestinal crypts.

As early as PID 3, changes were noted in the lymphocytic tissues, particularly the mesenteric lymph node. The most severe lesions were seen on PID 5. In contrast to the control group, a distinct junction could be seen between the cortex and medulla of the lymph nodes. This was due to the hyperplasia of reticuloendothelial cells (Fig. 15). The total number of small mature lymphocytes was greatly reduced. Many follicles with secondary reactive centers were present in the cortex. Some of the central portions of these follicles contained clumps of brightly eosinophilic proteinic material surrounding reticuloendothelial cells and proliferating lymphoblasts. The cortex and medulla were packed with lightly basophilic lymphoblasts containing large, pale-staining nuclei with one or two nucleoli. The cytoplasm of these cells was more abundant than that seen in the lymphocytes of the control group and was more eosinophilic. Mitotic figures were randomly scattered in the cortex. Occasionally small clusters of pyknotic lymphocytes were seen. Large numbers of reticuloendothelial cells were admixed among groups of proliferating lymphoblasts. Sometimes these groups of reticuloendothelial cells were isolated within the lymphoblastic cells. At other times they were confluent in long wide cords of cells which extended from the periphery to the center of the lymph node. The reticuloendothelial cells were greatly enlarged and many contained necrotic debris within their cytoplasm. The nuclei were

vesiculated and some had margination of the chromatin. The nucleoli were enlarged and brightly eosinophilic (Fig. 17). By PID 10 small lymphocytes were again numerous within the cortex. Follicles which contained a dark, outer zone of small mature lymphocytes were located around the periphery of the cortex. The central zone still consisted of the lymphoblastic type of cell with occasional reticuloendothelial cells. More lymphocytic cells were seen in the medulla at this stage of the disease. The reticuloendothelial cells were still prominent and many contained phagocytized hemosiderin in their cytoplasm. An occasional reticuloendothelial cell was seen which had phagocytized an erythrocyte.

The Peyer's patches in the control germfree animals were well developed but were not densely populated with cells (Fig. 8). A zone of small mature lymphocytes was present adjacent to the muscularis mucosae. The follicular cells were mostly lymphoblasts. They had larger, more vesicular, less basophilic nuclei with discernible nucleoli. Reticuloendothelial cells were seen only rarely in the control group. In the experimental group, by PID 5 the majority of the mature lymphocytes were no longer present (Fig. 9). Occasionally small foci contained one or two pyknotic lymphocytes. The lymphocytic cells were almost exclusively lymphoblasts except for a narrow zone of lymphocytes at the periphery of the Peyer's patch. The most striking change was hyperplasia and hypertrophy of reticuloendothelial cells similar to those in the thymus. The nuclei of these cells were enlarged and vesiculated. The nucleoli were greatly increased in size and in instances they accounted for

50% of the nuclear area. The cytoplasm contained clumps of darkly basophilic material which was suggestive of phagocytosis of necrotic lymphocytes. The intercellular space contained small deposits of eosinophilic, fibrillar material. By PID 10, the outer zone again contained mature lymphocytes. The central portion contained an admixture of lymphoblasts and reticuloendothelial cells. However, the reticuloendothelial cells had decreased in numbers so that they were no longer the predominant cell type.

Histologically the lymphocytic tissue of the spleen had similar changes to those seen in the thymus and lymph nodes. The splenic follicles in the control cats were composed primarily of mature lymphocytes. Reticuloendothelial cells were not a prominent histological feature. In the experimental group, however, by PID 3 the mature lymphocytes already were partly replaced by larger lymphoblasts. In addition, reticuloendothelial cells had begun to proliferate within the central portion of the splenic follicles. By PID 5, many reticuloendothelial cells were present throughout the splenic follicles. Most of these cells were greatly enlarged with the nucleus making up the larger proportion of the cell. Phagocytized debris was present within these cells. By PID 10, hemosiderin was also found in the cytoplasm of the reticuloendothelial cells. The splenic follicles again contained mature lymphocytes. Some follicles even had narrow outer zones of lymphocytes.

The tonsils, bronchial lymph nodes, and mandibular lymph nodes were also examined. These contained similar lesions but they were not as prominent as those seen in the thymus and mesenteric lymph nodes.

The bone marrow of the control and experimental groups did not differ significantly in the degree of cellularity. A significant difference in myeloid/erythroid ratio (Fig. 18) was not seen until PID 4. The M:E ratio fell to within normal limits again on PID 6 but significantly differed again on PID 8-10. The range of myeloid cells in the control group was 53-54%. In the experimental group the range for the myeloid cells was 52.75% to 59.75%. Differential counts revealed that the increased number of myeloid cells was due to an increase in the metamyelocytes and granulocytes. The erythroid series of cells did not have a significant decrease in any one classification of cells. Rather the total number of erythroid cells was simply decreased proportionately to the increased total number of myeloid cells. It is interesting to note that the germfree cat has higher values for the primitive blast forms of both the myeloid and erythroid series than has been reported for the conventional cat (5, 12, 19, 20).

Discussion

Experimental feline infectious enteritis in the germfree cat was not the fulminating disease with a high mortality rate as is reported in the literature for the conventional cat (6, 7, 12, 13, 16, 17, 21). All of the cats in this study which were inoculated with the infectious enteritis virus had a relatively short clinical course and were beginning to recover by PID 7. The severe leucopenia which occurred in all infected germfree cats was due to absolute lymphopenia with absolute neutropenia occurring a few days later. Panleucopenia has been reported in almost all spontaneous cases of the disease.

Fig. 18. The myeloid-erythroid ratio of the bone marrow of 14 germfree cats inoculated with feline infectious enteritis virus. The heavy black bar and dotted line represent the standard deviation and mean values of five control germfree cats. The relative increase of myeloid cells first occurred at the same time as the neutropenia (Fig. 3) with a second rise after peripheral blood values had returned to normal.

Fig.18

The severe thymic atrophy has not previously been reported. In our experimentally infected cats this was the only consistent gross lesion. The atrophy was due to almost complete depletion of the lymphocytes. This lesion along with hyperplasia of the reticuloendothelial cells occurred also in other lymphocytic tissues and was prominent in the spleen, lymph nodes, and Peyer's patches. Ultrastructural studies of the mesenteric lymph nodes confirmed that the large reticuloendothelial cells are macrophages phagocytizing damaged lymphocytes (8). These results indicate that the lymphocytes may be the target cells for the virus.

Reticuloendothelial cell hyperplasia was expected as it has been reported previously (13, 16, 17). The nucleoli of some of these reticuloendothelial cells were greatly enlarged but had all the other characteristics of nucleoli with the Feulgen reaction and acridine orange fluorochrome staining. It is possible that the intranuclear inclusion bodies reported by others may occur under some conditions since nuclear inclusions occur fleetingly in less than .1% of cells in tissue cultures (9, 10, 11, 15). None were observed in our material.

The myeloid/erythroid ratio of the bone marrow of the germ-free cat differs from that of the domestic cat reported in the literature (5, 12, 19, 20). The M:E ratio of the experimental cats in our study was significantly higher than that of the control group but it was still much lower than the 3.3 reported by Lawrence et al. (12) for cats which were beginning to recover from the disease. The fact that the percentage of blast forms in both the myeloid and

erythroid series is elevated in the germfree cat may account for the low absolute neutrophil count in the peripheral blood. Perhaps the germfree cat in the absence of any microbial flora has a decreased stimulus for granulocyte maturation. After several days post-inoculation with the virus, the number of more mature myeloid forms in the bone marrow increased.

Since the virus consistently produced lesions of infectious enteritis in the small intestine of conventional cats from our breeding colony, the absence of enteric lesions in the germfree cat was surprising. No degeneration or necrosis of the mucosal epithelial cells occurred in the experimental group. No inclusion bodies were found in the nuclei of the epithelial cells of the crypts. The enteric lesions in conventional cats with this disease are probably caused by the microbial flora of the intestinal tract. It is possible that the loss of lymphocytes results in depression of the immune mechanism. Such a mechanism has yet to be demonstrated. On the basis of our finding the term feline panleucopenia appears more appropriate than feline infectious enteritis for this disease.

Summary

In the absence of other microorganisms the virus of feline infectious enteritis produced a mild, non-fatal clinical disease. A biphasic temperature rise occurred on PID 2 and 4. All of the cats began to recover by PID 7. Absolute lymphopenia occurred on the first day followed by absolute neutropenia on PID 4. The leucocyte counts were within the normal range by PID 10. Severe atrophy of the thymus was the only macroscopic lesion produced.

Depletion of mature lymphocytes and reticuloendothelial cell hyperplasia occurred in all other lymphocytic tissue. On the basis of microscopic examination and histochemistry the intranuclear inclusion bodies in reticuloendothelial cells were interpreted as enlarged nucleoli. No inclusion bodies considered to be of diagnostic significance could be demonstrated. There was no evidence of enteritis grossly or microscopically.

CHAPTER III

THE INFLUENCE OF FELINE INFECTIOUS ENTERITIS VIRUS ON RENEWAL OF ILEAL EPITHELIUM IN THE GERMFREE CAT

Introduction

The disease feline infectious enteritis was named because of the striking lesions in the intestine of infected cats. When germ-free cats were experimentally infected, however, no lesions could be demonstrated in the intestines macroscopically, microscopically (6), or electron-microscopically (3). To corroborate these findings we have investigated the effects of feline infectious enteritis virus on the kinetics of intestinal cell turnover in germfree cats.

Abrams et al. (1) proved that the presence of a bacterial flora in the gastrointestinal tract significantly altered the rate of intestinal epithelial renewal in the mouse. Their data suggested that bacteria may act to accelerate the rate of loss of cells from the extrusion zone at the tips of the intestinal villi. Using tritiated thymidine as a radioactive label, they found that labeled epithelial cells reached the extrusion zone in the ileum of conventional mice in somewhat over 2 days while 4 days were required in the ileum of germfree mice.

Leshner et al. (4) reported that the generation-cycle time for the duodenal crypt cells of germfree mice was significantly lengthened over that of conventional mice. The increased duration

was due to an increase in the stages of synthesis and predivision of the generation cycle. Their findings concerning the epithelial renewal rate of the intestinal mucosa of germfree mice confirmed those of Abrams' group.

The objective of this study was to determine whether feline infectious enteritis virus altered the renewal rate of the ileal intestinal epithelium of the germfree cat.

Materials and Methods

Six 22-week-old germfree cats were used for this study. The cats were raised according to the methods of Rohovsky et al. (5). All cats were maintained in flexible plastic isolators. Specimens from the isolators were cultured weekly to demonstrate sterility. All animals were fed the same sterile diet. The cats were divided into two groups of three animals which were housed in separate isolators. The experimental group was inoculated intraperitoneally with 2.0 ml of a 10% infective splenic suspension in Hank's solution with antibiotics. The splenic suspension was made from a conventional cat which had been experimentally infected with feline infectious enteritis virus. The stock of virus had been obtained from Dr. John Gorham, Washington State University, Pullman, Washington. The control group was treated in a similar manner with a splenic suspension which had been harvested from a healthy cat from our specific pathogen-free breeding colony. Aliquots of both suspensions of spleen had been cultured for sterility and passaged in feline kidney cell cultures to insure that no cytopathic feline viruses were present.

On the third post-inoculation day both groups were injected with the radioactive material. Sterile aqueous tritiated thymidine¹ with a specific activity of 14.0 c/mm and a concentration of 0.5 mc/ml was used. Each cat was injected intramuscularly with 2.5 mg Sernylan² (phencyclidine hydrochloride) which immobilized them in 5 to 8 minutes. Each cat was then injected intracardially with 1.0 mc of tritiated thymidine. They were subsequently necropsied at intervals of 1, 24, and 48 hours following inoculation of the radioactive label.

At necropsy, the small intestine was opened, pinned to a block of paraffin and fixed in 10% buffered formalin. Sections of ileum taken 10 cm from the ileo-colic junction were imbedded in paraffin, serially sectioned at 6 μ , and stained for 2 minutes with 1% alcohol eosin. The slides were dipped in Kodak NTB-2 nuclear tracking emulsion³ according to the methods of Adamik.⁴ These coated sections were exposed in light-tight black boxes for 5 weeks at 4 C. They were subsequently developed with modifications of the method of Jofte (2) using the following times: (1) 6 minutes Kodak D19 developer, (2) 15 seconds Kodak acid stop bath, (3) 2 minutes Kodak rapid fixer, and (4) 4-minute wash in running distilled water. All solutions were maintained at a temperature of 21 C. The radio-autographs were then stained for 45 seconds with Harris' hematoxylin and mounted in piccolyte mounting media.

¹Schwarz BioResearch, Inc., Orangeburg, New York.

²Parke, Davis and Company, Detroit, Michigan 48232.

³Eastman Kodak, 343 State Street, Rochester, New York.

⁴E. B. Adamik, Schwarz BioResearch, Inc., Mountain View Ave., Orangeburg, New York.

The renewal rate of the intestinal epithelium was evaluated quantitatively according to the methods used by Abrams et al. (1). The crypts and villi were measured in terms of the numbers of epithelial cells. Only crypt-villus units which included in continuity, the bottom of the crypt, the lumen of the crypt, a continuous crypt-villus junction, and the tip of the villus, were counted. The mean number of labeled cells was determined from 40 ideally sectioned crypts per animal. Cells were considered to be labeled if three or more silver grains were present over their nuclei.

Results

By counting the total number of epithelial cells from the bottom of the intestinal crypts to the tips of the villi, the total lengths of the mucosal villi were determined. The epithelial cells of the crypts were also counted and subtracted from the total length of the mucosal villi to find the number of epithelial cells on the villi themselves. There was no statistically significant difference between the two groups for the total length of the mucosal villi, the length of the villi alone, or the length of the crypts (Table 2). The number of epithelial cells labeled with tritium were also counted from the base of the crypts to the tips of the villi (Table 3). Cells were counted to the leading edge of those with labeled nuclei in sections collected at one hour, 24 hours, and 48 hours following injection of the tritiated thymidine. At the end of one hour, labeled cells were found from the bottom of the crypt to approximately half way up the crypt (Fig. 19). In 24 hours, labeled

TABLE 2
NUMBER OF EPITHELIAL CELLS IN THE ILEAL MUCOSA OF GERMFREE
CATS WITH AND WITHOUT FELINE INFECTIOUS ENTERITIS

	Length of Crypts	Length of Villi	Total Length
Controls (3)	49.2 $\pm$ 1.2	110.1 $\pm$ 4.8	159.3 $\pm$ 4.7
Inoculated (3)	49.4 $\pm$ 2.4	110.6 $\pm$ 4.2	160.0 $\pm$ 4.2

TABLE 3
NUMBER OF TRITIUM-LABELED EPITHELIAL CELLS IN THE ILEAL MUCOSA OF
GERMFREE CATS AT INTERVALS AFTER INJECTION OF TRITIATED THYMIDINE

	1 hr	24 hr	48 hr
Controls	23.8 $\pm$ 1.9	51.4 $\pm$ 2.6	90.8 $\pm$ 2.3
Inoculated	23.0 $\pm$ 1.2	50.9 $\pm$ 2.5	90.6 $\pm$ 3.1

cells had migrated to the junction between the base of the villus and the top of the crypt (Fig. 20). In 48 hours, the labeled cells were located approximately 36% of the distance from the base to the tip of the villi. By use of the t test, no statistically significant difference was found between the infected and non-infected groups of germfree cats in the rate of migration and number of labeled cells.

Discussion

The results of this study further verify the finding that the feline infectious enteritis virus alone does not cause the intestinal lesions which occur in conventional cats with naturally

Fig. 19. Radioautograph of tritium-labeled epithelial cells in the ileal mucosa of a control germfree cat (K 1895) one hour following injection of the radioactive label. The labeled cells are located midway between the bottom and top of the intestinal crypt (arrow). H & E stain; X 500.

Fig. 20. Radioautograph of tritium-labeled epithelial cells in the ileal mucosa of a control germfree cat (K1896) 24 hours following injection of the radioactive label. The labeled cells have migrated to the top of the crypt (arrow). H & E stain; X 500.

Fig.19

Fig. 20

occurring feline infectious enteritis. The cellular kinetics were studied on the third, fourth, and fifth days after inoculation when the clinical signs and the lesions in the lymphocytic tissues were most severe. The length of the crypt and villus was not significantly altered in germfree inoculated cats. Also, the rate of migration of epithelial cells labeled with tritium did not vary between the two groups of cats. These findings are contrary to what might be expected from the histological lesion in conventional cats. The possibility that the cellular kinetics might vary at other stages of disease cannot be excluded.

Summary

Three germfree cats previously inoculated with feline infectious enteritis virus and three germfree control cats were injected with tritiated thymidine. The length of the villi and the renewal rate of ileal mucosal cells which were labeled with tritiated thymidine were equal in both groups. Feline infectious enteritis virus did not alter the turnover time of epithelial cells in the ileum of the germfree cat.

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