

## THE VIRUS OF INFECTIOUS FELINE AGRANULOCYTOSIS\*

### I. CHARACTERS OF THE VIRUS: PATHOGENICITY

By J. T. SYVERTON, M.D., J. S. LAWRENCE, M.D., R. J. ACKART, M.D.,†  
W. S. ADAMS,† D. M. ERVIN,† A. L. HASKINS, JR.,† R. H. SAUNDERS, JR.,†  
M. B. STRINGFELLOW, M.D.,† AND R. M. WETRICH†

*(From the Departments of Bacteriology and Medicine, The University of Rochester  
School of Medicine and Dentistry, Rochester, New York)*

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Infectious feline agranulocytosis is a malady of cats only recently described (1-3).<sup>1</sup> This infection at its height can be readily recognized and differentiated from other feline diseases by blood studies which make apparent the characteristic profound granulocytopenia, a less pronounced leucopenia, and a marked relative lymphocytosis in the absence of thrombopenia and appreciable anemia. The cytological pictures of the bone marrow and peripheral blood are essentially similar to those which have been reported for human agranulocytosis. An additional characteristic pathological feature is the presence of intranuclear inclusion bodies of Type A (Cowdry) in the intestinal epithelial cells and in the reticular cells of the lymphoid tissue. Detailed descriptions of the clinical, hematological, and pathological findings have been made (1-3).

It was found, early in these studies (1) that the infective agent of infectious feline agranulocytosis has properties common to characteristic viruses. However, most of the data from experiments designed to yield information about this virus were reserved for detailed presentation and now comprise the substance of two papers. The experiments described in this paper deal with the nature, properties, and pathogenicity of the virus. The second paper, which follows, presents the results of experiments that deal with the immunological relation of the virus of infectious feline agranulocytosis to other viruses. As the experimental work reported in these two papers was done concurrently and the results are mutually supplementary, these papers are presented together. All materials and methods are described in the first paper. The second paper includes a discussion of the facts reported in both papers, a consideration of these findings in relation to several papers which unquestionably deal with the same disease and virus (4-6), and a bibliography (the references are numbered serially throughout the two papers).

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† Student Fellow in Medicine.

<sup>1</sup> The references for this paper and the succeeding paper are numbered serially throughout both, the bibliography being given at the end of the second paper.

240 cats with feline agranulocytosis have been observed in this laboratory during the past 4 years. The general characteristics of the disease are briefly as follows: An incubation period of from 4 to 7 days (usually 5 or 6) following exposure to the infective agent merges into the stage of clinical disease, which is characterized by listlessness, inappetence, and a prone position. Vomiting, diarrhea, and nasal and ocular discharges occur most irregularly. Death may intervene at any time after the 5th day following exposure. Oral or perianal lesions have not been noted. A desire for food is the best index of recovery, which usually requires only 5 or 6 days. The mortality rate approximates 50 per cent. The typical hematological changes mentioned above occur on from the 6th to 8th day after exposure. The most marked pathological findings are present in the bone marrow, lymphoid tissue, and intestinal mucosa. The bone marrow when examined at the height of the disease shows a hypoplasia and an absence of differentiation of the myeloid cells. The erythroid cells and the megakaryocytes are generally present in normal percentages. The reticuloendothelial cells of the spleen and lymph nodes show evidence of proliferation, and intranuclear inclusion bodies (Cowdry's Type A), as evidence of specific virus activity, are not infrequently present in the cells of the gastrointestinal mucosa, the lymphoid tissues, and bronchial mucous glands.

#### *Methods and Materials*

*Cats.*—410 domestic cats were utilized for passages and to determine the presence of active virus in test and control materials. They were also used in immunity tests.

It became immediately apparent when we began our studies of feline agranulocytosis that the highly infectious nature of the inciting agent and the natural prevalence of the disease among cats from thickly populated districts made it essential to select and maintain each experimental animal with great precautions. Accordingly, after the first 13 passages of the infective agent in series in cats (1), we limited as often as possible our supply to kittens from farms where a history of illness or death among their resident cats was denied. (Even then we not infrequently encountered animals that were immune to infection by the feline virus, as some of our protocols show.) Many farms, free from the disease, provided several litters each year. We found that it was to our advantage to bring the animals personally from the farm to our isolation quarters in order to avoid any possibility of exposure to the infectious agent. The cats on arrival were separated into groups of from 3 to 6 animals, which were maintained as entirely separate units under rigid isolation for from 12 to 92 days in rooms widely separated from the animal house and from each other. Each cat was housed in a single metal cage. During the period of isolation, the blood picture of each cat was followed by leucocyte and differential counts, which were made at from 1 to 3 day intervals (almost regularly every 2nd day). Many of the animals also had rectal temperatures taken. Only those animals that appeared normal throughout the period of isolation were used for experimental purposes. Following exposure to any material under test for infectiousness, each cat had blood studies made daily. Finally, most of the animals without overt evidence of illness after exposure to the virus were subsequently tested for active immunity by the injection of a massive challenge dose of virus of known pathogenicity or by exposure to animals with the disease. In

many of the experiments, 1 or 2 normal animals were maintained as controls under the same environmental conditions, but they were not handled except to have blood counts taken.

At times, a single experiment, and therefore a single group of cats, answered several of the questions under investigation in this study on the nature and properties of the etiological agent. Thus one experiment might yield the following information about the infective agent: its pathogenicity by a given route of inoculation; its distribution within the body of the host; and its filterability through a given candle, as determined by its pathogenicity following filtration. Accordingly, some of the 410 experimental cats employed may be referred to several times.

*Other Animals.*—120 albino Swiss mice, 54 inbred albino guinea pigs, 24 pure-bred New Zealand white rabbits, and 6 stock hybrid rabbits, 8 ground squirrels (*Citellus richardsonii* Sabine), and the chorio-allantoic membranes of chick embryos were tested for their susceptibility to the virus.

The animals were used in groups of from 2 to 4 when they were employed to determine species susceptibility to the virus, and in groups of from 2 to 6 when blind serial transfers of tissue were effected. The blood picture of each animal was followed for several days before injection.

*Virus of Infectious Feline Agranulocytosis.*—32 strains of virus have been employed. The original strain was derived from hepatic tissue and carried by rapid serial passage for 13 generations before being glycerinated (1). Subsequent to this first experience, we have used in our studies strains isolated from liver, spleen, intestinal mucosa, feces, urine, respiratory washings, and blood.

One strain of the virus of malignant panleucopenia (4, 5)<sup>2</sup> and 3 strains isolated from the tissues of cats diagnosed clinically as feline enteritis were studied<sup>2</sup> and found to be indistinguishable pathologically, clinically, and immunologically from the virus of infectious feline agranulocytosis.

*Other Materials Used for Inoculation.*—The viruses of hog cholera,<sup>2</sup> fox encephalitis,<sup>2</sup> B virus infection, herpes (HF strain), vesicular stomatitis, equine encephalomyelitis (Western type), and lymphocytic choriomeningitis were employed in attempts to learn if any relationship to the causal agent of infectious agranulocytosis could be demonstrated.

*Preparation of Virus Suspensions for Inoculation.*—The tissues, which were used to provide the virus of feline agranulocytosis, were obtained from animals immediately after they died spontaneously or were killed by chloroform or ether at the height of the disease (as determined by the characteristic profound granulocytopenia). The suspensions containing each of the other viruses were prepared from glycerinated tissues. When liver, spleen, brain, or lung was utilized, the tissue was thoroughly

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<sup>2</sup> We are indebted to F. W. Schofield and A. W. Bain of the Ontario Veterinary College, Guelph, Ontario, and to W. A. Hagan and W. S. Monlux of the New York State Veterinary College at Cornell University for glycerinated specimens of liver and spleen from cases diagnosed clinically as infectious feline enteritis; to J. F. Enders and W. A. Hammon of Harvard University Medical School for tissues and immune serum from cases diagnosed as malignant panleucopenia; to R. E. Shope for hog cholera virus contained in whole blood; and to R. G. Green for fox encephalitis virus.

trituated with alundum<sup>3</sup> in Locke's solution to yield a 10 per cent suspension. Meat extract broth was substituted for Locke's solution when filtration experiments were contemplated. (Before trituration, tissues preserved in glycerin were first washed three times in Locke's solution to remove excess glycerin.) This suspension was centrifuged horizontally at 1500 R.P.M. for 30 minutes, and the supernatant fluid directly, or after filtration, was used undiluted or in decimal dilutions to inoculate animals.

The procedure outlined above necessarily had to be modified when intestinal mucosa, blood, or body secretions and excretions were employed. Unmodified whole blood and urine were used. On the other hand, nasopharyngeal washings, intestinal mucosa, and feces (10 per cent by weight) were suspended in meat extract broth in a flask containing glass beads and shaken for 60 minutes in a Camp six-flask shaking machine operated by a  $\frac{1}{2}$  horse power electric motor. The fluid suspension that resulted was centrifuged at 2500 R.P.M. for 1 hour, and the supernatant liquid withdrawn and filtered through a Berkefeld "V," "N," or "W" candle, or a Seitz E-K disc. The filtrate was used to inoculate animals.

*Filtration.*—Berkefeld candles of "V," "N," and "W" porosity, and Seitz E-K discs were employed. New candles were used for the earlier experiments and reused in later experiments. All candles were cleaned by the successive passage of saturated potassium permanganate, saturated oxalic acid, and water, and sterilized by autoclaving. Filtration was effected by negative air pressure obtained by attaching the filter to a water system. (A water bottle and mercury manometer were inserted between the filter and the water system.) The negative pressures in centimeters of mercury routinely employed for filtration were as follows: "V" candles, 10 cm.; "N" candles and Seitz E-K discs, 20 cm.; and "W" candles, 30 cm. *Serratia marcescens* was employed to test the impermeability of the filters to microbial agents. When a filter was found to be pervious to this bacterium, the results were not accepted in evidence of the filterability of the causative agent.

*Preservation of Virus.*—50 per cent glycerin buffered to a pH of 7.2 with phosphates, and freezing followed by dessication were the methods used for preservation of the virus.

*Cultures.*—In our earlier bacteriological studies, each virus suspension was used to inoculate a wide variety of media which included Douglas's broth, blood agar plates, Loeffler's media, Fletcher's media, media for growth of the pleuropneumonia group (7), deep meat tubes, and a modified Bordet-Gengou media for incubation under aerobic, anaerobic, and microaerophilic conditions. In later bacteriological studies only Douglas's broth and blood agar plates were inoculated with heart's blood, liver, and spleen, and incubated under aerobic and anaerobic conditions.

*Pathological Examination.*—Autopsies were performed on most of the animals whose death resulted spontaneously or from the purposeful use of an anesthetic. Tissues were fixed in Zenker's (5 per cent acetic acid) fluid, sectioned, and stained according to Giemsa's method or by means of hematoxylin and eosin.

<sup>3</sup> Alundum, an electrically fused crystalline alumina, prepared by the Norton Company, Worcester, Massachusetts, was used because of its excellent "cutting" qualities.

## RESULTS

*Properties of the Feline Infective Agent*

It became apparent early in these studies that the causative agent was not readily cultivable, and that it satisfied criteria which are accepted as characterizing a virus (1). Nevertheless, the extreme contagiousity of the disease and the high incidence of immunity among stock adult cats led us to question if our positive transmission experiments employing filtrates for the inoculation of 24 animals (1), and the two positive transmission experiments employing filtrates for the inoculation of 4 animals, as reported by Hammon and Enders (4), constituted adequate evidence for the unequivocal filterability of the causative agent. Because of this reasonable doubt, we made every effort to recover a cultivable agent and thereby to eliminate bacteria, fungi, or pleuropneumonia-like organisms.

*Bacteriological Studies.*—The blood and visceral tissues were cultured by employing a wide variety of both common and special liquid and solid media (as described above), with incubation under aerobic, anaerobic, and microaerophilic conditions.

It was found that extraneous contaminants were present only rarely and most irregularly. Moreover, microscopic study of sections and smears prepared from all affected tissues have not disclosed recognizable bacteria or parasites, excepting in the tissues from the gastrointestinal and respiratory tracts.

It is apparent, therefore, that the results of the bacteriological studies supported other evidence which showed that the causative agent was a filterable agent.

*Filterability.*—The agent readily traverses Berkefeld candles of “V,” “N,” and “W” grades, and Seitz E-K discs.

Typical feline agranulocytosis resulted, (a) in 26 of 31 cats that were inoculated with Berkefeld “V” filtrates representing suspensions prepared in seven experiments from liver, spleen, intestinal mucosa, feces, lung, or respiratory washings; (b) in 7 of 10 animals inoculated with Berkefeld “N” filtrates representing suspensions prepared in two experiments from hepatic tissue; and (c) in 12 of 13 recipients of Berkefeld “W” filtrates prepared in four experiments from hepatic tissue.

It would appear from the foregoing results that the virus traverses Berkefeld “W” candles more readily than either Berkefeld “V” or “N” candles. A more likely explanation, however, is that the difference in results can be ascribed to the presence of immune animals among the recipients of the Berkefeld “V” and “N” filtrates. However, that may be, because of the ready filterability of the agent through Berkefeld “W” candles, the results of these filtration experiments have been interpreted to suggest that the virus is of relatively small size, probably  $35\mu$  or less. Obviously, of course, ultrafiltration experiments in which graded collodion membranes of known average pore diameter

are employed must be carried out before acceptable information on this point will be forthcoming.

*Resistance to Glycerol and Drying.*—The infective agent of feline agranulocytosis is similar to other viruses in its resistance to glycerol and to desiccation when frozen.

The virus, as contained in affected hepatic tissues, and kept in 50 per cent glycerol buffered at 7.2 for from 7 to 138 days, induced the characteristic disease in 4 of 11 cats employed in three experiments when the glycerinated tissue washed thrice in Locke's solution and prepared for inoculation was injected by the subcutaneous or intraperitoneal routes.

The virus can be preserved by drying while in the frozen state.

The virus, as contained in a 10 per cent suspension of hepatic tissue, was frozen at approximately  $-80^{\circ}\text{C}$ . in a mixture of cellulose and solid carbon dioxide, and dried by high vacuum distillation from the frozen state. The containers were then sealed under vacuum and stored at refrigerator temperature. For use, the desiccated material was resuspended in sterile water, and 5 ml. were inoculated intraperitoneally into each of 4 cats. Two of these 4 animals developed typical feline agranulocytosis, one on the 8th and the other on the 9th day after injection.

From this experiment it was concluded that the infectivity of the virus is not appreciably affected by desiccation *in vacuo*.

#### *Pathogenicity by Different Routes of Inoculation*

Cats of all ages have been found to be susceptible to infection when virus is administered by a variety of routes of inoculation.

*Intraperitoneal Route.*—Passage of the virus in cats has ordinarily been effected by the intraperitoneal route. This route seemed the most desirable because of the ease of administration and the assurance that all susceptible animals would contract the disease.

After having remained well during the 12 to 92 day period of isolation, 34 groups of cats, containing 127 animals in all, were injected intraperitoneally with 5 ml. of virus in suspension. Of the 84 cats in the 21 groups that received unfiltered material, 49 had the typical disease; of 22 cats in 7 groups that received a Berkefeld "V" filtrate, 17 had the typical disease; of the 8 animals in 2 groups that received a Berkefeld "N" filtrate, 7 had the typical disease; and of the 13 cats in 4 groups that received Berkefeld "W" filtrate, 12 animals had the typical disease.

The results that were obtained when virus was introduced by the intraperitoneal route give ample evidence that cats are highly susceptible to infection by this route. On the other hand, the large number of cats that was recorded as not having developed the disease (35 or 84) can be ascribed to the

presence of immune animals among the recipients, and to the inclusion of only such cats as had unequivocal hematological evidence of the disease. It should be noted that many of the cats found to be "refractory" to infection were injected with unfiltered suspensions of the virus. Some of these cats were unselected stock adult cats and, therefore, undoubtedly had been naturally exposed to the infectious agent. As immature cats usually were employed in filtration experiments, on the other hand, a much greater percentage of the cats yielded unequivocal evidence of the disease.

In an effort to obtain a clue as to the natural mode of infection, virus was experimentally introduced by peripheral routes, which might indicate whether the virus was transferred in nature by droplet infection, contaminated food or water, or insect transmission. Circumstantial evidence based on the extraordinary spontaneous communicability of the infection by cage, body, and room contact made it seem possible that any one or all of the aforementioned vehicles were active. The intranasal route was first investigated, for it seemed to be the most probable route for natural infection.

*Intranasal Route.*—The introduction of the filterable agent by this route resulted in typical disease.

A single group of cats containing 6 animals was inoculated under light ether anesthesia by dropping 0.1 ml. of a tissue suspension containing virus into both nares. Two of the 6 animals had characteristic hematological findings 9 and 10 days later, respectively.

This single experiment made it clear that cats are susceptible to infection when virus is inoculated intranasally. When virus is introduced by this route, however, it is most difficult to control its spread. Accordingly, no further experiments employing the intranasal route were undertaken.

*Gastrointestinal Route.*—In a preliminary experiment, a 10 per cent fecal suspension was introduced by means of a rubber tube into the stomachs of 3 cats. Two of the 3 animals came down with the characteristic disease 4 and 17 days later.

From the results of this first experiment, it appeared that cats are vulnerable to infection by virus contained within the gastrointestinal tract. It was thought advisable, therefore, to carry out a second experiment to confirm the results of the first experiment and to test diluted fecal material for its infectivity.

Samples of a fecal suspension representing 5 dilutions,  $10^1$ ,  $10^4$ ,  $10^5$ ,  $10^7$ , and  $10^8$ , were introduced through a rubber tube into the stomachs of as many cats. Only the 2 animals that received the  $10^1$  and  $10^3$  dilutions contracted the disease.

The production in cats of typical feline agranulocytosis following the introduction by stomach tube of a fecal suspension was accepted as presumptive

evidence that feces from diseased cats contain virus, and that the virus can survive the gastric acidity to be absorbed from the gastrointestinal tract. It was recognized, however, that virus might have contaminated oral, nasopharyngeal, tracheal, or esophageal tissues when the tube was being inserted into, or withdrawn from, the stomach. Accordingly, a third experiment was designed to eliminate the possibility of fortuitous infection by introducing the virus directly into stomachs exposed surgically.

Six cats in a single group were exposed to infection by depositing within the gastric lumen 5 ml. of a Berkefeld "V" filtrate prepared from a 10 per cent liver-spleen tissue suspension of known infectivity. This was accomplished by the surgical exposure and inoculation of the stomach of each cat,<sup>4, 5</sup> according to the following procedure. Observing rigid surgical asepsis, the peritoneal cavity was opened by a right rectus incision and the stomach was exposed by bringing it out of the wound. After gauze had been packed at the juncture of the lowermost portion of the extricated stomach and the incision, two traction sutures of silk were placed in the anterior surface of the stomach wall. The first cat was inoculated by inserting a 2 inch hypodermic needle (24 gauge) through the stomach wall and injecting 5 ml. of filtrate. Because of the possibility that the serosal surface of the stomach might be contaminated when the needle was withdrawn, the technique was modified so as to eliminate any possibility thereafter of leakage or serosal contamination. The modification in technique was effected after the stay sutures had been placed when a ½ inch incision was made in the gastric wall and the edges of the wound picked up with mucosal clips and separated. This permitted the needle of the syringe to be inserted into the stomach without its coming into contact with anything. Each of the remaining 5 cats had 5 ml. of virus suspension deposited within the lumen of its stomach. The mucosal opening was then closed by two layers of inverting silk sutures, while the anterior wall was kept elevated by the stay sutures. The viscus was replaced, and the abdominal wall was closed with silk sutures.

Four of the 6 recipients came down with agranulocytosis 7 days later.

The production in this third experiment of feline agranulocytosis by the introduction of virus directly into stomachs that had been exposed surgically confirmed the results of the two preceding experiments by showing that virus is infectious when introduced into the gastrointestinal tract. Moreover, this experiment went further by eliminating accidental contamination by virus of the mucosa proximal to the lining of the stomach. When the results of the three experiments are considered collectively, it becomes apparent that one possible vehicle for the transfer of the infective agent is contaminated food.

*Cutaneous Route.*—The cutaneous route is important only if a biting insect be implicated in the natural spread of the disease. However, because the

<sup>4</sup> It is a pleasure to thank Dr. Herman E. Pearse and Dr. Robert L. Sewell of the Department of Surgery for performing these surgical operations.

<sup>5</sup> Ether anesthesia was employed for all operative procedures.



possibility of arthropodal transmission of the virus should be entertained, at least until the natural mode of infection has been settled, a single experiment was undertaken to test the cutaneous route as a possible avenue of infection.

One group containing 6 cats was injected intracutaneously with virus contained in a liver suspension. The virus suspension was diluted  $10^3$  and injected into the skin on the left flank, 0.1 ml. to each of 2 cats, 0.05 ml. to each of 2, and the other 2 were pricked by the tip of a needle that had touched the virus suspension. Of these 6 animals, only 2 came down with agranulocytosis—1 that received 0.05 ml. and 1 that received virus by needle prick. When these animals were tested for immunity by the injection of a massive dose of virus, all were immune as shown by the absence of evidence for infection.

The results of this single experiment make it clear that the virus is infective when introduced into the skin in small amounts. If virus be present in the blood of cats throughout the latter part of the incubation period and the phase of clinical disease, (which was proven in experiments that follow), a possible mode for transmission of the disease is by arthropodal vectors.

*Distribution of the Filterable Agent in Tissues, Body Fluids, and Excretory Products*

The marked hematological changes that characterize the disease early suggested blood as a source of virus. Accordingly, tests were performed to determine the presence of virus in the peripheral blood; to relate the presence of virus in the blood stream to the stage of the disease; and to provide source virus for further experimentation.

Blood samples from 16 animals were tested in 13 experiments for the presence of virus. These samples were used as whole blood (either directly or after treatment with potassium oxalate, sodium citrate, or heparin), lyophilized serum, or defibrinated blood for the intravenous (femoral vein), intraperitoneal, or intracardiac inoculation of 43 cats.

As it was found that the route of inoculation and the treatment of the blood before inoculation (as described above) were without apparent effect on the outcome, the results of all of the experiments are considered together. Thus, in summary, 17 of the 43 recipients came down with the typical disease. Of these animals, 6 were used to establish the presence of virus in the blood stream, 12 to relate the presence of virus in the blood stream to the stage of the disease, and 25 to provide source virus for further experimentation. Worthy of further comment, moreover, is the experiment in which the presence of virus in the blood was related to the stage of the disease. In this experiment, 3 cats were bled on the 2nd, 3rd, 5th, and 9th days after inoculation. Immediately after each bleeding, the blood was oxalated, pooled, and injected intraperitoneally in 5 to 10 ml. amounts into each of 3 cats. It was found that virus was present in the blood on the 2nd, 3rd, and 5th postinoculation days. The results

for the 9th day, were inconsequential, however, for all 3 cats in this group were immune.

These experiments show that virus is present in the blood stream, and that its infectivity is but little affected by heparin, sodium citrate, or dessication *in vacuo* when frozen. As blood, therefore, constitutes a readily available source of virus, it was used in later experiments. Moreover, the presence of virus in the blood stream throughout the preclinical period, when related to the demonstrated infectivity of virus inoculated by the cutaneous route, makes it apparent that the experimental criteria for possible arthropod transmission are satisfied.

The presence of virus in the blood of animals with agranulocytosis suggested that the infectious agent is widely distributed in the tissues. Accordingly, lung, spleen, and liver, as representative tissues, were tested for the presence of virus.

In a single experiment, a Berkefeld "V" filtrate, prepared from pulmonary tissue, was used for the inoculation of 6 cats, 3 intraperitoneally and 3 subcutaneously. All 6 had typical agranulocytosis from 6 to 8 days later, and 5 of the 6 died.

Splenic tissues from 2 animals were used for the intraperitoneal injection of 2 groups containing 7 cats. Of these 7 animals, 5 exhibited evidence of the typical disease.

Hepatic tissues from 15 cats were used in 17 experiments for the inoculation of 80 animals. Of these 80 cats, 44 had typical feline agranulocytosis.

These results give ample evidence for the infectivity of pulmonary, splenic, and hepatic tissues. Because of the ready availability and abundance of hepatic tissue, this material was used frequently and for a variety of purposes. It is for these reasons that such a large number of animals was injected with hepatic-tissue suspensions, and therefore, are included in the present report. The observations, which relate to changes in the dosages and methods of administration of the virus, are considered in the section that deals with "Pathogenicity by different routes of inoculation."

The question next arose as to whether virus is present in the eliminatory products of the body. Accordingly, respiratory secretions, feces, and urine were investigated.

Respiratory secretions were tested first because the high incidence and epizootic nature of the spontaneous disease and the ready communicability of the infection suggested that the malady is spread naturally by droplets of nasal spray. However, the almost complete absence of nasal secretions in cats at the height of the illness, led us to add mucosal scrapings from the respiratory passages and turbinates to the respiratory washings.

The material, which was tested for virus, was obtained from a single cat by mixing the meat extract broth washings from its nasal passages with the mucosal scrapings

from its nasal passages and turbinates. This mixture was triturated in broth and alundum, centrifuged, and filtered through a Berkefeld "V" candle to yield a filtrate which was used for the intraperitoneal inoculation of 3 cats. Two of the 3 animals had the disease 7 days later.

The results of this single experiment show that virus is present in the respiratory passages at the height of the disease, and suggest, therefore, that nasal secretions constitute one vehicle for the natural spread of the virus. Since we showed in earlier experiments that the cat is susceptible to infection by the intranasal route, it becomes apparent that the respiratory passages alone can serve as the portal both of exit and entry for the virus.

In an attempt to demonstrate virus in the feces, five experiments were carried out. In three of these experiments, the fecal suspensions were introduced parenterally, and in the other two, the fecal suspensions were deposited within the stomach by means of a rubber tube.

Seven cats, in groups of 2, 2, and 3, representing three experiments, were injected with Berkefeld "V" filtrates. Of these animals, 6 had typical feline agranulocytosis on the 5th day after injection, 1 on the 6th day. Four of the 7 died.

In the fourth experiment, each of 3 cats was given 5 ml. of a 10 per cent fecal suspension by stomach tube. Two of these animals developed the disease, 1 by the 4th day after inoculation and the other by the 7th day.

Each of the 5 cats, which were employed in the fifth experiment, received 5 ml. of one of the following dilutions of a Berkefeld "V" filtrate:  $10^1$ ,  $10^3$ ,  $10^5$ ,  $10^7$ , or  $10^8$ . Of these 5 animals, the cats that were given the  $10^1$  and  $10^3$  dilutions came down with agranulocytosis.

These five experiments show that abundant virus is present in fecal suspensions. Indeed, it would have been surprising if the characteristic pathological changes in the intestinal mucosa were not associated with the presence of virus in the feces. Nevertheless, we realized, of course, that the virus present in the feces could have its origin in nasal secretions, which had been swallowed, and therefore, that the associated occurrence of pathological changes in the intestinal mucosa and of virus in feces merely suggested the intestinal mucosa as a source of virus. In an attempt to obtain further evidence on this problem, two additional experiments were undertaken to determine if virus is present in the mucosal cells of the ileum.

In the sixth experiment, the ileum of a single moribund cat was removed, washed carefully to remove all of the intestinal contents, and scraped superficially to remove the surface epithelium. These scrapings were used to prepare a Berkefeld "V" filtrate, according to the technique outlined, and the filtrate was injected intraperitoneally into each of 3 cats. All 3 had the typical disease 7 days later.

Employing the same procedure for the seventh experiment, the mucosal lining of the ileum from another cat at the height of its disease was used to prepare a Berkefeld

"V" filtrate for the intraperitoneal injection of a group of 6 cats. Two of the 6 animals were ill with the disease 5 and 7 days later.

The results of the foregoing seven experiments show that virus is present in both the fecal content and mucosal lining of the intestine. When this evidence is considered in relation to the results of preceding tests, in which it was shown that cats are readily susceptible to infection by virus introduced into the stomach by tube or through surgical exposure, it becomes apparent that the gastrointestinal route also can act as a natural portal both for the entry and exit of the virus.

It seemed possible that virus might be excreted in the urine. Three experiments were carried out to test the possibility.

Urine was withdrawn suprapubically by syringe from the bladder of animals killed at the height of their disease. It was used without further treatment, in doses of from 2 to 5 ml. intraperitoneally, for passage to groups of 3, 4, and 5 cats, respectively, in three experiments. These experiments were carried out at intervals of several months.

None of the first group of 3 cats showed evidence of disease, following the injection of urine, but these same animals contracted the disease 20 days later when injected with a Berkefeld filtrate of fecal suspension. On the other hand, all of 4 animals in the second experiment and 3 of the 5 animals in the third experiment had agranulocytosis in from 5 to 11 days after a single injection of urine.

The results of these three attempts to demonstrate virus in the urine of cats with feline agranulocytosis suggest that virus is present irregularly in the urine of cats at the height of the disease. The demonstration of virus in two of three attempts, however, indicates that contamination by urine is another mechanism that must be considered in the natural spread of the disease.

#### *Susceptibility of Other Species*

Hammon and Enders (4) have found the rabbit, guinea pig, ferret, and mouse to be insusceptible to infection by the feline virus, and Kikuth, Gönner, and Schweickert (6) (employing an infective agent which appears to be identical with that described by Hammon and Enders, and by ourselves), found the dog (3 pups and their mother), *rhesus* monkey, rabbit, hamster, canary, hedgehog, and rat to be insusceptible. The small number of animals that they employed and their failure to use "blind" serial passages, except for brain material in mice, however, led us to carry out further tests to determine the susceptibility of common laboratory animals.

All animals were isolated for at least 7 days before blind serial transfers were carried out at 7 day intervals. Blood counts and temperatures were taken bidaily. The virus suspensions, which were used to initiate each passage transfer, came from a cat that had exhibited a typical agranulocytic blood picture and inclusion bodies, and had yielded virus of proven infectivity on transfer to 6 normal cats. Because it was felt that the host cell-virus relationship might manifest different affinities on transfer

in the tissues of species removed from the cat, the susceptibility of 3 types of tissues and their capacity for carrying the feline virus were tested by from 3 to 6 blind serial transfers. The source materials for transfer were brain tissue, hepatic and splenic tissues, and intestinal washings inclusive of the mucosa. The passage series employing brain tissue was initiated by injecting a 10 per cent hepatic-splenic suspension both intracerebrally and intraperitoneally, and thereafter using brain tissue for passage. The hepatic-splenic tissue suspensions and Berkefeld filtrates of intestinal washings were injected intraperitoneally.

*Rabbits.*—Four groups, each containing 6 New Zealand white rabbits, were used. Of the first group of 6 rabbits injected with virus of feline origin, 4 were given a 10 per cent hepatic-splenic suspension (2 intraperitoneally, 5 ml.; 1 intracerebrally, 0.25 ml.; and 1 both intracerebrally, 0.25 ml.; and intraperitoneally, 5 ml.). Although none of these animals showed signs of infection, each of these 3 “blind” passages was carried for 3 more transfers in series. The intracerebral series was maintained by both intracerebral and intraperitoneal injection of brain tissue from the preceding generation, the hepatic-splenic tissue series by the intraperitoneal injection of these same tissues from animals of the preceding generation, and the intestinal content series by the injection of Berkefeld filtrates prepared from the intestinal contents of the preceding generation. When none of these animals showed any evidence of infection, each passage series was terminated by making the final transfers to normal cats, which had been maintained under isolation. The cats remained well, as measured by the absence of (*a*) alterations in the blood picture, (*b*) clinical findings of disease, (*c*) temperature changes suggestive of disease, and (*d*) histological changes in the ileum, thereby indicating that no virus was present in the tissues under test. Moreover, the susceptibility of these same cats was affirmed from 13 to 20 days later, when all contracted the typical disease on injection with a virus suspension of known infectivity.

From this experiment we conclude that the rabbit is refractory to infection by the virus of feline agranulocytosis. Not only were two attempts to pass virus directly from the cat to the rabbit unsuccessful, but also attempts to enhance the pathogenicity of the feline agent for the rabbit, by using 3 types of material (hepatic-splenic tissues, brain, and intestinal washings) for “blind” serial passages through 4 successive transfers in rabbits, failed to yield evidence of activity. Moreover, the failure of these materials to elicit any evidence of infection when returned to the feline host strongly indicates that the virus is incapable of setting up even an inapparent infection in the rabbit.

The next three experiments were carried out with only slight modifications to test the susceptibility of 3 other species of animal, the guinea pig, the white mouse, and the ground squirrel (*Citellus richardsonii* Sabine).

*Guinea Pigs.*—In the first test, each of 4 guinea pigs was injected intracerebrally (0.1 ml.) and intraperitoneally (2 ml.) with a feline hepatic suspension of proven infectivity. None of the animals showed any evidence for infection.

The second test was designed and carried out in a fashion similar to the “blind” serial passage transfer in rabbits, which was described in the foregoing experiment. Three series of 4 passages each were effected, employing hepatic-splenic tissues, brain

tissue, and intestinal washings, respectively, as vehicles for the infective agent in the attempted maintenance and carriage of the virus. Twelve guinea pigs in 3 groups of 4 were used for each passage. The hepatic-splenic tissue passages and the brain-tissue passages were initiated with a feline hepatic-splenic tissue suspension (2 ml. intraperitoneally and 0.1 ml. intracerebrally), and the intestinal washings passage series with a Berkefeld "V" filtrate of feline intestinal washings (0.1 ml. intranasally and 2 ml. intraperitoneally). In the subsequent 3 passages, for which guinea pig materials were used, the anatomic sources of the inocula, dosages, and routes of inoculation remained the same. Total leucocyte and differential counts were taken 1 to 3 days before injection and on the 3rd and 6th days after injection. When the animals were killed on the 7th day after injection, the suspensions for passage were prepared by using tissues from all 4 of the animals used in each series. After the fourth passage, the passage material from each series was used for the injection of 2 cats, and pieces of the lower ileum were removed for histologic study.

No evidence for infection was observed in any of the 48 guinea pigs or 6 cats employed. If any virus was transmitted after the initial passage of infective feline tissues, therefore, the amount was insufficient to become established in the foreign host.

*Mice.*—Six successive transfers at 7 day intervals in each of 3 series were effected before the passage material, under test for its ability to carry virus, was returned to a feline host. For each passage, 3 groups of 6 mice of from 20 to 40 days of age were used as recipients for hepatic-splenic tissues, brain tissue, and intestinal washings, respectively. The two passage series employing hepatic-splenic and brain tissues were initiated by using a feline suspension of hepatic-splenic tissue, and the passage series employing intestinal washings was initiated with a filtrate of feline intestinal washings. For the brain tissue passage series, 0.1 ml. was injected intracerebrally and 2 ml. were injected intraperitoneally; and for the hepatic-splenic and intestinal washings series, 2 ml. were injected intraperitoneally.

A fourth and final passage series was carried out using groups of 6 mice for the passage of pulmonary tissue every 7th day for four successive transfers. The first group of mice was injected with a virus suspension of feline origin, and successive groups of mice received a 10 per cent suspension of pulmonary tissue prepared from the lungs of all of the 6 mice, which had been used in the preceding passage. Each mouse received 0.1 ml. intranasally and 0.5 ml. intraperitoneally.

On completion of each passage series, a suspension of the tissue under test was used to inject cats.

Of the 132 mice used in the four transfer series, the 18 mice used in preliminary experiments, and the 8 cats used to test the infectivity of mouse tissues representing the final passage, none gave any evidence, in the form of clinical, hematological, or histopathological findings, that the virus of feline agranulocytosis was infectious for mice.<sup>6</sup>

<sup>6</sup> These experiments inadvertently served another purpose, for they gave good evidence that the inbred Swiss albino strain of mouse, which is reared for use in this laboratory, was free from the viruses known to cause inapparent infections in laboratory mice, *viz.*, infectious ectromelia (8); lymphocytic choriomeningitis (9); the virus pneumonias, as described by Dochez, Mills, and Mulliken (10), Gordon, Freeman, and Clampit (11), Horsfall and Hahn (12), and Nigg (13); and spontaneous encephalo-

*Ground Squirrels.*—Four successive “blind” serial transfers in gophers were made. The 2 gophers that were employed in each transfer received virus by the intracerebral (0.1 ml.), intranasal (0.1 ml.), and intraperitoneal (1 ml.) routes. The 10 per cent feline splenic-hepatic suspension previously described was utilized to initiate the series, and thereafter, hepatic and splenic tissues from the 2 gophers in each preceding passage were used to prepare suspensions for the inoculation of the next generation in the passage series. When the final passage was terminated, representative portions of brain, liver, spleen, and intestinal contents were obtained from both gophers, and used to prepare suspensions. The suspension of intestinal contents was filtered through a Berkefeld “V” filter and the resultant filtrate was mixed with equal amounts of the unfiltered suspensions of brain, liver, and spleen. Each of 2 cats received 5 ml. of this mixture intraperitoneally.

None of the gophers or cats employed in this experiment gave any evidence for infection by the feline agent.

It is worthy of note that two attempts to establish the virus on the chorio-allantoic membrane of the developing chick failed to give any evidence locally for the pathogenicity of the virus, and, further, tissue suspensions prepared from these chorio-allantoic membranes failed to yield virus when they were returned to susceptible cats.

It is apparent from the results of the experiments above that our attempts to produce infection in species other than the cat were uniformly unsuccessful. From 4 to 6 “blind” serial passages were made in rabbits, mice, guinea pigs, and gophers, employing the intracerebral, intranasal, and intraperitoneal routes of injection for suspensions of brain, liver and spleen, and intestinal washings, respectively, for each species. The possibility that an inapparent infection was initiated and maintained by serial passages was ruled out when susceptible cats did not react to the injection of any of the tissues removed from the animals employed in the last passage transfer.

#### SUMMARY

Thirty-two strains of an infectious filterable agent, with properties that establish it as a virus, have been isolated from a malady of cats. This disease can be readily recognized and differentiated from other feline diseases by blood studies, which make apparent the characteristic profound leucopenia and marked relative lymphocytosis in the absence of thrombopenia and appreciable anemia. (Because the cytological pictures of the bone marrow and blood are essentially similar to those which characterize human agranulocytosis, we have

myelitis of mice (Theiler's disease) (14). Because murine encephalomyelitis may not become manifest for 30 or more days, 3 mice, representing the fourth to sixth successive mouse passage in each series, were permitted to live for from 30 to 40 days after the passages at 7 day intervals had been terminated. None of these mice showed any evidence of abnormality either before or after their death.

named the disease under study "infectious feline agranulocytosis.") The cytological reaction to the presence of the virus is further characterized by proliferation of the reticuloendothelial cells of the lymph nodes and spleen, and by the formation of intranuclear inclusion bodies in the cells of the gastrointestinal mucosa, lymph nodes, and bronchial mucosa.

The etiological agent, the virus of infectious feline agranulocytosis, is pathogenic for cats when given by the oral, intragastric, cutaneous, subcutaneous, intraperitoneal, intravenous, and intranasal routes; it can be recovered at the height of the disease from the blood, spleen, liver, lung, intestinal mucosa, nasal secretions, nasal mucosa and turbinates, feces, and urine. The virus appears to be limited in its pathogenicity to the feline species. We found that a variety of animals, as represented by albino Swiss mice, guinea pigs, domestic rabbits, and ground squirrels (*Citellus richardsonii* Sabine), failed entirely to react to the injection of massive doses of virus. Repeated attempts at infection of these animals regularly failed when the intranasal, intraperitoneal, subcutaneous, and intramuscular routes of inoculation were employed for single doses. The same was true when from four to six transfers in "blind" serial tissue passages were made. Moreover, attempts to propagate the virus on the chorio-allantoic membrane of the developing chick were unsuccessful.

The significance of the facts is discussed in the paper that follows.