Experimental ocular cryptococcosis
Preliminary studies in cats and mice

Paule Blouin and Robert M. Cello

Ocular cryptococcosis was produced in cats by the intracarotid injection of Cryptococcus neoformans. Infected eyes developed a progressive, multifocal chorioretinitis which was comparable to that found in the naturally occurring feline disease. The severity of the ocular disease, the development of infection in the fellow eye, and the degree of systemic involvement were shown to be related to the number of organisms inoculated. Mice infected intracerebrally with the same organism developed optic nerve meningitis. The pathogenesis of the ocular lesions thus produced is discussed.

Key words: experimental cryptococcosis, feline, ocular, pathogenesis, optic nerve meningitis, Cryptococcus neoformans, chorioretinitis

Cryptococcosis is a worldwide disease of man and animals caused by the encapsulated yeast Cryptococcus neoformans. The majority of human infections are thought to result in a self-limited pulmonary disease, but dissemination of the organism to other parts of the body does occur. Meningitis and meningoencephalitis are the most common sequelae of such dissemination and are responsible for the majority of deaths attributed to this disease.

Intraocular manifestations of cryptococcosis have been reported and appear to be of two types: those occurring as the result of extension of a meningeal infection and those having a hematogenous origin. The first of these mechanisms appears to be the most common. In 12 of 36 patients with cryptococcal meningitis in one study, chorioretinal involvement was detected in only two. In their description of ocular manifestations of cryptococcosis, De Buen and associates mention that papilledema is found in approximately two thirds of patients with meningeal disease. Similarly, in a review of 110 cases seen in Australia, although 40 patients had papilledema, optic atrophy, ophthalmoplegia, or blurred vision, none were found to have anterior uveitis or chorioretinitis.

In contrast, reports of intraocular cryptococcal infections proved by histology or culture are rare. Since the first such case reported in 1940, 21 cases have been described, including an instance of exogenous infection following transplantation of an infected corneal button.

Intraocular cryptococcosis appears to occur more frequently in cats than in man. Of the 33 reported feline cases, six had infections of the globe. This probably does not reflect the true incidence, since many reports do not state whether or not the eye was examined, and such an examination is often neglected in veterinary practice. At the Veterinary Medi-
Fig. 1. Characteristic fundic lesions in a naturally occurring case of feline cryptococcosis.

Clinical Teaching Hospital, University of California, Davis (VMTH, UCD), 18 cases of feline cryptococcosis were seen during a 10%-year period. Ocular examination was performed on 15 of these. While five animals had papillitis, seven had multifocal, circular areas of chorioretinitis (Fig. 1) which were remarkably similar in appearance. The location and distribution of these lesions suggested a hematogenous origin and prompted a study to determine whether they could be reproduced experimentally by the intra-arterial inoculation of \textit{C. neoformans}.

This report describes the results of such a study and compares the effects of three different dosages of organisms injected in the common carotid artery of normal cats. Additionally, it describes the ocular lesions seen in mice which were infected intracerebrally to assess the virulence of the organism.

Materials and methods

\textbf{Cultures.} An isolate was obtained from a clinical case of feline cryptococcosis at the VMTH, UCD. Identification of the organism as \textit{C. neoformans} was confirmed according to criteria previously described.\textsuperscript{15} Culture was always performed on Sabouraud's dextrose agar without cycloheximide. After multiple passages at both 20° and 36° C, Sabouraud's agar slants were inoculated and incubated for 72 hr at 36° C, then covered with mineral oil and refrigerated at 4° C. A suspension of organisms for the infection of experimental animals was prepared by inoculating plates with material from the refrigerated stock cultures. After four serial passages of 72 hr at 36° C, the colonies were gently scraped off the agar with a glass rod and rinsed three times with physiologic sterile saline (PSS). The number of yeast cells in the final suspension was determined by hemocytometer counts. In addition, colony-forming units (CFU) were counted after 48 and 72 hr of incubation at 36° C.

\textbf{Mice.} One hundred and forty-five 8-week-old male Swiss-Webster mice with an average weight of 25 gm were used for the mouse virulence studies. They were divided into groups of six or less per cage, kept at 20° C with 12 hr of cyclic light, and given commercial mouse food and water ad libitum. All mice were inoculated intracerebrally with 0.03 ml of suspension, using a tuberculin syringe and 27-gauge needle. The 17 animals that died within the 72-hr period after inoculation were arbitrarily eliminated from the study because their deaths were thought to be caused by the procedure itself. The remaining 128 mice were distributed as indicated in Table I. Control animals included mice inoculated with PSS only and those inoculated with a suspension of killed cryptococci which had been heated in a water bath for 1 hr at 63° C. Dead mice were collected twice a day for 30 days, at which time all survivors were sacrificed. One eye of each animal was minced and cultured for the presence of \textit{C. neoformans}, and the other was placed in 10% buffered formalin. Cultures and India ink smears of the brain were also performed.

\textbf{Cats.} Seven male and four female adult cats with normal eyes were obtained from local animal shelters and observed for 1 to 2 weeks. During this period they were vaccinated against feline panleukopenia and tested by a fluorescent antibody technique to ensure that they were free of feline leukemia virus. All animals were housed in individual cages at 20° C with 12 hr of cyclic light. Water and commercial dry cat food were given ad libitum.

Intra-arterial inoculation was accomplished under dissociative anesthesia induced with ketamine HCl after systemic premedication with atropine sulfate. The left common carotid artery was surgically exposed, and 1 ml of cryptococcal suspension was injected via a 25-gauge needle. Blood was then allowed to flow through the artery for 10 sec with the needle in place, after which the vessel was ligated.

The cats were randomly distributed into three groups of three each and one group of two controls. Group I was infected with $8 \times 10^5$ CFU, group II with $8 \times 10^6$ CFU, and group III with...
Table I. Schedule of mouse inoculation and results

<table>
<thead>
<tr>
<th>Group No. *</th>
<th>No. of mice</th>
<th>No. of CFU in inoculum</th>
<th>No. of yeast cells in inoculum</th>
<th>Deaths</th>
<th>Positive brain cultures</th>
<th>Positive eye cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>2.9 \times 10^6</td>
<td>9.6 \times 10^6</td>
<td>5</td>
<td>5 (71%)</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>2.9 \times 10^5</td>
<td>9.6 \times 10^5</td>
<td>10</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>2.9 \times 10^4</td>
<td>9.6 \times 10^4</td>
<td>20</td>
<td>18 (82%)</td>
<td>18 (82%)</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>2.9 \times 10^3</td>
<td>9.6 \times 10^3</td>
<td>19</td>
<td>17 (77%)</td>
<td>17 (77%)</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>2.9 \times 10^2</td>
<td>9.6 \times 10^2</td>
<td>8</td>
<td>8 (73%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>2.9 \times 10^1</td>
<td>9.6 \times 10^1</td>
<td>10</td>
<td>8 (73%)</td>
<td>5 (45%)</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>2.9 \times 10^0</td>
<td>9.6 \times 10^0</td>
<td>6</td>
<td>4 (33%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>1a</td>
<td>5</td>
<td>-</td>
<td>9.6 \times 10^4</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>4</td>
<td>-</td>
<td>9.6 \times 10^2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3a</td>
<td>4</td>
<td>-</td>
<td>9.6 \times 10^2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4a</td>
<td>4</td>
<td>-</td>
<td>9.6 \times 10^2</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5a</td>
<td>5</td>
<td>-</td>
<td>9.6 \times 10^1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6a</td>
<td>5</td>
<td>-</td>
<td>9.6 \times 10^1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mice of groups 1a through 6a inoculated with heat-inactivated suspension. Mice of group C inoculated with physiologic sterile saline.
† Cultures not performed on one mouse.
‡ Two mice died of wounds inflicted by the others.

Table II. Ocular findings, cat study

<table>
<thead>
<tr>
<th>Cat</th>
<th>Time of Sacrifice(^a)</th>
<th>Time of onset of anterior uveitis(^b)</th>
<th>Time of onset of fundic lesions(^c)</th>
<th>No. of fundic lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left eye</td>
<td>Right eye</td>
<td>Left eye</td>
<td>Right eye</td>
</tr>
<tr>
<td>Controls*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pc-1</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pc-2</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-1</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-2</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-3</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-4</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-5</td>
<td>32</td>
<td>-</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>P-6</td>
<td>32</td>
<td>14</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-7</td>
<td>31</td>
<td>30</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>P-8</td>
<td>32</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>P-9</td>
<td>31</td>
<td>11</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

* Received 1 ml sterile supernatant or 1.1 \times 10^9 heat-killed organisms.
† Inoculated with 8 \times 10^5 CFU.
‡ Inoculated with 8 \times 10^6 CFU.
§ Inoculated with 8 \times 10^7 CFU.
\(^a\) No. of days after infection.
\(^b\) Anterior uveitis prevented visualization of the fundus.

8 \times 10^8 CFU. One control received 1 ml of sterile filtered supernatant obtained after centrifugation of the suspension, while the other control received approximately 1.1 \times 10^9 heat-killed organisms. This was the hemocytometer count corresponding to the 8 \times 10^8 CFU dosage.

All eyes were dilated daily with 1% atropine sulfate and examined by direct and indirect ophthalmoscopy. Biomicroscopy of the anterior segment was performed every 2 to 3 days with the Kowa SL-1 slit-lamp biomicroscope. External and fundic photographs were taken at approximate intervals with the Kowa RC-2 fundus camera. Serum samples were collected from all cats once during the preliminary period of observation and on the day of sacrifice. These were tested for the presence of cryptococcal antigen using the latex slide agglutination method (Crypto LA kit, International Biological Laboratories, Rockville, Md.) All cats were sacrificed 31 or 32 days after in-
Infection by an intravenous overdose of barbiturate. The eyes were enucleated within 10 min of death and fixed in either 10% buffered formalin or Bouin’s fixative. They were then processed for light microscopy, and 8 μm sections were stained with hematoxylin and eosin or Mayer’s mucicarmine stains. Samples of brain, lung, liver, spleen, and kidneys were cultured in duplicate on Sabouraud’s dextrose agar and incubated for up to 3 weeks at 36° C. Other sites were also occasionally cultured for the presence of *C. neoformans.* Quantitative determinations were not performed.

Results

Mouse virulence studies. The results of the mouse virulence studies are summarized in Table I. Of the 95 animals inoculated with living organisms, 78 (82%) died. *C. neoformans* was recovered from the brain of 70 (90%) and from the eyes of 66 (85%) of the dead animals. Most of the mice died after a progressive illness during which they acquired a characteristic dome-shaped skull. An India ink preparation made from the brain of a dead mouse revealed a sizable increase in yeast cell diameter and in the width of the polysaccharide capsule when compared with preinoculation measurements. Six (18%) of the control mice died during the 30-day observation period. However, none of the deaths could be attributed to cryptococcal infection, since all brain and eye cultures were negative.

One eye from each of eight animals that had died of cryptococcal infection was studied by light microscopy. The mice selected for this purpose spanned the whole range of dosages used in the experiment, and all had positive cultures from the brain and from the fellow eye. Due to severe autolytic changes and processing artifacts, it was difficult to evaluate the extent and type of inflammatory reaction, but cryptococci could easily be located with the mucicarmine stain. Organisms were present in the optic nerve meninges of six of these eyes (Fig. 2). Although sections of the other two eyes failed to include the optic nerve meninges of an intracerebrally infected mouse. (Mayer’s mucicarmine, ×2000.)
nerve, cryptococci were found in the posterior episcleral connective tissue. In addition, a cryptococcal panophthalmitis was present in one eye, and yeast cells were seen in the retina of another.

**Cat studies.** None of the cats died in the 32-day period during which they were observed. The surgical sites were completely healed 10 days after intracarotid inoculation. Ocular findings are summarized in Table II. Ocular lesions were not detected in the two control animals or in the cats inoculated with $8 \times 10^5$ CFU (group I). Three to five days after infection, two cats in group II ($8 \times 10^5$ CFU) developed rapidly progressive multifocal chorioretinal lesions in their left (P-5, P-6) and right (P-6) eyes. Initially these were seen as multiple pinpoint opacities located under the retinal vessels and scattered throughout the tapetal fundus (Fig. 3). During the ensuing 12 to 23 days, each lesion enlarged into a well-defined, round, gray opacity ranging from 0.5 to 1.5 disc diameters in size (Fig. 4). Many of these elevated the retinal vessels (Fig. 5). Eventually most lesions coalesced to form large dark patches with indistinct borders suggestive of severe diffuse chorioretinitis. Lesions located in the nontapetal fundus were more difficult to detect because of the large amount of pigment in the choroid and retinal pigment epithelium. They appeared grayish-white and opaque, and progressed at the same rate as

![Fig. 3](image1.png) Cat P-6, left eye, 6 days after infection. Numerous small chorioretinal lesions are scattered throughout the tapetal fundus.

![Fig. 4](image2.png) Cat P-6, right eye, 16 days after inoculation. Single large chorioretinal lesion superior to the optic nerve head.

![Fig. 5](image3.png) Cat P-5, left eye, 23 days after infection. Multiple raised chorioretinal lesions.
the tapetal lesions. After 9 days, the right eye of cat P-5 developed a single gray lesion, 0.25 disc diameter in size, which appeared to regress during the course of the experiment, in contrast to the fellow eye, in which the lesions became increasingly severe. Anterior uveitis, manifested by flare and cells in the aqueous humor, was noted in the left eye of cat P-6 14 days after infection. After 2 days, a fibrin clot formed in the anterior chamber. This resolved spontaneously 2 days later.

Histologic examination of eyes removed 32 days after infection showed a moderately severe chronic iridocyclitis and multiple large foci of chronic chorioretinitis (Fig. 6) in both eyes of P-6 and in the left eye of P-5. The choroid and retina contained large numbers of lymphocytes and macrophages with only rare epithelioid cells, neutrophils, or plasma cells. Many macrophages and scattered neutrophils infiltrated the subretinal space at the sites of severe chorioretinal inflammation, causing focal elevations of the retina. Chronic optic neuritis was detected only in the left eye of cat P-6. Cryptococci could not be demonstrated in the anterior segment, optic nerve, or optic nerve meninges, and only a few were seen in the peripheral vitreous and choroid. In contrast, they were numerous in the subretinal space, both free and within macrophages (Fig. 6). Organisms were also present in necrotic areas of the retina associated with subretinal exudate and underlying choroiditis. The third cat (P-4) in group II inadvertently received most of the inoculum outside the carotid artery and failed to show evidence of ocular involvement.

The three cats in group III developed severe, bilateral, multifocal chorioretinal lesions (Fig. 7) which initially looked like those described in the previous group but progressed to a total retinal detachment in two left eyes (P-8, P-9) and diffuse involvement of the fundus in the others (Fig. 8). Anterior uveitis was seen in all three left eyes and in one right eye (P-7). Ocular involvement was

Fig. 6. Chronic chorioretinitis in the tapetal area of cat P-6. Cryptococci are most numerous in the subretinal exudate. (Hematoxylin and eosin, ×500.)
particularly severe in the left eye of cat P-8, in which an acute, painful anterior uveitis was present 24 hr after infection. The intense exudative reaction in the anterior chamber combined with a miotic pupil, which had developed in spite of topical atropine, prevented visualization of the fundus until the eye was sectioned. This animal also developed an extensive ulcerative blepharitis 27 days after infection from which large numbers of cryptococci were cultured. Gross examination of the globe revealed a total exudative retinal detachment.

Histologically, both eyes of each cat in this group were severely affected. In two left eyes (P-8, P-9) there was a total retinal detachment with very large numbers of organisms in the subretinal exudate, vitreous, choroid, and retina (Fig. 9). Retinal necrosis was prominent. Cryptococci were detected in the iris and ciliary body and associated with a chronic inflammatory reaction. They were also found in the substance of the optic nerve in both eyes and in the optic nerve meninges of one. The remaining four eyes had extensive chorioretinitis, with the majority of organisms located in the subretinal space as in group II. Although these had chronic inflammatory cells infiltrating the iris and ciliary body, cryptococci could not be detected in the anterior uveal tract.

Extraocular lesions occurred only in cats of group III. They consisted of distortion and swelling of the left nostril and upper lip and appeared in all three cats of this group between 16 and 30 days after infection. Left frontal sinusitis was also observed at necropsy. Table III summarizes the results of cultures and serum antigen titers taken from these animals at the time of death. C. neoformans was isolated from the left nostril, left conjunctival sac, brain, and lung of all three cats, from the right nostril, right conjunctival sac, and left frontal sinus of two, and from the liver, spleen, and kidney of one. All serum cryptococcal antigen titers were negative before infection but reached very high levels at the end of the experiment (1:32,768 in two cats, 1:8,192 in the third).

C. neoformans was recovered only from the brain of one cat (P-5) in all the other groups. Serum antigen titers remained negative in cats of groups I and II and in the saline-inoculated control. A weakly positive (1:2) antigen titer was detected in the post-experiment sample of the control which had
Fig. 9. Cryptococci in a section of necrotic, detached retina, cat P-9. (Mayer’s mucicarmine, ×2000.)

Table III. Results of cultures and serum antigen titers taken at time of death in cats inoculated with $8 \times 10^8$ CFU

<table>
<thead>
<tr>
<th>Cat</th>
<th>Left nostril</th>
<th>Right nostril</th>
<th>Left conjunctiva</th>
<th>Right conjunctiva</th>
<th>Left frontal sinus</th>
<th>Brain</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Cryptococcal antigen titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-7</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1:32,768</td>
</tr>
<tr>
<td>P-9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>1:8,192</td>
</tr>
</tbody>
</table>

been inoculated with $1.1 \times 10^9$ heat-killed cryptococci.

Discussion

The development of an animal model of intraocular cryptococcosis provides the means to study the pathogenesis of the disease and evaluate the results of antifungal therapy. With such a model, the progression or regression of the lesions can be readily monitored, and photographic documentation of all stages of the disease is possible in the live animal. The use of the cat for this model has many advantages: it is relatively inexpensive, easy to handle, and has a high incidence of chorioretinal lesions in the naturally occurring disease. Like all subprimate species, it has the major disadvantage of possessing an eye which has anatomic features different from those of man. Most important of these are the presence of a tapetum, the lack of a fovea, and a very different vascular supply. Such differences may account for the high incidence of intraocular lesions in natural feline cryptococcosis as compared to the human disease.

Cryptococci can reach the eye by four possible routes: direct extension from the para-
nasal sinuses, via the optic nerve meninges, or hematogenous dissemination, either as part of a generalized infection or with localization to the eye in the absence of other clinical manifestations. Of these, meningeal and hematogenous spread are considered to be the two most likely routes of ocular infection. Grieco and associates have postulated that since cryptococcal uveitis is often accompanied by meningeal involvement, it probably results from spread along the optic nerve meninges. In the case they report, it could not be determined whether the infection first started in the choroid or retina. The latter site was favored because of the affinity of C. neoformans for the meninges.

In the naturally occurring feline disease, the multifocal nature of the chorioretinal lesions and their histologic appearance suggested a hematogenous spread of organisms to the choroid, and it was hoped that this phenomenon could be duplicated experimentally. Although highly artificial, the intracarotid route of inoculation was selected because it provided a way to shower the eye with organisms without disturbing the integrity of the globe. This has been successfully accomplished with Histoplasma capsulatum in the primate and rabbit and with bacteria in the dog. In addition, the difficulty of inducing lesions of cryptococcosis in the cat by a variety of other routes has been demonstrated.

Although arterial anastomoses are described in the cat between both sides of the head via the carotid plexus and the circle of Willis, it was postulated that the ipsilateral eye, being closer to and in more direct communication with the inoculation site, would develop more lesions than the opposite eye. This hypothesis was confirmed experimentally. Fundic lesions were regularly produced in the cats of groups II and III by the injection of cryptococci in the left carotid artery and were consistently more numerous in the left eye than in the right eye. The lesions in the higher dosage group contained a much larger number of organisms, particularly in the retina and choroid. This would seem to indicate that severity of infection is directly related to the number of organisms reaching the globe. Although cryptococcal organisms were also found in the anterior segment of the two most severely affected eyes, they were not detected in the others in spite of the presence of an inflammatory reaction. A similar phenomenon has been described in toxoplasmosis.

A unique feature of the group of cats infected with the highest dosage of organisms was the development of rhinitis and blepharitis on the inoculated side. The time of onset and the fact that cryptococci were cultured from the conjunctival sac suggest the possibility that these developed from an extension of nasolacrimal system infection.

The incidence of involvement of the optic nerve meninges was high in intracerebrally infected mice. In contrast, this was detected only once in the cat experiment as part of a panophthalmitis. This suggests that optic nerve meningitis develops by extension from the nervous system, while ocular infection has a hematogenous origin. Since the cat has a high incidence of ocular lesions in the natural disease, it can be postulated that in that species, hematogenous dissemination is a frequent occurrence.

This experiment did not demonstrate the specific site in which cryptococci first localize, since the eyes were sectioned only when the lesions were well advanced. At that time, organisms were most consistently found in the subretinal space. The ocular response to cryptococcal infection was chronic inflammation with a minor granulomatous component. Giant cells were not detected, and epithelioid cells were only rarely present. In addition, the intensity of the inflammatory reaction varied greatly from one eye to the other. It is difficult to draw conclusions regarding the relationship between the type and number of inflammatory cells and the severity of the lesions, since the eyes were not studied sequentially. Further reports will include a more detailed description of the lesions which are present at varying times after infection.

The results of the latex agglutination test for detection of serum cryptococcal antigen
were interesting. Although the three cats in group III acquired a very high antigen titer, the animals in group II remained negative despite the fact that two cats had progressive ocular lesions and one of these also had a positive brain culture. One possible explanation is a lack of sensitivity of the latex agglutination test, which is reported to detect 0.025 micrograms of polysaccharide per milliliter of serum. Another is that very little capsular antigen is released from the eye into the general circulation. Definitive conclusions must await studies involving a larger number of animals. In any event, the fact that the test yielded negative results in these two animals indicates that it cannot be used to exclude the possibility of an active ocular cryptococcal infection. Based on this study and on previous clinical observations, the fundic lesions in feline cryptococcosis are unique, and their presence strongly suggests the diagnosis of the disease, even in the face of a negative latex agglutination test.

REFERENCES