BIOLOGY OF THE INFECTIOUS AGENT OF FELINE INFECTIOUS PERITONITIS

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PREFACE

Feline infectious peritonitis is an interesting disease to study not only because it is a fairly recently characterized disease whose infectious agent has not been isolated but also because it offers opportunities to study a rather unique host-parasite relationship.

I would like to give special thanks to Dr. Ralph G. Buckner, Small Animal Clinic, Department of Medicine and Surgery, for his sustained interest in this research problem, for his continued cooperation in helping with clinical procedures, procuring clinical specimens and animals for research purposes and assistance in directing the handling of the experimental anaimals.

For their help and encouragement during my academic work and the planning and execution of this research project, I would like to express my appreciation to my graduate committee who in addition to Dr. Buckner are Dr. Merwyn L. Frey, Department of Veterinary Parasitology and Public Health, Dr. Elizabeth T. Gaudy, Department of Microbiology, and Dr. Robert A. Patnode, Department of Microbiology and Immunology, Health Sciences Center, University of Oklahoma, Oklahoma City.

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INTRODUCTION

Diseases of cats have received less attention than the diseases of animals of greater economic importance consequently there are many aspects of feline diseases yet to be explored.

Feline infectious peritonitis (FIP) is a disease which has been described only recently. It is characterized most commonly by the presence of ascites and persistent fever, which does not respond to antibiotics, followed by depression and death. Recently lesions involving the eye, brain, and other organs have been attributed to FIP.

Since FIP was first described as a distinct disease entity it has received considerable attention from the clinician in describing the various clinical aspects of the disease, from the pathologist in characterizing the lesions, and from various workers in attempting to isolate an infectious agent, to observe the agent by electron microscopy, or to reproduce the disease in experimental animals. However, some of the basic aspects of the disease have not been studied. The primary reason for this is that the infectious agent has not been isolated and experimental animals other than the cat have not been found.

The present study pertains to the biology of the disease. Specifically, the following research objectives were undertaken:

Development of a system for studying the production of infection and disease in experimental animals.

Characterization of the infectious agent.

Study of certain aspects of the host-parasite relationship in FIP.

Study of microorganisms associated with the disease.

REVIEW OF LITERATURE

Natural Infections

Character of Disease

Feline infectious peritonitis was first described as a disease entity in 1963 by Holzworth (9) when she briefly characterized it under the name chronic fibrinous peritonitis.

In 1966, the infectious nature of FIP was shown when ascitic fluid from a cat with FIP was injected into experimental cats and the disease was reproduced (32). When records of necropsy cases from previous years were examined, it was found that there were no cases showing similar pathology before 1953 in Massachusetts (17), 1954 in California (25), or 1962 in Ohio (32). Following the original report of the infectious nature of FIP, there has been an increasing number of reports of cases from various areas of the United States. Since 1970 the natural disease in domestic cats has been reported in various parts of the world including Canada (22), England (10), Ireland (8), Switzerland (23), and Japan (11).

A cat with FIP will typically have a history of a persistent fever (39.3° to 41.1°C), gradual anorexia, depression and weight loss (32), and late in the disease, emaciation. On examination the mucous membranes often appear pale and the ascites is denoted by a full, soft, distended abdomen. Occasionally the cat may show diarrhea, icterus, or pleural effusion with marked dyspnea. Variant forms of the disease

such as meningoencephalitis, panophthalmitis, bilateral endogenous granulomatous uveitis, and nephritis may be present in addition to or in place of the peritoneal form (20). Cats with FIP are usually characterized clinically as being unresponsive to antibiotics and the disease ends fatally or the cat is killed in the moribund stage.

Feline infectious peritonitis is usually considered to be an almost universally fatal disease in spite of intensive treatment (17, 25). There are however a few scattered citations in the literature of cats recovering following treatment with tylosin and prednisolone (3, 17) and lavage with flumethasone and chloromycetin succinate (19). An occasional cat is thought to recover spontaneously (31).

Epidemiology

Feline infectious peritonitis has been reported on many occasions to have occurred in multiple cat households (7, 9, 25) and in catteries (7, 9, 32) as well as in relatively isolated households. There has been to date no report of experimental cats acquiring the disease by contact and the method of natural transmission is not known at this time (29).

While cats of all ages are susceptible to FIP, young cats have usually been found to have the highest incidence (9, 18, 25). Wolfe and Griesemer (32) found that of the cats with FIP which they studied, 79% were under 2 years of age, while 61% of all cats seen during the same time period were under 2 years of age. Among the 71 cases seen in the Boston area, 50% of the cats with FIP were less than 1 year old but when these results were compared with those of all cats presented to the same hospital during the same time period there was no significant

difference (17). A kitten that died of FIP at 23 days of age may be the youngest reported case of naturally acquired feline infectious peritonitis (15), while cats as old as 15 years have been reported to have the disease (26).

A greater number of male than female cats naturally infected with FIP has been found (3, 7, 17, 32). The percentage has varied from 70% (17) to 81% (32) male and was shown to be significantly more than the percentage of male cats in the total population of those hospitals. In one study, however, no sex predilection was found when compared with the total population of cats seen during the same time period (25).

While some authors have found a larger percentage of cats with FIP to be Siamese (17, 32, 33), other authors have found no difference in predisposition by breed (18, 25). Since purebred cats are more apt to receive attention from veterinarians than others and since cats raised in groups are known to have an increased incidence of FIP on occasion, this situation is difficult to interpret.

Species of Felidae other than the domestic cat, such as the lion (2, 17), leopard, and jaguar (17), have been found naturally infected with FIP.

Many cats with FIP have a history of having been treated by a veterinarian for wounds, abscesses, or respiratory or other diseases. These cats have often been liberally treated with a variety of antibiotics (9, 17) or corticosteroids (17). Perhaps the increased incidence of FIP in recent years can be correlated with this increase in treatment; however, it would be hard to prove and difficult to know whether the recent increased incidence is not just due to more careful reporting of the disease once it was fully characterized.

Experimental Infections

Production of Disease

There have been varying reports on the susceptibility of experimental cats to infection with FIP. Ward and Pederson (24) using cats of 6 weeks to 6 months of age, found that all 15 of their experimental animals developed FIP. However, most research workers have shown that only two-thirds to three-fourths of the experimental animals they used developed the disease (1, 7, 11, 32, 33). The various ages or sexes of the animals which did not develop the disease were not given, but Wolfe and Griesemer (33) stated that they found there was no age, sex, or breed difference in experimental cats and that germfree and conventional cats are equally susceptible to FIP and that the disease is the same in both.

Many different tissues and routes of inoculation have been used successfully in the production of disease in experimental animals. Ascitic fluid, whole blood, urine, spleen, liver, and omentum have been used (7). Disease has been produced by the intraperitoneal, intravenous, subcutaneous, and oral routes (7), and additionally, Ward (27) reported positive results with the intracerebral, intrathoracic, and intranasal routes.

Study of the Infectious Agent

Attempts to identify the causative agent of FIP by isolating aerobic (7, 11, 32) and anaerobic (11, 32) bacteria, mycoplasma (7, 11, 32), and fungi (32) from tissues and fluids of cases of FIP have all proven unsuccessful. Isolation of the agent by yolk sac (11, 32) and

chorioallantoic membrane (11) inoculations of embryonated chicken eggs has also been unsuccessful. Many laboratory animals such as mice (7, 11, 27), guinea pigs (11, 27), rats (7), rabbits (27), and hamsters (11) have been utilized in unsuccessful attempts at production of disease or transmission of the agent.

Tissue culture methods have generally proven unsuccessful in the attempt to isolate the etiologic agent of FIP. Primary feline and monkey kidney cells, HeLa cells, human amnion and human embryonic lung cells (32) have been used in cell cultures as have feline fetal fibroblasts, canine sarcoma, and mouse peritoneal macrophages (27).

Wolfe and Griesemer (32) found that primary tissue cultures of kidney and spleen cells from a cat with naturally acquired FIP demonstrated the absence of microorganisms after 2 subpassages. Hardy (7) had the same results using cells from the omentum and spleen. However, Low, et al. (13) reported on the isolation of a cell-vacuolating agent from kidney cell cultures prepared from 4 of 6 domestic cats with both naturally and experimentally acquired FIP. The intraperitoneal and intravenous inoculation of experimental cats and kittens with the supernatant and cells from these cultures did not produce the disease. The agents as seen in electron micrographs were in the cytoplasm of the cell. They were described as being 50 nm in diameter in the extracellular spaces and as having a trilaminar membrane with an overall diameter of 90 nm. Riggs, et al. (16) reported isolating what may be the same agent from lymph nodes of domestic cats during a survey of animal neoplasms. Whether the agent is related to FIP or is an adventitious agent is not known.

Cells from the omentum and spleen from cats with both natural and

experimental FIP were grown and passed serially as monolayers and then the supernatant fluid was passed through a 220 nm average pore diameter (APD) filter before testing it in cats (7). The cats did not develop FIP. Peritoneal macrophages from cats with FIP have also been cultured with no agent found (26).

Special stains such as periodic acid-Schiff, Giemsa, Kinyoun's acid fast, Gram's and Grocott have been used on histological sections in order to detect the presence of microorganism but all these efforts were unsuccessful (27, 32).

Ascitic fluid and spleen homogenate have been filtered through an APD 200 to 250 nm filter and then used to produce disease in cats (7, 27, 28, 33, 34). From these experimental cats virus-like particles in mesothelial cells and macrophages from lesions in the omentum, mesentery, spleen and liver have been identified in electron micrographs (7, 27, 28, 34). These virus-like particles have never been seen in natural cases of FIP (7, 27, 28, 34). These virus-like particles were described as budding into the endoplasmic reticulum or cytoplasmic vesicles (27, 28, 34). Morphologically the particles were described as being between 70 and 100 nm in diameter. Club-shaped projections were seen protruding from the particles and therefore the particles were considered to be Corona-like because of the similarity to the Coronavirus 224E of human respiratory tract origin and the mouse hepatitis virus.

Pathology of Naturally Acquired FIP

Peritoneal Form

The ascitic fluid which usually characterized the peritoneal form of the disease is "voluminous, clear to slightly cloudy, viscous and

may clot on exposure to air" (18). It has a high protein level and low cellular content.

Robinson et al. (17) described early stages of the disease as showing light gray fibrinous exudate over the peritoneum and mesenteric membrane or showing discrete, white, granular foci on the surface of such visceral organs as the spleen and liver. In cases of longer standing, organization of the fibrinous deposits occurred. Fibrous adhesions adhering to lobes of the liver or between the liver and spleen may be seen. Discrete foci may be seen on the omentum in early cases while the omentum becomes necrotic, gelatinous, and contracted into a ball in more chronic cases.

Extraserosal Form

Montali and Strandberg (14) presented a good summary of the extraserosal lesions in FIP. They described the pyogranulomatoid lesions in the kidneys, lungs, eyes, and leptomeninges. Phlebitis was said to be common and they thought that the extraserosal form might progress by the vascular route. A homogenate of spleen and kidney from one of their cats showing only extraperitoneal lesions was injected IP or IV into kittens and produced the typical peritoneal form of FIP.

Pathology of Experimentally Produced FIP

Peritoneal Form

The pathology of the experimental and natural disease were found to be similar except for the greater amount of focal necrosis and less exudation in the experimental cats inoculated by the intraperitoneal

route (25, 29). Extraserosal lesions may occasionally be seen in these cats but these lesions are never as extensive as in cats inoculated by the subcutaneous route.

Estraserosal Form

Ward et al. (29) found that when cats were inoculated subcutaneously they developed necrotic foci in various organs such as the liver, spleen, lungs, omentum, and lymph nodes as well as at the site of inoculation. Of 9 cats developing experimental FIP following subcutaneous inoculation only 2 developed ascitic fluid and marked omental lesions in addition to the extraperitoneal forms.

Feline infectious peritonitis has been produced in experimental cats by inoculation of infectious material by many different routes with varying results. Intrapleural inoculation produced granulomatous pleuritis, intracerebral inoculation produced granulomatous lesions in the brain, intravenous inoculation produced systemic lesions, and intranasal inoculation produced diffuse granulomatous pneumonia (29).

MATERIALS AND METHODS

Production of Disease in Cats

Cats

With the exception of a cat that had recovered from FIP, all mature cats, i.e. those over 1 year of age, were obtained from the Enid, Oklahoma, animal pound and all kittens 5 to 10 weeks of age were donated singly or in litters by local residents. All cats were of mixed breeds and no attempt was made to record the predominant breed types. Care and housing of the cats met the standards of the United States Department of Health, Education, and Welfare as set forth in "Guide for the Care and Use of Laboratory Animals" (24). Inoculated animals were separated from uninoculated cats by being kept in a separate room in another part of a large building. The rectal temperature and general health status of each animal were usually recorded daily. Using the method recommended by the manufacturer, the kittens were vaccinated with inactivated panleukopenia vaccine[a] on arrival and every 2 weeks thereafter until they were 12 weeks old when all of them had received at least 2 vaccinations.

Control cats and kittens were inoculated with either 2 ml of ascitic fluid subcutaneously (SQ) or 0.5 to 20 ml intraperitoneally (IP). If signs of FIP developed, the cats were killed in the terminal stage

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[a]Felocine[R], Norden Laboratories, Lincoln, Nebr. 68501.

of the disease. If asymptomatic, they were observed for 60 to 90 days after inoculation and then killed.

All kittens were 8 to 16 weeks old when they were injected with the inoculum. Five kittens were used singly in preliminary trials and received from 0.5 to 2.5 ml of inoculum intraperitoneally. For the other trials 2 kittens were used per trial. Kittens of each pair, consisting of 1 male and 1 female, were matched approximately by weight and age. Unless the kittens were used for controls, they were inoculated IP with 0.5 ml to 1 ml of inoculum per kitten. The kittens were killed either 4 days after commencement of a high fever (40°C) or in the terminal stages of the disease, or they were observed for 30 days before being killed if asymptomatic. For the purpose of these experiments the incubation period was considered to be the time period from the day of inoculation until the first day the cat's temperature exceeded 39.7°C. Disease was determined by temperature rise for 4 days or more, as well as the presence of gross lesions at necropsy.

One mature cat and 2 kittens were treated with prednisolone acetate,[b] 22 mg/kg/day, given intramuscularly (IM) for 4 days preceding injection with the infectious material and again the day of the injection. On the sixth and seventh days prednisolone was given at the rate of 11 mg/kg and 5.5 mg/kg, respectively.

The recovered cat (R 43) was brought to Dr. Ralph G. Buckner, Small Animal Clinic, Department of Medicine and Surgery, for diagnosis, treatment, and later certain experimental procedures. A synopsis of of this cat's clinical record is as follows:

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[b]Merticortelone[R] acetate, Schering Corporation, Bloomfield, N. J. 07003.

Day 1. Cat first examined by a veterinarian.

Day 14. Laporatomy and 3 lavages, each consisting of 300 ml of 1:4000 chlorhexidine[c], followed by several 300 ml saline washes.[d]

Day 69. Serum obtained for neutralization tests. Temperature first returned to normal at that time.

Day 172. Splenectomy.

Day 308. Challenge of the cat with standard FIP inoculum.

Day 317. Cat killed in the moribund condition.

Mature cats and kittens are numbered consecutively as they appear in the thesis with each test group being given a prefix to identify the cats referred to in the Discussion section. Cat numbers, inocula, and treatments of inocula appear in the Appendix.

Inocula

Inoculum number 1 was undiluted ascitic fluid from a cat with naturally acquired FIP. The ascitic fluid had been collected during a laporatomy performed under the direction of Dr. Buckner. Storage of this and all other inocula was at -70°C in an electric freezer. The inoculum was used in 10 to 20 ml amounts for the IP injection of 2 mature cats (M 1 and M 2). It was also used in a combined dosage of 8 ml IP and 2 ml SQ in a third cat (M pred 3).

Inocula 2 and 3 consisted of the spleen and ascitic fluid, respectively, obtained at necropsy from a cat with naturally acquired FIP. A portion of the spleen (4.2 g) was suspended in phosphate

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[c]Novasan[R], Fort Dodge Laboratories, Fort Dodge, Iowa 50501

[d]Method suggested by Dr. John Hahn, Laboratory Animal Veterinarian, St. Louis University School of Medicine, St. Louis, Mo., in a personal communication to Dr. Buckner.

buffered[e] saline to a final concentration of 33% w/v and then homogenized by agitation for 2 minutes in a household blender. It was then used immediately as inoculum (number 2) in doses of 2 to 2.5 ml for IP injection of 3 kittens (K pred 4, K pred 5, and K 6). The undiluted ascitic fluid (inoculum number 3) was used in 0.5 ml and 1 ml quantities for IP inoculation of 2 kittens (K 7 and K 8, respectively).

Kitten K 8 was used as the source of inoculum number 4. The kitten was killed 4 days following temperature rise above 40°C. The liver and spleen were collected, pooled, homogenized, and diluted to a 33% concentration as described above. The mixture was then centrifuged at 10,000 x g for 5 minutes and the supernatant was placed in 1.5 ml quantities in screw-capped glass tubes and stored at -70°C. For use this homogenate was thawed by holding the tubes under cold running tap water and the inoculum was diluted to a final concentration of 20% w/v with phosphate buffered saline. The homogenate was then centrifuged at 15,000 x g for 15 minutes and the supernatant was used for inoculation. Five-tenths milliliter of this material was referred to as the standard inoculum.

Inocula were desired to determine the presence or absence of the infectious agent of FIP in cats during the early stage of the disease, during the middle of the clinical course of the disease, and just before death. In order to prepare these inocula, 6 kittens were inoculated with the standard inoculum. Two of these kittens were killed on the first day their temperature rose to 40°C or higher (4 days post inoculation), 2 kittens were killed 4 days after their initial

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[e]All phosphate buffered saline used for this research study was 0.85% NaCl dissolved in 0.01 M phosphate buffer at pH 7.4.

temperature rise (6 days post inoculation), and the 2 remaining kittens were killed when they became moribund (13 days post inoculation). The spleens were collected from each pair of kittens, pooled, and an inoculum prepared in the same manner as the standard inoculum. These inocula were referred to as number 5 (early disease inoculum), number 6 (mid-term disease inoculum), and number 7 (late disease inoculum), respectively.

The source of inoculum number 8 was cat R 43 which had recovered from FIP after laporatomy and chlorhexidine lavage. Following this treatment the cat showed a gradual clinical improvement over a 2 month period and approximately 5 months after the laporatomy the spleen was removed in order to determine if it might be infectious when used as an inoculum for kittens. A sample (6.4 g) of spleen was homogenized, centrifuged, and diluted using the method described for the standard inoculum.

Two room and 2 cage control kittens which will be described later were used to determine if the infectious agent of FIP could be transmitted from inoculated to uninoculated kittens and produce infection or infection and disease. The spleens were used as a source of inoculum to rule out the possibility that only infection and subclinical signs of disease might have been produced. The control kittens were killed and then the spleens of each pair were collected, pooled, homogenzied, and processed in the same manner as the standard inoculum. The inoculum prepared from the room control kittens was labelled number 9 and that from the cage control kittens number 10.

Method of Euthanasia

All cats were killed by intrathoracic injection of sodium pentabarbitol solution[f] (2.2 ml/kg body weight).

Method of Necropsy

After death, animals were dipped in a benzalkonium chloride[g] solution (1:2500) as a method of disinfection, and the excess fluid was drained from the animal. Standard microbiological methods of necropsy and aseptic collection of tissues were used. Liver, spleen, omentum, and mesenteric lymph nodes were collected and cultured immediately as described later. Appropriate samples were prepared for fluorescent antibody technique studies by quick freezing them on dry ice cubes covered with aluminum foil held in a styrofoam container and then storing the samples in screw-capped glass tubes at -20°C. Tissues saved for future transmission studies were frozen in sterile, plastic specimen bags at -70°C. A record was kept for each animal of the gross appearance of the ascitic fluid, abdominal peritoneum, visceral peritoneum, liver, spleen, omentum, kidneys, mesenteric lymph nodes, thoracic fluid, pleura, lungs, and pericardium.

Characterization of the Infectious Agent

The effect of the following physical and chemical agents on the causative agent of FIP was determined as well as the ability of the

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[f] Beuthanasia[R], Burns-Biotec Laboratories, Inc., Oakland, CA 94621.

[g] Roccal[R], Winthrop Laboratories, Division of Sterling Drug, Inc., New York, N.Y. 10016.

agent to replicate in mice.

Filtration

The standard inoculum used in this experiment was filtered through a cellulose acetate filter with an average pore diameter (APD) of 200 nm.

Ether Extraction

The standard inoculum was mixed with anesthetic grade ether at a final concentration of 20% ether and held 18 hours at 4°C. The bulk of the supernatant was aspirated off and the remaining ether was removed by evaporation under 22 kg negative pressure for 20 minutes.

pH Stability

The standard inoculum was diluted 1:1 with MEM[h] tissue culture medium without added carbonate and the pH was lowered to pH 5 with 1 M Tris HCl.[i] The mixture was then refrigerated at 4°C for 18 hours. The mixture was not neutralized before injection. To account for the dilution, 1 ml was used for inoculation of each kitten.

The above procedure was also used for the pH 3 stability determination, except that the pH was lowered to 3 with the use of 0.1 M HCl.

Heat Stability

One milliliter aliquots of the standard inoculum contained in 9 by

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[h]MEM Eagle, Earle's Base, BBL[R], BioQuest, Cockeysville, MD 21030.

[i]Trizma HCl Reagent Grade, Sigma Chemical Company, St. Louis, MO 63178.

74 mm rubber stoppered tubes were held in a water bath at either 50°C for 6 minutes or 56°C for 30 minutes. The tubes were cooled to room temperature under cold tap water immediately after removal from the water bath.

Replication in Mice

Newborn to 24 hour old suckling mice were obtained from an inbred mouse colony maintained in the Department of Parasitology and Public Health. The experimental groups each consisted of 10 litter mates. Group 1 was given 0.01 ml of the standard inoculum intracranially and 0.03 ml IP and observed for 4 days. Group 2 was inoculated with the standard inoculum in the same manner as Group 1 and at the same time was given 0.01 ml prednisolone acetate IM (0.025 mg) and observed for 4 days. Both groups of mice were then killed by exposure to chloroform and the brain, liver, and spleen were harvested by aseptic means (Pass 1). Immediately after collection the organs from each group were pooled and diluted with phosphate buffered saline to 20% w/v, homogenized using a mortar and pestle, cultured for bacteria, and stored in a screw-capped tube at -70°C. After neonatal mice became available (3 to 11 days) this material was thawed in cold running tap water and used for the inoculum for the next group of mice (Pass 2). The above procedure was repeated for Pass 3.

Mice dying within 24 hours were discarded since death was arbitrarily attributed to trauma from the injection or to bacterial infection acquired during the injection procedure. Mice dying before day 4 were necropsied. The liver, spleen, and brain were collected, cultured for bacteria and fungi using methods detailed later, and then stored at

-70°C. If these cultures were negative the tissues were pooled with those of the rest of the individuals of their group. If the tissues contained bacteria or fungi they were discarded.

Certain Aspects of the Host-Parasite Relationship

Relationship Between Stage of Illness and Presence of the Infectious Agent

In order to test for the presence of the infectious agent of FIP in kittens during the early stage of the disease, during the middle of the clinical course of the disease, just before death, and in a recovered cat, kittens were inoculated with Inocula 5, 6, 7, and 8.

Relationship Between Disease and the Concentration of the Infectious Agent

The standard inoculum was diluted 1:10, 1:100, and 1:1000 with phosphate buffered saline. Two kittens were inoculated with 0.5 ml from each dilution.

Site of Replication of the Infectious Agent in the Cat as Determined by the Fluorescent Antibody Technique

Serum presumed to lack immune factors associated with FIP was obtained from a healthy cat before inoculation with the standard inoculum. Serum obtained from the same cat from which Inoculum 2 was obtained, a cat with naturally acquired FIP, and serum obtained from another cat, with naturally acquired FIP which was euthanitized in the

moribund stage of the disease, were used separately for the fluorescent antibody technique. All sera were conjugated with fluorescein isothiocyanate according to the methods of Corstvet and Sadler (4). Fluorescein conjugated rabbit IgG to feline IgG[j] was used for the indirect fluorescent antibody technique.

Frozen tissue sections were prepared from the liver and spleen from a normal kitten as well as liver and spleen from the cat from which Inoculum 2 was obtained. In addition frozen tissue sections were prepared from the liver of cats from which Inocula 5, 6, and 7 were prepared, which represented cats in the early stage of the disease, the middle of the clinical course of the disease, and the late stage of the disease, respectively. Sections were cut, fixed, and stained according to previously described methods (4).

Neutralization

Serum was obtained from a cat presumed to be susceptible to FIP before inoculation with the standard inoculum (preinoculation serum), from a cat late in the course of the clinical disease (late serum), and from the recovered cat (recovered serum). The serum from the recovered cat was taken when its temperature first returned to normal which was 69 days after it was initially presented to a veterinarian with clinical signs of FIP.

Each serum sample was inactivated at 56°C for 30 minutes, mixed 1:1 with the standard inoculum, and allowed to remain at room temperature with occasional agitation for 30 minutes before injection into kittens.

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[j]Microbiological Associates, Inc., Bethesda, MD 20014.

To compensate for the dilution factor, each kitten was inoculated with a 1 ml amount.

Challenge of the Recovered Cat

Three hundred and eight days after the cat was first presented to a veterinarian with signs of FIP it was challenged using the standard inoculum. The recovered cat had been splenectomized on Day 172 (136 days earlier).

Controls

The two kittens used as room controls were placed separately in cages adjacent to cages with inoculated kittens. These cages had wire mesh on the front and left side and solid metal on the right side and back so there was no direct contact between kittens. The room control kittens were cared for in the same manner as the infected kittens and in no special order. The room control kittens were killed after 30 days and their spleens were processed as described previously.

Two kittens were used for cage controls. Each kitten was placed in a cage with an infected kitten. Thirty days after its cage mate developed a high temperature (40°C or higher) the cage control was killed and the spleen harvested and processed as described previously.

Two kittens and 4 mature cats were maintained in the normal colony for the duration of the experimental work as controls to detect any adventitious infections resulting in disease.

Two mature cats which had not been splenectomized were challenged at the same time as the recovered cat. The male cat weighed 22 kg and the female cat weighed 11 kg.

Microorganisms Associated with FIP

As mentioned previously, the liver, spleen, omentum, and a mesenteric lymph node were cultured for bacteria and fungi immediately after being collected from the necropsied kittens. Standard microbiological methods and keys were used for the isolation and identification of organisms.

Media

Brain heart infusion agar[k] containing 5% citrated bovine blood, 10% horse serum, and 1% Albimi yeast autolysate[l] was inoculated and then cross streaked with a Micrococcus sp. The inoculated medium was placed in an atmosphere of 5 to 8% CO_2 (candle jar) and incubated at 37°C.

Medium for the propagation of Mycoplasma spp. was prepared from PPLO agar[k] with 10% horse serum and 1% Albimi yeast autolysate added and the pH adjusted to 7.6. This medium was cross streaked with Micrococcus sp. after inoculation and incubated in a moist atmosphere of 5 to 8% CO_2 (candle jar).

Sabourard dextrose agar[k] contained in plastic petri dishes was used for the isolation of fungi. The agar was streaked then the dish was sealed with masking tape and incubated at room temperature.

Thioglycollate broth[k] (5 ml per screw-capped tube) was incubated at 37°C for 1 week after inoculation. If growth was macroscopically evident, the broth was streaked on brain heart infusion agar and

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[k]Difco Laboratories, Detroit, MI 48233.

[l]Pfizer Ind., New York, N.Y. 10017.

incubated aerobically as above or anaerobically in a sealed jar which contained a disposable gas generator envelope.[m]

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[m]Gas Pak[R], BBL, Div. Becton, Dickinson & Co., Cockeysville, MD 21030.

RESULTS

Production of Disease in Cats

In an attempt to produce disease and to obtain a consistent inoculum, Inoculum 1 was injected into 2 mature cats (M 1 and M 2). As shown in Table 1, the 2 cats did not develop clinical signs or gross pathological lesions of FIP during a 60 day observation period. The same inoculum was found to produce signs of the disease, starting at 40 days after inoculation, in one cat (M pred 3) which had been pretreated with prednisolone.

Later, when this method of prednisolone pretreatment was used on 2 kittens (K pred 4 and K pred 5) followed by injection of Inoculum 2, FIP was produced after a 3 day incubation period. Inoculum 2 was then injected into a kitten (K 6) which had not been pretreated with prednisolone and this kitten developed FIP after only a slightly longer incubation period than in K pred 4 and K pred 5.

Since Inoculum 2 which was a spleen homogenate had produced disease readily in a kitten which was not pretreated with prednisolone, it was decided to try Inoculum 3 which consisted of ascitic fluid from the same cat as the spleen homogenate of Inoculum 2 in 2 kittens (K 7 and K 8). This also produced clinical disease in a short time (5 days and 3 days, respectively). Kitten 8 was killed 3 days after the temperature exceeded 40°C (7 days after inoculation) and the standard inoculum (Inoculum 4) was prepared from the spleen of this kitten. This standard

TABLE 1--Methods of Production of Experimental Feline Infectious Peritonitis (FIP)

Cat Number	Sex	FIP Inoculum Number *	Amount Injected IP (ml)	Clinical Disease	Incubation Period ** (Days)	Total Days Temperature Exceeded 40°C	Post Inoculation Day Killed Moribund
			Mature Cats (over 1 year old)				
M 1	M	1	10	No	8†	0	60‡
M 2	F	1	20	No	---	0	60‡
M pred§3	F	1	8 + 2 SQ	Yes	55//	1	64
			Kittens (8 to 16 weeks old)				
K pred 4	M	2	2.5	Yes	3	9	12
K pred 5	F	2	2.5	Yes	3	9	12
K 6	F	2	2	Yes	7	13	21
K 7	M	3	0.5	Yes	5	4	8#
K 8	F	3	1	Yes	3	3	7#
K 9	M	4	0.5	Yes	5	5	13
K 10	F	4	0.5	Yes	5	6	12

*Inocula 1, 2, and 4 were splenic homogenates diluted to a final concentration of 1:5 with 0.01 M phosphate buffered saline pH 7.4. Inocula 1, 2, and 3 were from naturally infected cats. Inoculum 3 was undiluted ascitic fluid from the same cat as inoculum 2. Inoculum 4 (the standard inoculum) was from cat K 8, so it is pass 2 of Inoculum 2.

**Time from inoculation to the day the temperature first exceeded 39.7°C.

†Temperature rose on day 8 only, no other signs of clinical disease.

‡Killed at 60 days, no gross pathological lesions.

§Prednisolone acetate, 22 mg/kg/day, IM for 4 days preceding injection with the standard inoculum and again the day of the injection and PID 1, and 11 mg/kg on PID 2 and 5.5 mg/kg on PID 3.

// Clinical signs of depression and rough coat starting 41 days after inoculation.

#Killed 4 days after temperature exceeded 40°C and before it reached the moribund stage.

inoculum was tested in kittens K 9 and K 10 using 0.5 ml inoculated intraperitoneally so that these results could be compared with results from subsequent experiments in which the inoculum was subjected to various chemical and physical agents.

The cat (M pred 3) and the kittens (K pred 4 - K 10) which developed clinical signs of FIP also showed characteristic gross lesions of FIP at necropsy (Figure 1). Of the lesions which occurred, 3 were used as criteria for determining whether or not disease was produced in all subsequent experimental cats. These were (1) deposits of granular, gray-white exudate intermittently present on the serosa of the abdominal viscera, (2) vascular engorgement in the omentum, and (3) discrete granular serosal elevations. These 3 types of lesions were present in all cats which had FIP whether they had a short clinical course of the disease or a longer more chronic course as in the case of the mature cat M pred 3. Other lesions seen in some but not all cats with FIP were: the presence of ascitic fluid; discrete or diffuse white foci just under the liver capsule or scattered throughout the parenchyma of the liver; a contracted, gelatinous omentum containing numerous lesions; and serosal lesions most obvious over the gall bladder, urinary bladder, and colon. Lesions on the diaphragm were rarely seen except in 2 kittens injected with a mixture of the standard inoculum and serum from a recovered cat. These two kittens will be detailed later. Lesions in the pleural cavity were never seen in the experimental cats.

Characterization of the Infection

The results of the experiments in which the standard inoculum was subjected to various physical or chemical treatments before injection

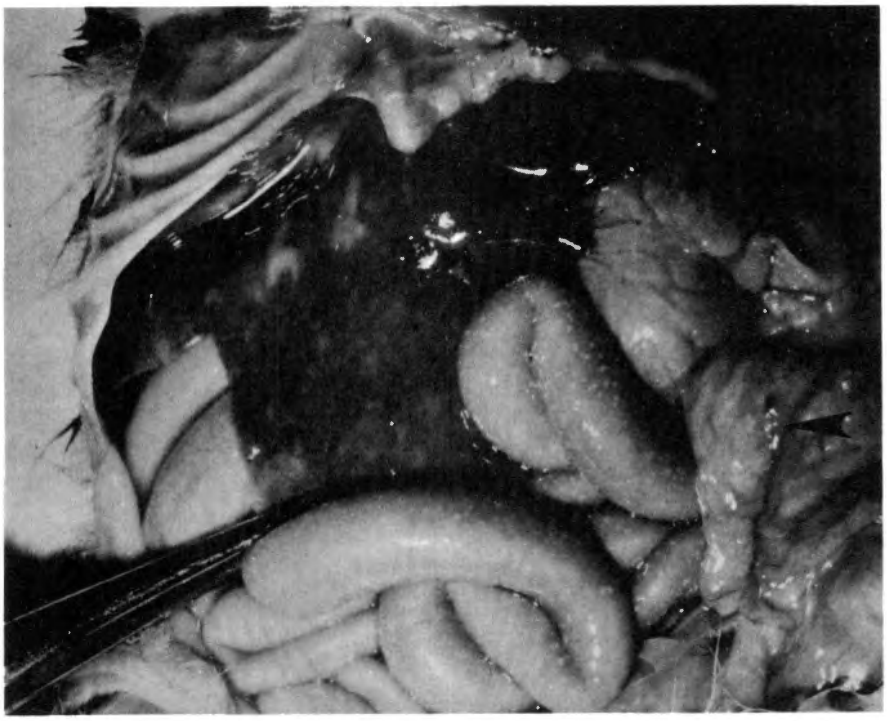

Figure 1--Viscera of a kitten (K 10) inoculated intraperitoneally with the standard inoculum. The kitten was killed in the moribund stage and shows emaciation associated with the musculature over the ribs and whitish focal lesions under the capsule of the liver. These lesions range from less than 1 to several centimeters in diameter. The serosal surface of the intestines shows white, discrete, elevated foci. The omentum (arrow) is seen as a contracted, thickened, edematous, adherent mass.

into kittens are summarized in Table 2. From these results it can be said that under the conditions of these experiments the infectious agent was found to pass through a filter with an APD of 200 nm, was not infectious after treatment with ether, was infectious after exposure to pH 5 but not 3, and produced variable results of exposure to heat. The infectious agent in the standard inoculum was stable at 4°C for 18 hours (as shown by the fact that the inoculum adjusted to pH 5 produced FIP). In addition, the standard inoculum produced disease in experimental cats after storage at -70°C for at least 1 year.

To characterize the infectious agent further, its ability to replicate in neonatal mice treated with corticosteroid was determined. None of the mice in the 3 passages of the inoculum in Group 1 (standard inoculum) or Group 2 (prednisolone and the standard inoculum) showed neurological signs, ascites, rough coat, or gross lesions at necropsy.

Certain Aspects of the Host-Parasite Relationship

The results of the tests to determine the presence or absence of the infectious agent at various stages of the disease are summarized in Table 3. The infectious agent was present in Inoculum 5 (the early disease inoculum), Inoculum 6 (the mid-term disease inoculum), and Inoculum 7 (the late disease inoculum). The spleen from the recovered cat (inoculum 8) did not produce clinical signs of disease.

Kitten ED 23 (Table 3) did not appear sick during the 30 day period of observation although his temperature rose to 39.7°C on the ninth day after inoculation, respectively. On necropsy gray fibrinous exudate was seen on the serosal surface of the stomach only and engorgement and a minimal number of discrete foci were seen in the omentum. This was

TABLE 2--Infectivity of the Standard Inoculum After Exposure to Various Physical and Chemical Processes

Animal Number	Sex	Inoculum* Treatment	Clinical Disease	Incubation Period**	Total Days Temperature Exceeded 40°C	Post Inoculation Day Killed Moribund
F 11	M	Filtered, 200 nm	Yes	6	5	9
F 12	F	Filtered, 200 nm	Yes	13	4	29
E 13	M	Ether	No	9†	1	30‡
E 14	F	Ether	No	---	---	30‡
pH 15	M	pH 5	Yes	6	3	8
pH 16	F	pH 5	Yes	6	3	8
pH 17	M	pH 3	No	---	---	30‡
pH 18	F	pH 3	No	---	---	30‡
H 19	M	56°C, 30 min	Yes	5	2	6
H 20	F	56°C, 30 min	No	---	---	30‡
H 21	M	50°C, 1 hr	Yes	3	4	7
H 22	F	50°C, 1 hr	No	---	---	30‡

*This was the standard inoculum.

**Time from inoculation until the temperature exceeded 39.7°C.

†Temperature of 40°C on PID 9, no other clinical signs of disease.

‡If no clinical signs of disease were observed the kitten was killed at 30 days post inoculation.

TABLE 3--Infectivity of Homogenates Prepared From Spleens Collected From Kittens During Various Stages of FIP

Animal Number	Sex	Inoculum	Clinical Disease	Incubation Period (days)*	Total Days Temperature Exceeded 40°C	PID** Killed Moribund
ED 23	M	5 (Early disease)	No†	9‡	0	30§
ED 24	F	5 (Early disease)	Yes	11	3	13
MD 25	M	6 (Mid-term disease)	Yes	2	3	4
MD 26	F	6 (Mid-term disease)	Yes	8	3	12
LD 27	M	7 (Late Disease)	Yes	6	1	8
LD 28	F	7 (Late Disease)	Yes	6	3	8
RD 29	M	8 (Recovered)	No	---	---	30§
RD 30	F	8 (Recovered)	No	---	---	30§

*Time from the day of inoculation until the temperature exceeded 39.7°C.

**Post inoculation day.

†FIP diagnosed at necropsy. Lesions included engorged omentum with discrete plaques on the upper half of the omentum and on the serosa of the anterior half of the stomach.

‡Temperature of 39.7°C on day 9 only.

§If signs of disease did not develop, the cat was killed at PID 30.

the only cat in the experiment which showed lesions consistent with FIP at necropsy but did not show definitive clinical signs of disease.

Varying the concentration of the standard inoculum before it was injected into kittens caused noticeable differences in production of disease (Table 4). The inoculum produced disease in cats when a 1:10 dilution was inoculated but not when a 1:100 dilution was inoculated.

The site of replication of the causative agent of FIP was studied by using the fluorescent antibody test. For this purpose sections of liver and spleen from a cat which had had naturally acquired infection and disease were compared with those from a cat thought to be susceptible to FIP. The tissue sections made from the tissues taken from the susceptible cat were negative for specific immunogens by both the direct and indirect fluorescent antibbdy technique. The indirect test showed more nonspecific staining than the direct test but no definite areas of fluorescence were seen with either. The direct test used with the infected tissues showed discrete fluorescent particles in the cytoplasm of most cells in the parenchyma of the liver. There was also some nonspecific fluorescence. Sections of liver from cats killed in the early, middle, and moribund stage of the disease as well as sections of nondiseased liver were surveyed for the infectious agent by using the fluorescein conjugated serum from the cat that had acquired the infection and disease from its environment. Faintly fluorescent but discrete particles, often ringing the nucleus, were seen in the cytoplasm of parenchymal cells in all sections of liver taken from the cats with FIP but none in the sections from the nondiseased cat. The cells with the fluorescent particles were diffusely scattered throughout the section

TABLE 4--Relationship Between Disease Production and Concentration of Inoculum

Animal Number	Sex	Inoculum* Dilution	Clinical Disease	Incubation Period ** (Days)	Total Days Temperature Exceeded 40°C	PID† Killed Moribund
Conc 31	M	1:10	Yes	8	3	11
Conc 32	F	1:10	Yes	7	3	11
Conc 33	M	1:100	No	---	---	30‡
Conc 34	F	1:100	No	---	---	30‡
Conc 35	M	1:1000	No	8§	1	30‡
Conc 36	F	1:1000	No	---	---	30‡

*This was the standard inoculum.

**Time from inoculation until the temperature exceeded 39.7°C.

†Post inoculation day.

‡If clinical signs of disease did not develop, cat was killed at PID 30.

§Temperature of 39.9°C on day 8, 41.1°C on day 9, normal temperature for the rest of the days. No lesions of FIP at necropsy.

and did not appear to be confined to cells in or surrounding what appeared to be discrete areas of parenchymal cell abnormality.

Table 5 summarizes the results of the neutralization studies in which the standard inoculum was added in a 1:1 ratio to 3 different sera. The standard inoculum and serum without specific immune factors to FIP were injected into kittens N Pre 37 and N Pre 38 and the standard inoculum and serum obtained from a kitten in the late stage of the disease were injected into kittens N LD 39 and N LD 40. These 4 kittens all developed FIP to the same degree as seen in kittens K 9 and K 10 (Table 1), which were injected with the untreated standard inoculum. However, injection of a mixture of the standard inoculum and serum from the recovered cat into kittens N R 41 and N R 42 produced a disease with a longer incubation period, with a more moderated temperature curve, and of a more chronic form. The temperature of N R 41 was 39.9°C for 1 day only and over 39.4°C on only 4 days and the temperature of N R 42 was 40.8°C for 1 day only and over 39.4°C for 7 days. These kittens had many hard, elevated lesions on both the visceral and parietal peritoneum (Figure 2), abundant ascitic fluid, and white foci resembling necrosis under the liver capsule and scattered through the hepatic and splenic parenchyma. The lesions in the diaphragm (Figure 3) were especially noticeable in these 2 kittens. These lesions showed many linearly-arranged necrotic foci and plaques adhering to the peritoneal surface of the diaphragm. The majority of kittens with experimental FIP seen in these experiments showed lesions on the diaphragm only rarely.

The recovered cat was challenged to see whether or not it was immune to FIP. The inoculum given was the standard inoculum. This

TABLE 5--*In vivo* Neutralization of the Infectious Agent of FIP

Animal Number	Sex	Type Serum Added to Inoculum*	Amount of Inoculum (ml)	Clinical Disease	Incubation Period** (Days)	Total Days Temperature Exceeded 40°C	PID† Killed Moribund
N Pre 37	M	Preinoculation	1.0	Yes	5	4‡	8‡
N Pre 38	F	Preinoculation	1.0	Yes	3	6‡	8‡
N LD 39	M	Late Disease	1.0	Yes	5	4‡	9‡
N LD 40	F	Late Disease	1.0	Yes	3	5‡	9‡
N R 41	M	Recovered	1.0	Yes	10	0	19
N R 42	F	Recovered	1.0	Yes	8	1	19
R 43§	M	Inoculum Alone	0.5	Yes	6	5	10
M C 44//	M	Inoculum Alone	0.5	Yes	6	7	14
M C 45//	F	Inoculum Alone	0.5	Yes	4	1	10

*Standard inoculum used alone or mixed 1:1 with 1 of 3 sera.

**Time from inoculation to first day temperature exceeded 39.7°C.

†Post inoculation day.

‡Killed 4 days after temperature exceeded 40°C which was before it reached the moribund stage.

§Recovered cat, mature.

// Mature cats which served as controls for the pathogenicity of the inoculum for mature cats.

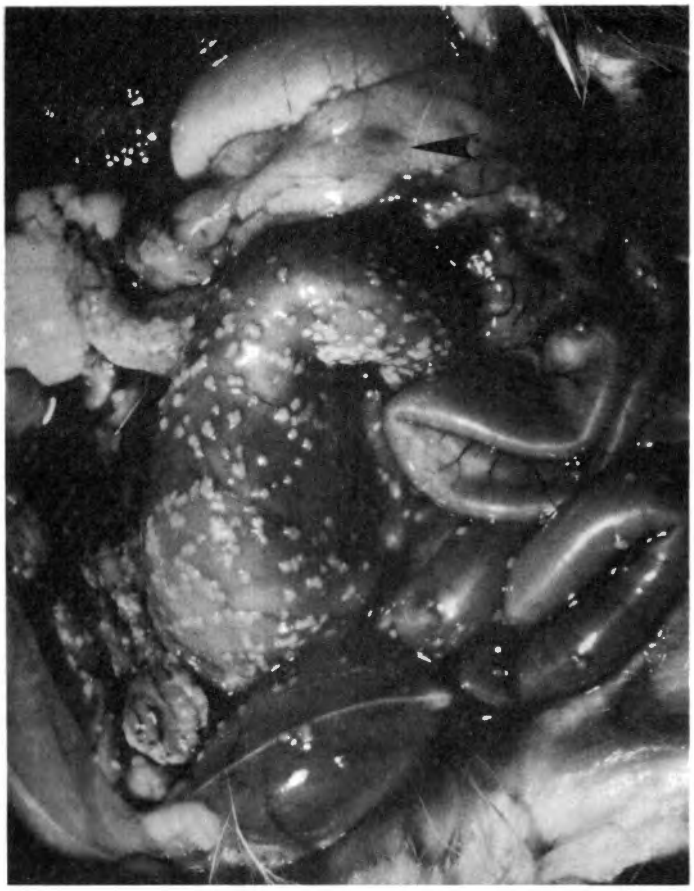

Figure 2--Viscera of a kitten (N R 42) inoculated intraperitoneally with a mixture of the standard inoculum and serum from a recovered cat. The kitten, which was killed in the moribund stage, had white, sharply defined, serosal elevations with subjacent hyperemia over the serosal surfaces. These lesions were especially prominent over the colon. The omentum (arrow) can be seen as an edematous mass, hyperemic, and containing white focal lesions.

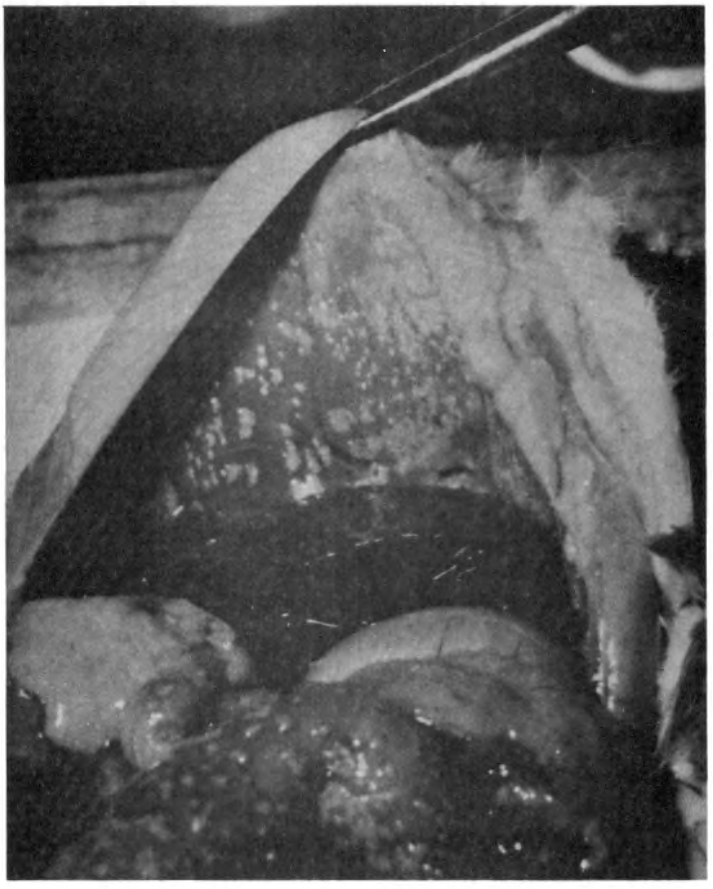

Figure 3--Diaphragm of a kitten (N R 42) inoculated intraperitoneally with a mixture of the standard inoculum and serum from a recovered cat. The diaphragm has been exposed to show the linearly oriented, white, elevated, plaque-like lesions.

recovered cat had been splenectomized approximately 4.5 months previously. As a basis for comparison, 2 supposedly susceptible mature cats with intact spleens were inoculated with the standard inoculum. The results of these experiments are summarized in Table 5. The recovered cat (R 43) displayed the same degree of susceptibility to the infectious agent in the inoculum as did the 2 susceptible mature cats (M C 44 and M C 45).

The infectious agent of FIP did not spread from inoculated to noninoculated kittens and produce infection or infection and disease. None of the room (RC 46 and RC 47) or cage (CC 48 and CC 49) controls which were included in the various experiments displayed any clinical signs or gross pathologic lesions. Furthermore, Inocula 9 and 10 which were composed of splenic homogenate from the room and cage control kittens, respectively, failed to produce infection and disease when inoculated into susceptible kittens. This was apparent because of the lack of clinical signs or gross pathologic lesions 30 days after inoculation in these kittens (Inoculum 9 was inoculated into kitten T RC 50 and T RC 51; inoculum 10 was inoculated into kittens T CC 52 and T CC 53). Four noninoculated control kittens and 2 noninoculated mature cats maintained in the normal colony did not develop FIP or any other disease during the course of these experiments.

A summary of the study designed to measure sex differences in susceptibility to FIP is presented in Table 6. The 2 male kittens which developed FIP when their female test mates did not were both involved in the study of the effect of heat treatment on the standard inoculum. The sex differences in the mature cats were not included in Table 6 since they were not matched by age, weight, or possible previous exposure to

TABLE 6--Sex Differences of Paired Kittens Injected With the Standard Inoculum

Differences Shown	Number of Kittens	Animal Numbers
Male More Susceptible Than Female		
Male died, female no disease at 30 days	2	H 19, H 21
Male had a shorter incubation period	2	F 11*, MD 25**
Male had short, sharp temperature rise, no gross lesions of FIP at necropsy; female had no temperature rise or gross lesions of FIP at necropsy	2	E 13†, Conc 35‡
Total males showing difference	6	
Female More Susceptible Than Male		
Female fatal disease, male short, sharp temperature rise and no gross lesions of FIP at necropsy at 30 days post inoculation	1	ED 24§
Total females showing difference	1	

*F 11 had an incubation period of 6 days while F 12 (the female test mate) had an incubation period of 13 days.

**MD 25 had an incubation period of 2 days while MD 26 (the female test mate) had an incubation period of 8 days.

†Short, sharp temperature rise on day 9 only.

‡Short, sharp temperature rise on days 8 and 9 only.

§ED 24 (female) had temperature over 39.7°C starting on PID 11, killed moribund on PID 13, while ED 23, the test mate, had a one day temperature rise of over 39.7° on day 9.

other cats with FIP. However the difference found in susceptibility between mature female and male cats is noteworthy. Inoculum 1 which was injected into 1 male and 1 female mature cat (Table 1) produced a temperature rise in the male but not in the female, however no gross pathologic lesions of FIP were present at necropsy in either cat. When M 44 and M 45 (Table 5) were inoculated with the standard inoculum, M 45 (the female) showed a shorter incubation period. M 45 was approximately one half the weight and size of M 44 and the previous exposure of these cats to FIP was not known.

Microorganisms Associated with FIP

Only rarely have microorganisms been isolated from cats with FIP and these organisms have been considered to be nonpathogens or post mortem invaders (11, 25, 32). In spite of this it seemed worthwhile to culture the tissues of the cats used in these experiments to determine if any of them died from a bacterial infection complicating FIP or to determine if any bacteria or fungi are regularly associated with FIP.

Mycoplasma spp. were not isolated from any of these cats. Bacteria and fungi were not consistently isolated. In fact the majority of specimens cultured for bacteria and fungi produced no growth at all. The organisms isolated from tissues were a nonhemolytic Corynebacterium sp. isolated from the liver of N LD 39; Hormodendrum sp. isolated from the liver, spleen, and omentum of M 3; and Aspergillus sp. from the liver and spleen of one of the cage control kittens (CC 49), and from the liver, spleen, omentum and mesenteric lymph node of one of the kittens (T CC 52) which had been inoculated with inoculum 10 (this

inoculum was a splenic suspension obtained from the pool of the spleens of the 2 cage controls CC 48 and CC 49).

The Hormodendrum sp. was isolated most consistently on the PPLO medium without inhibitors with fewer colonies being found on Sabourard's agar medium. On the PPLO medium the colonies were small, translucent, and usually did not revert to the typical mycelial form until transfer to Sabourard's agar medium. The small translucent colonies serially transferred on PPLO medium would occasionally produce black yeast like colonies which would revert to the mycelial stage when transferred to Sabourard's agar medium. The individual cells from the Hormodendrum sp. colony growing on the PPLO medium appeared to be only faintly Gram negative, stained red with Giemsa's stain, and were coccoid in shape. The Aspergillus, sp. on the other hand never produced abberrant colonies or individual vegetative forms on PPLO or Sabourard's agar medium.

DISCUSSION

One of the major concerns of this investigation was to develop a system of animal experimentation which would produce FIP consistently in a reasonably short time and with the results readily capable of interpretation and comparison, either clinically, or at necropsy, or both.

The system as developed consisted of inoculating 0.5 ml of 20% homogenate of spleen collected during the acute stage of illness intraperitoneally into young kittens.

By using young kittens obtained at weaning or shortly thereafter it was thought that the kittens would have had a minimum chance of previous exposure to FIP, would be in the most susceptible age group, and could be considered to be a consistent group of animals as opposed to adult cats obtained from an animal pound, which varied by age, weight, and may have had some previous exposure to FIP. It was thought that by pairing the kittens by sex as well as weight and age, some concept of sex differences in experimental FIP could be determined.

The small volume of inoculum (0.5 ml) was chosen because it would produce the least amount of response to foreign protein but still produce disease after a relatively short incubation period. Since the volume used for inoculation was small, all kittens used in the investigation and inoculated with the standard inoculum received an inoculum from the same splenic homogenate. The spleen was chosen as the source of the inoculum because it was shown to contain infectious material

and it could be processed so that an inoculum with a homogeneous consistency was obtained. The ascitic fluid was discarded as a source of inoculum because of the possibility of large amounts of antibody being present and also the fact that the viscous nature of the ascitic fluid made it difficult to homogenize and thus produce a uniform inoculum.

One of the most important aspects of the system was the collection of the spleen at the height of the acute stage of the disease and not at a late stage of the disease process as was the case in the first inoculum tested. Many investigators have used tissue from cats diagnosed to have FIP at necropsy as inocula for experimental animals but have been unable to produce disease 100% of the time. It was thought that by collecting the spleen during the acute stage of the disease the infectious agent would be present in the largest number.

By using the intraperitoneal route of inoculation, a consistent disease pattern was produced and the gross pathological lesions were readily interpreted at necropsy. Ward, et al. (29) have shown that when FIP is produced by injecting infectious material by the intraperitoneal route the omentum is contracted into a thick mass with foci of necrosis over the peritoneal serosal surfaces while similar material inoculated subcutaneously produced foci of necrosis at the site of inoculation and usually at one or more other sites such as the omentum, liver, lungs, brain, eye, bone marrow, etc. However, they found that the only consistent site of the lesion following the subcutaneous route was at the site of inoculation. Since the concern of this investigation was not the pathogenesis of the disease, the intraperitoneal route of inoculation, with its more obvious appearance of gross lesions seemed

to be the route of choice.

Tissue homogenates as inocula and ascitic fluid from 2 naturally infected cats were compared as to their ability to produce disease in mature cats and kittens. The inoculum (Inoculum 1) produced clinical disease only in a mature cat which had been pretreated with prednisolone and not in 2 mature cats which had not been pretreated. The second inoculum (Inoculum 2) produced clinical disease almost as rapidly in a kitten which had not been pretreated with prednisolone as in 2 kittens which were pretreated (Pass 1 of Inoculum 2). Pass 2 of Inoculum 2 produced disease with the same short incubation period in kittens as in mature cats. This suggests that when either a certain number of infectious particles or a virulent strain is used the mature cat is just as susceptible as the kitten. The reports that FIP occurs naturally much more frequently in cats under 1 year of age than in older age groups (9, 18, 32) may indicate that under natural conditions (1) the causative agent is more virulent for the kitten, (2) older cats have some form of resistance associated with age or acquired immunity, or (3) the kitten is more frequently exposed. The differences noted in these 2 inocula (Inocula 1 and 2) which may have been due to the concentration of the causative agent in the tissues taken from naturally infected cats may explain why various investigators have obtained different morbidity rates in experimental animals.

Since the 3 mature cats each received a large inoculum and did not develop diseases readily, it is possible that large amounts of antibody were present in the ascitic fluid (Inoculum 1) or that the large amount of foreign protein in both inocula stimulated phagocytosis to such an extent as to eliminate the infectious particles before they had a chance

to become established. However, other investigators have reported producing disease with inocula of the size (5).

Corticosteroids have previously been shown to enhance the production of various diseases, including those of viral etiology, in experimental and in nonexperimental animals. Corticosteroids are thought to produce these results by their anti-inflammatory effect, their capacity to depress the immune response, and their effect on interferon production (6). Corticosteroids are known to decrease phagocytosis and in addition they have been shown to stabilize lysosomal membranes so that digestion of engulfed particles is decreased. Any or all of these may explain why prednisolone enhanced the capacity of the first inoculum to produce disease.

The experiment involving the exposure of the standard inoculum to various physical and chemical processes was designed to reveal some of the basic characteristics of the infectious agent. Previous research workers (7, 28, 30, 34) have reported seeing virus-like particles in electron micrographs of tissues from cats experimentally infected with FIP. Since the same type of virus-like particles, which have been described as Corona-like, have apparently been described by 2 different authors, it was decided to establish tests which might help to determine whether or not the infectious agent is a Corona-like virus.

Since the infectious agent of FIP has not been cultivated in tissue culture or in embryonated eggs, the methods which could be utilized within the framework of the present model were limited. The results of various physical and chemical processes on the standard inoculum suggested that the infectious agent can be considered to be a virus. The agent was ether sensitive and capable of withstanding pH 5

for at least 18 hours at 4°C. This pH susceptibility is consistent with Coronavirus (12). The variation in the results of the temperature experiments may have been due to the presence of cellular material which protected the infectious particles enough to allow some particles to avoid being inactivated under the test conditions.

By the fluorescent antibody technique, antigen was detected in the cytoplasm of cells in the liver. To obtain more definitive results by this method, immune serum made by injection of a purified immunogen prepared from the causative agent of FIP would have to be used. The preparation of such immune serum must await the isolation of the causative agent of FIP.

The results of experiments designed to determine the presence or absence of the infectious agent during various stages of the disease showed that the infectious agent was present during the early, middle, and late stages of the disease. During the early stage of the disease (first temperature rise over 40°C, which was 4 days after inoculation), the presence of a smaller number of infectious particles in the inoculum may account for the delayed incubation period in one of the kittens and the subclinical case of FIP noted in the other kitten given this material. Results of fluorescent antibody studies suggested that antigen was also present in liver cells in all 3 disease stages.

If more kittens had been available it would have been desirable to kill kittens at 48 hour intervals after injection with the standard inoculum and use the homogenates prepared from the spleens from these animals as inocula. In this way a somewhat more accurate determination could be made as to how long after injection (1) the infectious particles become mature or (2) are present in large enough quantity to be infectious.

The injection of the homogenized spleen obtained from the recovered cat into test cats showed that either no infectious agent was present at that time (172 days after the first clinical signs) or that if the infectious agent was present it was present in insufficient quantity to produce clinical signs of disease.

Dilutions of the standard inoculum before injecting it into experimental animals showed that either a large number of infectious entities was necessary to produce disease or that the infectious agent was present in the standard inoculum in a small amount and was diluted out by the 1:100 and 1:1000 dilutions. Kitten Conc 35, which had been inoculated with the standard inoculum diluted to 1:1000 had a temperature rise on day 9 to 41.1°C. This temperature seems to indicate that infection was present. Since this kitten showed no gross signs at necropsy, it can be postulated that the kitten was able to contain the infection completely, if in fact there was one.

When various sera were mixed with the standard inoculum before being injected into kittens, complete neutralization of the infectious particles was not found. The standard inoculum plus the preinoculation serum (which was injected into kittens N Pre 37 and N Pre 38) gave approximately the same lack of effect as the standard inoculum plus the serum collected late in the course of the disease (which was injected into N LD 40 and N LD 41). The kittens injected with a mixture of serum from the recovered cat plus the standard inoculum (N R 41 and N R 42) showed a disease course with a slightly longer incubation period, a more chronic form, and, most noticeably, a more moderate fever curve. At necropsy these 2 kittens showed an obvious difference in the gross appearance of lesions as compared with kittens which were given the

standard inoculum alone. In these 2 kittens (N R 41 and N R 42) a much greater number of large, raised plaques over the visceral peritoneum was seen and this was most noticeable on the peritoneal surface of the diaphragm. In this series of tests, as in previous tests, the presence of cellular material in the standard inoculum may have protected a few of the infectious particles from neutralization.

At the time (308 days after receipt) that permission was finally granted to challenge the recovered cat (R 43), he was shown to be as susceptible to FIP as the control cats. R 43 had been splenectomized 136 days earlier, so this did not represent a challenge of a normal recovered cat but instead challenge of a cat whose reticuloendothelial and immune systems had been seriously compromized. The results obtained with the serum neutralization test (Table 5) indicate that the serum collected from the recovered cat 239 days earlier apparently had some neutralizing effect when mixed with the standard inoculum as seen in kittens N R 39 and N R 40 and yet after challenge there was not a delay in incubation period, elongation of time of clinical disease, or a decreased febrile course. This might indicate that some neutralizing antibody was present in the serum collected at day 69 and none was present at day 308. It would be interesting to ascertain how long an effective antibody level remained in a recovered cat and whether or not there is more than one serologic type of the infectious agent of FIP. Challenge of recovered cats with ascitic fluid (taken from them during the active stage of the disease) at various intervals after recovery might give an indication of the establishment and longevity of an effective immunity. Challenge of recovered cats with homologous and heterologous ascitic fluid should indicate whether or not there is a

difference in immunogens associated with the infectious agent of FIP.

Room controls and cage controls did not show clinical signs of disease during the course of the experiments. Perhaps if the control kittens had been left in a cage with a series of kittens showing clinical signs of FIP or had been left in the room with infected kittens for a much longer period of time they might have developed the disease. The main thing the controls showed is that either the infectious agent is not transmitted by airborn means or if it is the resultant infection does not produce disease.

The development of FIP more often in the male than in the female in kittens which were paired by sex (Table 6) seems to confirm the findings that natural cases of FIP occur more often in the male than in the female cat (3, 7, 17, 32). These differences are shown by (1) males dying while the females showed no disease, (2) males which had obviously shorter incubation periods than the female, and (3) males which had a 1 or 2 day short, sharp temperature increase but no disease while the females had no short, sharp temperature increase or disease. The 1 or 2 day temperature rise of kittens E 13 and Conc 35 occurred at day 8 or 9 after inoculation. This time period seems consistent with the development of disease. If the short temperature rise had been due to a manifestation of a response to foreign protein it would be expected that the temperature rise would have occurred much earlier.

It is of interest that, while the mature cats were not included in Table 6 since they were not paired by weight, age, or previous exposure to cat populations, the mature male M 1 developed a short, sharp temperature increase on day 8 after inoculation while M 2, the female, did not show any increase in temperature.

The only time that female kittens appeared to be more susceptible than males was in the case of ED 23 in which the male had an incubation period of 9 days as opposed to 11 days for the female. The male, however, did not develop clinical disease and was diagnosed as having FIP only at necropsy which was performed 30 days after inoculation. The female (ED 24) developed the more usual febrile course which consisted of temperature of over 40°C for 3 days and was killed at 13 days when the kitten was in the moribund stage.

It should be emphasized that in all of the experiments only 2 animals were used per group because of the shortage of experimental kittens. However certain trends appear to have been established and the results seem to indicate that the male kitten is more susceptible to experimental disease with the causative agent of FIP. Most of the instances in which the male showed the greater susceptibility were cases in which the total number of infectious particles might have been small. The treatments of the inocula for these kittens were heat treatment of 56°C for 30 minutes (H 19), or of 50°C for 1 hour (H 21), filtration (F 11), ether treatment (E 13), and dilution of the inoculum 1:1000 (Conc 35). The inoculation of the mid-term disease inoculum into MD 25 also showed the male more susceptible (shorter incubation period) yet it would be expected that this inoculum had approximately the same number of infectious particles as the standard inoculum. It is possible then that when the number of infectious particles is high both sexes are equally susceptible and that when the number is minimal the male is more susceptible than the female. Thus in nature the male kitten may be responding to a minimum number of infectious particles while the female is not.

Very few microorganisms were isolated from the tissues of the cats used in these experiments. The cats from which these isolation attempts were made had been killed either when the cat was in the middle of the course of the disease or when it was moribund. This would account in part for the lack of organisms isolated from the experimental cats as opposed to clinical cases of cats with FIP which are often necropsied several hours after death and thus in these latter cats the various enteric bacteria found in tissues could be an indication of postmortem invasion and not a contributing factor in FIP. The lack of culturable microorganisms as well as the inability to detect microorganisms by the use of various stains with tissue sections (27) would tend to substantiate the contention that the disease is caused by a virus.

The majority of cats and kittens used in these experiments were injected with an aliquot from a common inoculum. Very few bacteria or fungi were isolated from the tissues of these cats. The cat from which the aberrant colonial and morphologic form of Hormodendrum sp. was isolated had been inoculated with Inoculum 1. The cat from which Inoculum 1 was prepared was also found to harbor the aberrant form of Hormodendrum sp. This unusual form of Hormodendrum sp. has also been isolated from natural cases of FIP (21). Aspergillus sp. was isolated from a cage control kitten (CC 49) and also from one of the 2 kittens (T CC 52) which was inoculated with the spleen from this control cat. It is of interest that T CC 52 harbored the Aspergillus sp. for 30 days after inoculation without showing gross pathological lesions. It is also noteworthy that the Aspergillus sp. was not isolated from the test mate (T CC 53) of T CC 52 even though both received an aliquot from the same inoculum.

SUMMARY

A system for the consistent production of disease in experimental feline infectious peritonitis (FIP) was developed. This included the use of young test kittens injected intraperitoneally with a small quantity of splenic homogenate obtained from a kitten killed in the acute stage of the disease. Determination of disease by gross appearance of lesions at necropsy was found to be satisfactory under the test conditions.

The results obtained by subjecting a standard inoculum to various physical and chemical treatments suggested that the infectious agent of FIP was a virus since it was filtrable, sensitive to ether, resistant to pH 5 but not to pH 3, and at least partially inactivated at 50° and 56°C. The infectious agent was found to be present in tissues in the early, middle, and late stages of the disease and the infectious agent was present after the original inoculum was diluted 1:10 but not after dilution of the inoculum 1:100.

A cat which had had FIP and recovered clinically had some serum neutralizing antibodies shortly after clinical recovery. The spleen from this cat did not prove infectious when it was introduced into experimental cats. When the recovered cat was challenged it developed FIP with the same clinical course of the disease as that produced in the inoculated control cats.

Male kittens were shown experimentally to be more susceptible than female kittens to a lower concentration of the infectious agent but

both sexes were equally susceptible after inoculation with the undiluted standard inoculum. This tends to substantiate the reports of increased susceptibility of males in naturally occurring disease.

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APPENDIX

Summary of Data Obtained from Each Experimental Cat

Cat		Inoculum				
Number	Sex	Number	Type	Treatment	Results	Remarks
M 1	M	1	As. Fl.*	None	No disease	Fever PID** 8
M 2	F	1	As. Fl.	None	No disease	---
M pred 3	F	1	As. Fl.	None	FIP	Cat pretreated with prednisolone, Hormodendrum sp. isol.
K pred 4	M	2	Spl. homog.†	None	FIP	Kitten pretreated with prednisolone
K pred 5	F	2	Spl. homog.	None	FIP	Kitten pretreated with prednisolone
K 6	F	2	Spl. homog.	None	FIP	---
K 7	M	3	Asc. fluid	None	FIP	---
K 8	F	3	Asc. fluid	None	FIP	---
K 9	M	4	Spl. homog.	None	FIP	---
K 10	F	4	Spl. homog.	None	FIP	---
F 11	M	4	Spl. homog.	Filtered, 200 nm	FIP	Shorter incub. period‡ than F 12
F 12	F	4	Spl. homog.	Filtered, 200 nm	FIP	---

APPENDIX (Cont.)

Cat		Inoculum				
Number	Sex	Number	Type	Treatment	Results	Remarks
E 13	M	4	Spl. homog.	Ether	No disease	Fever PID 9
E 14	F	4	Spl. homog.	Ether	No disease	---
pH 15	M	4	Spl. homog.	pH 5	FIP	---
pH 16	F	4	Spl. homog.	pH 5	FIP	---
pH 17	M	4	Spl. homog.	pH 3	No disease	---
pH 18	F	4	Spl. homog.	pH 3	No disease	---
H 19	M	4	Spl. homog.	56°C, 30 min	FIP	---
H 20	F	4	Spl. homog.	56°C, 30 min	No disease	---
H 21	M	4	Spl. homog.	50°C, 1 hr	FIP	---
H 22	F	4	Spl. homog.	50°C, 1 hr	No disease	---
ED 23	M	5	Spl. homog.	Early disease inoculum	FIP at necropsy	Fever PID 9 only
ED 24	F	5	Spl. homog.	Early disease inoculum	FIP	---
MD 25	M	6	Spl. homog.	Mid-term disease inoculum	FIP	Shorter incubation period than MD 26
MD 26	F	6	Spl. homog.	Mid-term disease inoculum	FIP	---
LD 27	M	7	Spl. homog.	Late disease inoculum	FIP	---
LD 28	F	7	Spl. homog.	Late disease inoculum	FIP	---

APPENDIX (cont.)

Cat		Inoculum				
Number	Sex	Number	Type	Treatment	Results	Remarks
RD 29	M	8	Spl. homog.	Recovered cat's spleen	No disease	---
RD 30	F	8	Spl. homog.	Recovered cat's spleen	No disease	---
Conc 31	M	4	Spl. homog.	Diluted 1:10	FIP	---
Conc 32	F	4	Spl. homog.	Diluted 1:10	FIP	---
Conc 33	M	4	Spl. homog.	Diluted 1:100	No disease	---
Conc 34	F	4	Spl. homog.	Diluted 1:100	No disease	---
Conc 35	M	4	Spl. homog.	Diluted 1:1000	No disease	Fever PID 8 and 9 only
Conc 36	F	4	Spl. homog.	Diluted 1:1000	No disease	---
N Pre 37	M	4	Spl. homog.	Neut. with preinoc. serum§	FIP	---
N Pre 38	F	4	Spl. homog.	Neut. with preinoc. serum	FIP	---
N LD 39	M	4	Spl. homog.	Neut. with late dis. serum	FIP	Corynebacterium sp.
N LD 40	F	4	Spl. homog.	Neut. with late dis. serum	FIP	---
N R 41	M	4	Spl. homog.	Neut. with recovered serum	FIP	Longer incub. period, more chronic course
N R 42	F	4	Spl. homog.	Neut. with recovered serum	FIP	Longer incub. period, more chronic course.
R 43	M	4	Spl. homog.	None	FIP	---

APPENDIX (cont.)

Cat		Inoculum				
Number	Sex	Number	Type	Treatment	Results	Remarks
M C 44	M	4	Spl. homog.	None	FIP	---
M C 45	F	4	Spl. homog.	None	FIP	Shorter incub. period than M C 44
RC 46	M	None	---	Room-control	No disease	---
RC 47	F	None	---	Room-control	No disease	---
CC 48	M	None	---	Cage-control	No disease	---
CC 49	F	None	---	Cage-control	No disease	Aspergillus sp.
T RC 50	M	9	Spl. homog.	Test of room control	No disease	---
T RC 51	F	9	Spl. homog.	Test of room control	No disease	---
T CC 52	M	10	Spl. homog.	Test of cage control	No disease	Aspergillus sp
T CC 53	F	10	Spl. homog.	Test of cage control	No disease	---

*Ascitic fluid

**Post-inoculation day

†Splenic homogenate

‡Incubation period

§Neutralized with preinoculation serum

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