AN EXPERIMENTAL APPROACH TO THE STUDY OF INTRAOCULAR TOXOCARA CANIS

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INTRODUCTION

PARASITIC INVOLVEMENT OF THE HUMAN BY THE DOG ROUNDWORM, *TOXOCARA canis*, is a relatively new disease concept having been recognized only within the past 30 years. It is a subject that has stimulated ophthalmologists, pediatricians, veterinarians, and laboratory scientists as well as those in the field of public health and hygiene. The interest in this subject is reflected by the voluminous reports in the literature and in spite of the many advances in the clinical, pathologic and diagnostic evaluation of this disease there remain many unanswered questions. It is the purpose of this paper to analyze the existing information, consider the unanswered questions and to present an experimental approach to the study of intraocular Toxocara infection.

HISTORICAL BACKGROUND

OCULAR INVOLVEMENT

In 1950 Wilder[1] studied 46 eyes histopathologically at the Armed Forces Institute of Pathology (AFIP), mostly from small children and each from a different patient, which had been enucleated with clinical diagnoses such as retinoblastoma, pseudoglioma and panophthalmitis. These cases were selected since each had a fairly uniform history of a white pupillary reflex, and pathologic findings of eosinophilic abscesses and granulomatous changes, similar to those associated with helminth infections elsewhere in the body. Initial pathologic diagnoses included pseudoglioma, endophthalmitis and Coats' disease. Nematode larvae or their residual hyaline capsules diagnosed as third stage hookworm larvae were found in serial sections of 24 eyes, indeed, three larvae were found in one eye. Larvae were not found in the remaining eyes although the pathologic findings were otherwise similar to eyes containing larvae and probably had the same etiology. Lesions in the second eye of two patients suggested bilateral involvement.

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Wilder named the entity nematode endophthalmitis, and commented that it probably played an important and previously unrecognized role in childhood blindness in the US She postulated that larvae entered the eye via the ophthalmic and ciliary arteries with lesions usually occurring in the choroid, and spreading secondarily to the retina and vitreous. To support the hypothesis of choroidal distribution she quoted a case examined by Heath in which a larva was seen perforating the wall of a choroidal vessel. In 1956 Nichols[2] reported a histological study of five enucleated eyes obtained from the AFIP; in four eyes he identified second stage *T canis* larvae segments, which is a common ascarid or roundworm found in dogs. In the fifth eye he thought the organism was probably also *T canis* rather than a hookworm larva. It is unclear whether these five eyes were from the group previously studied by Wilder. These initial findings firmly established *T canis* as the etiology for some cases of nematode endophthalmitis.

Since these initial reports, others have confirmed that *T canis* infection caused similar pathologic changes.[3,4] Ashton[5] reported the clinical and histopathologic findings of four cases in 1960, all with a solitary type of retinal lesion not previously recognized. He commented on the potential importance of this entity and the need for serial sections of pathologic specimens to find larvae. The following year Duguid[6] performed a pathologic examination of six eyes with chronic endophthalmitis. *T canis* larvae were positively identified in two eyes and in the other eyes fragments thought to be *T canis* were found.

Duguid[6,7] discussed the clinical features of the two types of ocular lesions seen in association with Toxocara infection. In the first type a solitary granulomatous lesion is usually located on the temporal side of the posterior pole near the disc and macula. It is usually about one to two disc diameters in size, white, round and elevated with only minimal surrounding pigment changes and little reaction in the overlying vitreous. A crescent shaped dark area, possibly representing a larva, can sometimes be seen in the lesions and retinal folds often radiate out from the mass. Occasionally a retinal vessel both crosses the lesion and dips into it. The second type of lesion is a chronic endophthalmitis in which a clinical diagnosis may be difficult to make. Marked vitreous reaction with an associated retinal detachment is frequently seen, and a whitish mass is often present behind the lens with a cyclitic membrane and mild anterior uveitis. Both types of lesions are usually unilateral, impair vision to varying degrees, may have an associated strabismus, and often produce a white pupillary reflex. Such lesions, in the past, were frequently diagnosed as retinoblastoma.

Brett[8] described a patient with a white mass involving the ciliary body

with a mild to moderate vitreous and anterior chamber reaction. Because of the possibility of retinoblastoma the eye was enucleated and pathologic study of the globe[9] revealed a larva, thought to be *T canis*, in the lesion. Another case of peripheral retinitis with pathologic demonstration of a *T canis* larva was reported by Hogan et al[10] in 1965. These cases were believed to represent a third type of ocular lesion due to *T canis*.

Perkins[11] reported the clinical findings in 150 cases of uveitis in children. He described several cases of peripheral uveitis thought to be secondary to *T canis* and in the overall series thought that 10% might be due to this organism.

Ferguson and Olson[12] subsequently mentioned three ocular manifestations of Toxocara, and Ashton,[13] discussed these lesions and summarized the 158 cases published to that time. The most frequent lesion was chronic endophthalmitis (101 cases); solitary posterior retinal granulomas occurred next in frequency (54 cases); and peripheral retinitis had been noted in only three cases. He commented that the three types of lesions have the same basic reaction and vary only in severity and location. Brown[14,15] listed the reported cases of ocular *T canis*, gave a clinical review of the subject and commented on the geographic distribution of cases.

Maumenee[16] stated that peripheral lesions with associated vitreous strands and displacment of the retinal vessels and tissues toward the peripheral scar was the most frequent form of nematode endophthalmitis seen at the Wilmer Institute. Subsequently, Wilkinson and Welch,[17] and Welch[18] in reviews of 41 eyes with ocular lesions due to Toxocara found peripheral inflammatory masses in relatively quiet eyes to be the most common lesion in their series and emphasized the importance of this manifestation. Welch[18] commented on a case with a peripheral retinal lesion seen by him in 1960 in which a *T canis* larva was found histologically. This probably represents the first proven case describing the peripheral form of the disease. O'Connor[19] studied 20 uveitis cases and found nine similar to those reported by Wilkinson and Welch[17] having peripheral retinal masses joined to the disc by retinal folds. Under the retina he found a tube-like structure running from the area of the disc to the peripheral inflammatory mass and thought that this clinicopathologic finding might be specific for Toxocara infection.

Other ocular findings such as hemorrhages and exudates sometimes resembling Coats' disease have been reported. Macular lesions may occur[20] as well as diffuse retinal lesions with associated pigmentary changes, optic atrophy and narrowing of the retinal arteries.[21] A marked vitreous reaction following the death of a larva in the vitreous cavity has been observed[22] however I have seen a case in which a previously motile larva in

the anterior vitreous died one week after it was first observed and underwent disintegration without any significant, clinically observable vitreous reaction over a follow-up period of three years, suggesting that death of the organism does not always produce a significant intraocular inflammatory response. At least one case of optic neuritis secondary to *T canis* with pathologic confirmation has been reported[23] and another with primary involvement of the optic nerve, presumably due to *T canis*, has been observed.[24]

Although anterior segment involvement with *T canis* is uncommon, at least two cases with iris nodules and associated anterior uveitis and hypopyon have been observed[25,26] as well as a case of hypopyon associated with a peripheral retinal mass.[27] Motile larvae have been seen in the cornea[25,28] and in one of these three cases *T canis* was pathologically proven. The first case of a live larva in the lens has recently been reported.[29]

It is apparent from the foregoing that *T canis* larvae can invade most ocular tissues and produce a variety of manifestations with the three primary forms of involvement being: a solitary retinal granuloma in the posterior pole, a diffuse chronic endophthalmitis, and a peripheral inflammatory mass in an otherwise relatively quiet eye. It may well be that peripheral ocular lesions are the commonest type of nematode endophthalmitis.

Although ocular lesions due to *T canis* usually occur in children there have been several reports of infection in adults.[30-32] Therefore, this possible etiology must be considered in all patients, regardless of age, who present with ocular lesions of the type caused by this organism. Nematode endophthalmitis is usually unilateral and only rarely is more than one larva found in an eye.

SYSTEMIC INVOLVEMENT

In addition to the ocular involvement from Toxocara, there are also systemic findings associated with the infection. Two years after Wilder's report of nematode endophthalmitis, Beaver et al[33] reported findings in three children with chronic eosinophilia, cough, pulmonary infiltration, fever and hepatomegaly. Open biopsy of the liver was performed in all three patients and in one case portions of a larval nematode, identified as *T canis* or *T cati*, was found. This was the first report demonstrating *T canis* as the probable causative agent for this systemic syndrome usually found in young children. Because of the involvement of internal organs they proposed the term visceral larva migrans (VLM) to differentiate it from cutaneous larva migrans. Since this initial study, it has been shown that the

syndrome of VLM is usually caused by the migration of second stage *T canis* larvae throughout the body but in some cases it may be due to larvae of other nematodes. They can invade most tissues and in one case in which the distribution of larvae was studied at autopsy,[34] the organs most heavily involved were the liver, skeletal muscles and brain. Pulmonary signs and symptoms are related to movement of the larvae through the lungs, and encephalitis[35] as well as epilepsy and poliomyelitis[36] may, in some cases, be related to *T canis* infection. The clinical manifestations of VLM can vary widely. Some patients may be asymptomatic while others may have severe abnormalities and a few deaths attributed to *T canis* have been recorded. Generally, the disease course is benign with the common factor in most cases being an eosinophilia.

The cases with ocular infection due to *T canis* differ somewhat from those with the systemic infection of VLM. The ocular cases occur in older children and adults whereas VLM is most often seen in children under four years of age. Patients with eye lesions usually do not have systemic signs or symptoms and most laboratory tests including eosinophil counts are normal. A history of pica or geophagia is less frequent in patients with the ocular form of the disease and they often have not had close contact with dogs or cats. In at least two cases of liver biopsy proven VLM,[37,38] ocular lesions apparently developed four years after the systemic infection. There are several papers which discuss various basic and clinical aspects of Toxocara infection quite well and serve as good reviews of the subject.[39-44]

LIFE CYCLES—NATURAL VS ABNORMAL HOST

T canis, a nematode or roundworm, is commonly found in dogs and less frequently in other animals and has a worldwide distribution. The dog is a natural host and man is a transport or paratenic host in which there is no essential development of the larvae.[40] The worm has four larval stages and an adult stage.[45,46] In the second or infective stage, larvae of *T canis* average approximately 400μ in length and 18 to 20μ in diameter[2] (Fig 1A). Prenatal infection in puppies occurs in utero by transplacental transmission if the pregnant bitch is infected. Larvae migrate to the lungs and the trachea and hence to the small intestine where they mature into adults. The adult female worm deposits ova in the dog's intestine and these are passed in the feces in an unembryonated form (Fig 1B). Embryonation of the egg occurs in soil over several weeks to months, a period dependent upon conditions such as temperature and moisture. At the end of this time the embryonated egg contains an infective second stage larva (Fig 1C). The ova are very resistant to adverse conditions and may remain viable for years.

Human infection occurs when embryonated eggs are ingested and the

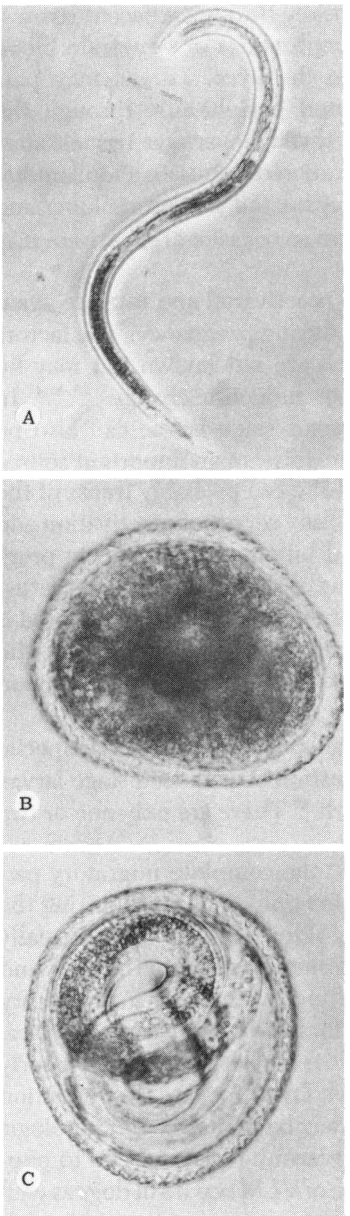

FIGURE 1
A: Toxocara canis, 2nd stage larvae. B: Toxocara canis, unembryonated egg. C: Toxocara canis, embryonated egg.

second stage larvae hatch out in the small intestine where they rapidly penetrate the mucosa. They may migrate locally through adjacent tissues, enter the lymphatics and go to regional lymph nodes or penetrate blood vessels and enter the portal circulation to the liver. Larvae may pass through the lungs and hence be distributed peripherally through the systemic circulation where they migrate through various tissues after leaving the blood vessels. This somatic migration can initiate an inflammatory response and the larvae may be encapsulated in a granuloma and either remain viable or be destroyed, while in an occasional case there may be minimal tissue reaction.

Surviving larvae in the dog may become reactivated and migrate again months or years later, especially in bitches during pregnancy. The factors stimulating migration in the pregnant bitch are not known but may be related to alterations in the immune system or endocrine changes.[39,42,47] In addition to transplacental transmission second stage larvae can also be passed through the colostrum but this is probably not an important source of infection. Once a bitch has been infected she can probably transmit the infection to multiple litters. I am unaware of any report of reactivation and migration of larvae in a previously infected human female during pregnancy resulting in clinical signs or symptoms or transmission to the fetus. The length of time larvae remain alive in tissues varies with the host and it has been reported that they have remained alive and infective in the monkey for at least 10 years.[40] However, it is not known how long they can survive in human tissues.

Adult *T canis* worms rarely develop in man and then only under special circumstances which would require the ingestion of advanced stage larvae from a puppy or possibly from a nursing bitch.[40] There are only one or two such cases reported in the literature.

In dogs, for reasons not fully understood, the complete migratory pattern of the larvae which ends in the gut occurs only in puppies during the first one to three months of life whereas in older dogs the larvae usually assume a somatic type migration to various tissues throughout the body and do not return to the gut. The explanation for this change in migratory pattern may be related to immunologic factors or possibly some physical change in the pulmonary vascular bed.[47] It is a well known, but poorly understood, fact that older dogs have a much lower incidence of infection compared to puppies and young dogs. This may be related to immunologic mechanisms, or the high incidence of transplacental transmission to puppies or other unknown factors. The syndrome of VLM occurs in dogs as well as humans with the kidney being the organ most frequently involved.[48] There appears to be only one report of histologically proven nematode

endophthalmitis in the dog.[49] The authors reported four cases, three of which had a solitary granuloma of the retina similar to those reported by Ashton and the fourth case had a granuloma of the optic disc. Similar lesions have occasionally been seen by veterinary opthalmologists on routine examination of dog fundi but these have not been pathologically proven to be Toxocara (Charles Martin, DVM, personal communication 1978).

DISTRIBUTION OF TOXOCARA

The prevalence of *T canis* in dogs throughout the world varies from 6% to 100% depending on the location, method of sampling and age of the dogs.[39,42] An examination of stool specimens from over 1000 dogs and cats by Woodruff,[51] revealed that approximately 15% of the dogs and cats in the London area were infected with *T canis* and *T cati*. Oldham[52] from autopsy studies found 6.4% of dogs to be infected with *T canis* and 8% of cats to be infected with *T cati*. Borg and Woodruff[53] gathered 800 samples of soil throughout England and approximately 25% contained ova of *T canis* or *T cati*. Portions of the samples were examined for viability and infectivity and essentially all of the organisms were alive and infective. This supports the concept that close contact with dogs and cats is not necessary to cause *T canis* or *T cati* infection as the worms are easily spread by contact with contaminated soil.

The prevalence of Toxocara infection in humans is not known[54] and is more difficult to determine for a variety of reasons.[55] *T canis* and not *T cati*, or other roundworms, appears to be the major etiology of VLM and nematode endophthalmitis in man. In a British survey of 485 healthy persons[41] approximately 2% gave a positive reaction to a Toxocara skin test indicating that they probably had a Toxocaral infection sometime in the past. *T canis* infection is found as frequently in cities as in the countryside, in developed as well as undeveloped countries and in areas of "good as well as bad hygiene." An evaluation of 73 animal hospital employees for possible *T canis* infection, using the ELISA test, revealed antibody to *T canis* in 11% of the persons tested.[56] No significant association could be shown between the percentage of positive reactors and dog ownership. In another study of kennel workers,[57] blood from 34 persons was examined for *T canis* antibodies using a fluorescent antibody test. All 24 employees who had been there between six months and 10 years had a negative reaction. Two of four persons employed over 10 years had a positive reaction. This study indicates that good personal hygiene probably prevents Toxocara infection even when working in highly contaminated areas.

DIAGNOSTIC STUDIES

A number of laboratory tests are available to evaluate *T canis* infection but many provide only indirect and non-specific information which makes diagnosis difficult. Examination of the stool for ova is, for practical purposes, always negative as the larvae rarely develop to an adult stage in humans. Most patients have a significant eosinophilia, usually during the acute migratory phase of the infection, which is a hallmark of the disease. Patients with ocular lesions usually do not have systemic symptoms, however, and there is typically no eosinophilia.

Skin testing was performed by Duguid[7] using antigen made from both larval and adult forms of *T canis*. Skin testing in rabbits and 12 patients suggested it provided a good method for the diagnosis of Toxocara infection. Woodruff[41,58] also developed a skin test that appeared to be rather specific with few false positive or cross reactions when tested in humans and experimentally infected animals. It has been used extensively in surveys of various populations. Collins and Ivey[59] in an experimental study of skin test reactions found that antigens made from larvae were more sensitive indicators of Toxocara infection than antigens made from adult worms. These findings differ from those of Woodruff who concluded that antigens made from adult worms were sensitive and specific indicators of Toxocara infection.

Huntley et al[60] studied a group of 51 patients thought to have VLM and compared them with a group of controls. In the infected patient group there was a hyperglobulinemia with significant elevation of the gamma globulins relative to the controls, while the hemagglutination and flocculation tests were positive to Toxocara antigens in only 30%. In another study Huntley[61] demonstrated a significant level of anti-gamma globulin factor in 26 of 59 children during the acute phase of their disease. Huntley[62] also found anti A and anti B titers to be higher in a group of patients with VLM as compared to controls. The rationale for this test is that *T canis* larvae contain components of human blood group substances which may produce a response in some patients infected with these larvae. Hogarth-Scott and coworkers[63] found significantly elevated titers of IgE in patients with VLM but not all studies have supported these findings.[44]

Another test that can be of value is the Fluorescent antibody test (FAT).[41] Woodruff found good correlation between this and the skin test used by him and noted that the positive skin test persisted for a longer period of time. The indirect hemagglutination test (IHA) and the bentonite flocculation tests (BFT) have also been used widely. Problems of cross reactivity, especially with Ascaris, have been identified and these tests may not be sufficiently sensitive. In a study of 237 patients by Krupp[64] using the IHA

with antigens from larvae and adult worms, antibodies to *T canis* were found in one-third of patients believed to have VLM.

Many of the tests for the diagnosis of Ascaris and Toxocara infection lack both sensitivity and specificity and non-specific tests cannot be reliably used for the diagnosis of VLM.[65] Two possible reasons were suggested for the difficulties with serologic diagnosis. First, there may not be any antibody in the serum during the chronic phase of the disease and, second, the antigens used for the test might not be sensitive enough. In an interesting report, Remky and Kraft[66] described two cases thought to have ocular Toxocara infection. The diagnosis was confirmed in one patient on the basis of positive antibody studies on an aqueous humor specimen, whereas in the second case the serum contained an elevated level of antibodies but none were found in the aqueous.

The development of more specific testing techniques such as the Enzyme-linked immunosorbent assay (ELISA) test[67-69] has significantly enhanced the capability of making a serologic diagnosis of Toxocara infection. Cypess et al[54] studied 10 patients with the ELISA test and found it to be very sensitive and useful in the diagnosis of VLM and the data suggest it can distinguish patients with Toxocariasis from those infected with other cross reacting parasites. Another study[43] found the ELISA test to be 78% sensitive and 92% specific in patients with presumed Toxocariasis. The use of larval secretory antigens in fluorescent antibody and hemagglutination tests[70] may be even more accurate than the ELISA test. Experimentally a positive titer can be obtained within four days after infection. Both the ELISA and secretory antigen tests use larval rather than adult worm antigen which significantly improves their accuracy over older tests which used only antigen from adult worms. It is still difficult to obtain enough antigen for testing purposes but new techniques to obtain secretory antigen[71] and the need for less material to perform this test as well as its accuracy may make it the test of choice in the future. In spite of all these advancements further refinement is necessary to obtain an unequivocal test.

Currently the only way to make a specific diagnosis of infection with *T canis* is to identify larvae in tissue sections.[13] Tissue reactions to larvae are fairly typical with inflammatory changes occurring in the areas of migration. The earliest response is usually an accumulation of eosinophils followed by other types of inflammatory cells including epithelioid and multinucleated giant cells. These may initially form an eosinophilic abscess, followed by a granuloma which is often surrounded by a dense fibrous, hyalinized capsule. The central area of the lesion, where the larva is usually found, frequently undergoes fibrinoid necrosis. The etiology of the

pathologic changes is not fully understood and may be due to physical damage from migration of the larvae, reaction to toxic larval products, immunologic phenomena or other unknown factors. Larvae may be destroyed by the tissue reactions or may remain intact and viable within the encapsulated lesion. Occasionally there may be only minimal reaction around the larvae.

Specific identification of larvae may be difficult as they are small and multiple serial sections are necessary to find them. In addition, changes in the larvae such as shrinkage from fixation and damage from the tissue reaction may occur, further complicating identification.[2,13,42] Specific identification of *T canis* larvae can be made, however, if a sufficient amount of the organism is present and especially if there is a cross section through the mid gut level.[2,42] Ashton[13] has commented that serial sectioning is not practical as a routine procedure and a presumptive diagnosis can often be made on the basis of the typical tissue reaction even though no larvae can be demonstrated. The diagnosis has been made in this manner in many of the reported cases.

In patients with suspected VLM in which it is important to make a definitive diagnosis, a liver biopsy can be done. This should be performed under direct visualization so that an area of granulomatous reaction can be obtained as needle biopsies may miss the lesions and give a false negative report.

TREATMENT MODALITIES

The treatment of *T canis* infection is difficult and there are no drugs which have been conclusively proven to be effective, especially for larvae in the tissues. A number of antihelminthic agents have been used[43,44,72-76] including: piperazine citrate (Antepar), diethylacarbamazine (Hetrazan), oxophenarsine hydrochloride (Mapharsen) and thiabendazole (Mintezol). Thiabendazole, a parasiticidal drug, is effective against the adult worm and inhibits egg production as well as interfering with larval development. Although it appears to have helped some patients, as evaluated by improvement in their clinical status and laboratory tests, there is no clear evidence that it is effective against larvae in tissues.[70] It is not known if thiabendazole penetrates the eye thus, even if effective elsewhere in the body, it may not be useful in the treatment of ocular lesions.[20] Local and/or systemic corticosteroids can be of value in reducing the inflammatory response and its damaging effects. If a larva is visualized and is not located in or near a vital structure such as the macula, it can be destroyed by photocoagulation.[77]

The best method of treatment, however, is prevention which requires

pet control.[47] Dogs, especially puppies, should be checked for worms at regular intervals and dewormed when necessary. There are many good antihelminthic drugs which will remove adult worms from the dogs but none that affect second stage larvae in the tissues of the dog. Young children should avoid contact with breeding bitches and their puppies until the latter are weaned. In addition, all dog feces should be destroyed as soon as possible and efforts made to prevent soil contamination.

EXPERIMENTAL STUDIES

Essentially all mammals and birds, which serve as intermediate hosts, can be infected with *T canis* larvae. There appears to be a typical tissue distribution of the larvae in each host. The organs most frequently affected are the liver, lungs, brain, kidneys and muscles.[40,78] A variety of animals have been experimentally infected with *T canis* larvae including: mice, rats, guinea pigs, hamsters, rabbits, pigs, monkeys and sheep.[79] A large proportion of larvae are found in the brain in mice, rats, hamsters and guinea pigs whereas in the rabbit and monkey many larvae are found in the liver and only a few in the brain. In the paratenic host only somatic migration occurs and this results in wide tissue distribution of the larvae.[42] Another feature of *T canis* infection is their encapsulation in tissue which can have a protective effect permitting long periods of infectiveness. Larvae have been known to remain alive and subsequently infective in monkeys for at least 10 years.[40]

There have been relatively few studies in monkeys, especially clinicopathologic reports, and none have elaborated on the ocular manifestations. Wiseman[80] infected three monkeys, and showed that there was a rapid and persistent rise in the eosinophil count after infection and the degree of rise of the eosinophils was directly related to the number of *T canis* eggs in the infecting dose. Observations of various clinical, laboratory and gross pathological changes have been made in macaque monkeys which were infected with large (100,000-400,000) oral doses of embryonated eggs.[81] Six of nine animals developed only mild symptoms and three developed neurologic abnormalities beginning approximately one week after infection. There was a rise in the WBC's and eosinophils over one to three weeks which gradually returned to normal in all but two animals over the next few weeks. A secondary rise was seen in these parameters about six to seven weeks after infection in the latter two animals. In four of the monkeys varying degrees of serum protein elevation, including beta and gamma globulin, were noted and remained elevated in three monkeys for the duration of the study. There was also a rise in the SGOT and SGPT during the first week after infection. Post mortem studies were done between

seven and 56 days following infection. Gross pathologic changes consisted primarily of granuloma formation and tissue destruction with hemorrhage. The greatest concentrations of larvae were found in the liver with lesser amounts in the central nervous system (CNS), lungs, kidneys and muscles. One larva was found in the left eye in each of two monkeys while no larvae were present in the eyes of the other seven. No description of ocular changes was given.

Olson[82] found no larvae in the intestine within three days after oral infection of mice with *T canis* eggs. Larvae were found, however, in the skeletal muscles and brain within three to four days after infection, demonstrating a rapid migration of larvae from the gut throughout the body. In mice previously sensitized to Toxocara there was a delayed migration through the liver and marked pathologic changes were noted in the liver as compared to non-sensitized mice suggesting a protective filtering mechanism in the liver in animals previously infected.

Olson[83] found *T canis* larvae in the eyes of mice as early as three days after oral ingestion of infective eggs and the number of larvae in the eyes increased for the first week after infection and then remained stable over a period of four months suggesting that once a larva invades the eye, migration stops yet the larva may remain viable within the eye for varying periods of time. The larvae invaded both the anterior and posterior segments of the eye at the same time but at necropsy more larvae were recovered from the posterior portion of the eye. Intraocular hemorrhages were first seen on day three (the same time as the larval arrival) and lasted approximately one month.

The number of larvae recovered from the eyes and the percentage of eyes infected increased when the dose of Toxocara eggs was increased. The authors postulated that the probability of ocular infection in humans, as in mice, may be related to the severity of the systemic disease. Olson[84] further studied two groups of mice, one which had never been infected and the other group which had been previously infected. Each animal was then given a comparable dose of *T canis* eggs. Iris hemorrhages were seen as early as day two in both groups but in the previously infected mice the bleeding sites tended to be pinpoint whereas they were more diffuse in the mice not previously infected. The hemorrhages increased in frequency during the first week which corresponds to the period of increased larval invasion. Ocular histopathologic studies revealed the retina was the site of 90% of the larvae but only 20% of the lesions. The lesions varied in both cell type and morphology. This suggests that the larvae migrated out of an area after producing a lesion. Prior infection with *T canis* did not appear to immunologically protect the eye from larval invasion but did appear to

enable the eyes to develop a more rapid response to a new larval challenge.

Donnelly et al[85] studied the effect of intravitreal injection of live and dead *Ascaris suum* larvae on the formation of IgE antibody within the guinea pig eye. Their findings suggest that IgE antibody may be produced locally within the eye and that live larvae induce a better response than dead larvae. This may be related to the ability of the live larvae to migrate or provide a different antigenic stimulus. They also found that IgE antibody does not leak from the peripheral circulation into the aqueous under normal conditions. Ninety percent of the aqueous samples contained IgE antibody one to two weeks after intravitreal injection of larvae. Live larvae were found in the lungs 16 days after intraocular injection indicating that larvae can migrate out of the eye in guinea pigs.

Thomas H. Pettit MD (personal communication, 1976) produced nematode endophthalmitis in the rabbit using different techniques and routes of infection and was able to achieve focal granulomas, similar to those seen in humans, with intravitreal injection of *T canis* larvae. Injection of larvae into the carotid artery or suprachoroidal space did not produce intraocular lesions. This is one of the few experimental studies correlating clinical and pathologic findings in the eye.

There have been two reports of experimental human infection with *T canis* involving a total of three patients.[86,87] Small doses of embryonated eggs (100-200) were given which produced a dramatic and prolonged eosinophilia but no significant clinical signs or symptoms developed in any of the patients over a follow-up period of four to 13 months.

In spite of a voluminous amount of research work on *T canis* and the various disease states it produces there have been relatively few studies pertaining to the eye except for clinical descriptions, usually of advanced disease, and pathologic reports. There are still many unanswered questions about the ocular aspects of the disease and this study was undertaken to try to answer some of them. One of the major goals was to establish a clinical model to study the natural course of the disease. In this way it might be possible to answer many questions concerning *T canis* infections, for example: to determine how the larvae enter and exit from the eye; why various ocular tissues react differently to the infection; whether larvae do become dormant in the eye or continue migrating out of the eye and to other tissues; determine why larvae apparently have a predilection for certain areas of the eye such as the peripheral retina; evaluate various laboratory tests and methods of treatment; and obtain a better overall understanding of the pathophysiology of the disease especially as it relates to the eye.

MATERIALS AND METHODS

Six out of eight young adult Owl monkeys (*Aotes trivirgatus*), three male

and three female, were infected. The monkeys are referred to in the text as M-1, M-2, etc. After arrival they were caged in groups and quarantined for 30 days. Baseline laboratory studies and eye examinations were then performed. The initial, as well as all follow-up, eye examinations were performed by the author, and most were done using ketamine anesthesia. Examinations included: external evaluation of the conjunctiva and pupils; slit lamp evaluation of the cornea, anterior chamber, iris, lens and vitreous using a KOWA (portable) or Zeiss slip lamp; a dilated fundus examination with direct and indirect ophthalmoscopes and the Hruby lens after pupillary dilation with 2½% phenylephrine and 1% tropicamide. Photographs were taken with a hand held KOWA camera and a Zeiss slit lamp camera. Baseline and follow-up laboratory studies include: A. Hematology—1. Complete blood count (CBC), (hemoglobin, hematocrit, RBC, WBC and differential count) 2. Circulating eosinophil count 3. Blood group and type and anti A and anti B antibodies. B. Chemistry—1. SGOT, SGPT, alkaline phosphatase 2. Protein electrophoresis 3. Immunoelectrophoresis. C. Serology—1. Enzyme-linked immunosorbent assay (ELISA) test 2. Indirect hemagglutination (IHA) test 3. Bentonite flocculation (BFT) test 4. Heterophile antibody. D. Parasitology—1. Examination of stool specimens for ova and parasites 2. Blood smear evaluation for parasites.

Laboratory studies were repeated as indicated throughout the experiment but some tests were not performed with equal frequency or on the same dates in each monkey. The hematology and chemistry tests, as well as the blood smears for parasites, were performed in the clinical laboratories of our hospital. The Hgb, Hct, RBC and WBC counts were done on a Coulter automated cell counter and the differential count with Wright stained smears per 100 cells. The circulating eosinophil counts were done in a hemocytometer using Phloxine B diluent in propylene glycol but due to technical difficulties their reliability was somewhat uncertain. Chemistry tests were done with the automated SMA 12, automated ABA 100 or automated ACA analyzers, protein electrophoresis by the cellulose acetate membrane method and immunoelectrophoresis by the neophelometric method with the automated Technicon analyzer. Blood grouping and typing as well as studies for anti A and B antibodies were performed in the blood bank at our institution. Stool examination for ova and parasites was done by a parasitologist in the Department of Cell and Molecular Biology using direct smears and the formalin-ether sedimentation technique. Serological studies were performed by the Parasitic Serology Section of the Division of Parasitology at the Center for Disease Control (CDC) in Atlanta, GA. The ELISA test technique is essentially the same as described by Cypess et al.[54] The hemagglutination (IHA), bentonite flocculation

(BFT) and heterophile tests were performed using standard techniques. Interpretation of the test titers by the CDC is as follows: A. ELISA— <1:16 = not significant, 1:16 or > = probably significant and 1:32 or > = significant; B. IHA— <1:64 = not significant and 1:64 or > = significant; C. BFT— <1:5 = not significant and 1:5 or > = significant; D. Heterophile— any titer especially a rising titer = significant.

T canis eggs were obtained from the distal uteri of gravid adult *T canis* worms which had been removed from the jejunum of a dog. These were initially placed in 0.5% formalin to retard bacterial growth and subsequently cultured at 29°C in petri dishes containing normal saline with 0.05% formalin (V/V) for at least 30 days. Infective (embryonated) eggs were then thoroughly washed 5 times by centrifugation in a large volume of water and then suspended in a small volume of water to be used for oral infection.

Second stage larvae were obtained by hatching fully embryonated eggs in vitro utilizing methods described by Olson and Jones[88] and de Savigny.[71] Washed embryonated eggs were incubated at 29°C in a 3.5% solution of benzalkonium chloride (V/V) for 15 minutes with periodic agitation and were then sedimented by centrifugation (800 × g for 2 minutes) and transferred to a sterile tube following which aseptic techniques were employed. The eggs were washed 6 times by sedimentation in lactated Ringers (USP, injection), pipetted onto a glass slide and a glass cover slip was placed over the monolayer. Pressure was then applied to the cover slip to crack open the egg shells thereby freeing the larvae. The freed larvae were pooled in Ringers solution containing penicillin (100 units/ml) and streptomycin (250 μg/ml). The pooled larval suspension was held overnight in the antibiotic Ringers solution at 37°C in a Baermann Apparatus containing eight layers of lens paper through which the larvae migrated and were subsequently collected on the other side the next morning. Larvae to be used for injection into the carotid artery were washed once with the antibiotic Ringers solution, counted and suspended in the same solution for injection. Larvae for intraocular injection were collected individually, washed five times in 10 volumes of antibiotic Ringers solution and suspended in an appropriate amount of the same buffer for injection. Only selected, microscopically viable larvae were used.

Infection of monkeys was performed in the following manner (Table I):

A. ORAL INFECTION (EMBRYONATED EGGS)

A nasogastric tube was passed to the stomach in an unanesthetized monkey. Embryonated eggs containing infective second stage larvae suspended in 3cc of normal saline were passed down the tube

TABLE I: INFECTION OF MONKEYS

Date	Monkey	Sex	Route	Side	Innoculum
8/28	1	F	Vitreous	OS	29 larvae
8/28	2	M	Vitreous	OS	15 larvae
8/28	4	M	NG* tube	—	5000 eggs
8/28	5	M	NG* tube	—	5000 eggs
8/28	6	F	Carotid	L	500 larvae
9/29 (32) @	1	F	Vitreous	OD	63 larvae
9/29	7	F	Vitreous	OS	75 larvae
10/13 (46) @	2	M	Vitreous	OD	35 larvae
11/22 (86) @ (54) #	1	F	Vitreous	OS	70 larvae
11/22 (86) @	5	M	Vitreous	OS	68 larvae

Information regarding infection of monkeys: NG* = nasogastric tube. ( ) @ = number of days following initial infection. ( ) # = number of days following second infection.

and the tube was then flushed with an additional 3cc of saline to clear the tube of eggs.

B. INTRACAROTID INJECTION (SECOND STAGE LARVAE)

The monkey was anesthetized with ketamine and the neck shaved, prepped and sterilely draped. The left carotid artery was surgically exposed and approximately 500 motile second stage *T canis* larvae were injected distally using a 30g needle. The wound was closed with 3-0 silk.

C. INTRAVITREAL INJECTION (SECOND STAGE LARVAE)

The monkeys were anesthetized with ketamine and 0.5% tetracaine drops for topical anesthesia and 2½% phenylephrine and 1% tropicamide drops for dilation of the pupil were instilled. The eye was fixated with forceps and penetration of the globe was made 3-4mm posterior to the limbus on the temporal side (at the 9 o'clock position in the right eye and 3 o'clock position in the left eye) with a 30g needle which was inserted 7-8mm directed towards the center of the globe. Between 0.1 and 0.2ml of vitreous was withdrawn (for ELISA testing) and motile second stage larvae in a similar volume of saline or balanced salt solution (BSS) were then injected. The first two vitreal injections (left eye of monkeys M-1 and M-2) were performed with a three-way stopcock on the needle and separate syringes for withdrawing vitreous and injecting the larvae. Because of dead space in this system possibly preventing total transfer of larvae into the eye, all subsequent injections were performed without the stopcock. This was accomplished by using two syringes, one for aspirating vitreous and the other to inject larvae, and these were

interchanged with the needle in place in the eye. A small amount of vitreous loss usually occurred at the perforation site during injection or after removal of the needle. In order ensure that most of the larvae entered the eye and to help tampanode the perforation site, 0.1cc of air was flushed into the vitreous cavity through the syringe and needle immediately following larval injection. Immediately after the injection, the syringe in which the larvae had been contained was flushed out, placing the contents on a glass slide. This was examined under a microscope and any remaining larvae were counted, and this number subtracted from the original amount to give a better approximation of the total injected into the eye. These procedures were followed for all injections except the left eye of M-1 and M-2 when the three-way stopcock was used.

Monkeys receiving vitreous injections of larvae were examined with the slit lamp and indirect opthalmoscope shortly following the injection. Following experimental infection, complete eye examinations, as in the baseline study, were performed at least one-two times per week, being more frequent in the early post-infection period. Lesions were documented with photographs whenever possible.

Monkey #7 (M-7) died 3 weeks after intravitreal injection of 75 larvae in the left eye. The right eye had not been infected and served as a control. Monkey #1 (M-1) was sacrificed with an overdose of pentobarbital 13 weeks after the first vitreous injection of 29 larvae in the left eye and one week after a second injection of 70 larvae into the vitreous of the left eye and nine weeks after injection of 63 larvae into the vitreous of the right eye. Monkey #5 (M-5) was sacrificed with an overdose of pentobarbital 13 weeks after oral infection with 5000 embryonated *T canis* eggs and one week after injection of 68 larvae into the vitreous of the left eye.

Post mortem examinations were performed on each monkey and all tissues were evaluated for gross pathologic changes with multiple slices through the various organs. Specimens of tissue containing suspicious lesions were taken from liver, lung, heart, kidneys, and skeletal muscle for histologic study and placed in 10% formalin for fixation. The entire brain was removed and examined grossly: following fixation in 10% formalin a more detailed study was performed by making multiple sections through all areas from which specimens were taken for histologic study.

Following enucleation, gross examination of the eyes was performed after which specimens of aqueous and vitreous were obtained for serologic testing. The eyes were then fixed in 10% formalin. After fixation the eyes were sectioned and the intraocular structures grossly examined before embedding in paraffin. Serial 8μ thick sections of all globes were then made.

Six consecutive sections (total of 48μ) were mounted on each slide and every third slide was stained with hematoxolin and eosin (H&E). Slides with serial sections around areas with lesions of the type seen with Toxocara infection were also stained and examined. The slides were reviewed with a general pathologist as well as a veterinary pathologist familiar with the morphology of *T canis* and tissue changes encountered in parasitic disease. They were also reviewed by a parasitic morphologist who specifically identified the organisms.

Monkeys #2, #4 and #6 are being followed to observe the long term effects of infection through the three different routes. Each has been observed a minimum of three months.

RESULTS

I. CLINICAL FINDINGS

A. NASOGASTRIC INFECTION

Seven days—Two monkeys, M-4 and M-5, both of which were infected with 5000 embryonated eggs by nasogastric (NG) tube, became mildly hyperactive approximately one week after infection and this increased somewhat over the following week and then progressively cleared in one to two weeks. No neurologic deficits were noted.

Nine days—A small superficial dot hemorrhage was noted in each eye of M-4. In the right eye it was immediately nasal to the disc and in the left eye the hemorrhage was a short distance inferior and temporal to the disc near an artery and questionably had a white center (Fig 2A). The remainder of the eye examination was normal in both monkeys.

Sixteen days—No change occurred in M-4 over the following week but there was an occasional cell and slight flare in the anterior chamber and a mild anterior subcapsular cataract in the right eye of M-5 as well as a small area of depigmentation in the macula which may not have been related to the infection. No vitreous cells were seen. The remainder of the examination in the right and left eyes were normal.

Eighteen days—A small hemorrhage with an associated whitish "exudate" was present along the inferior temporal vein in the mid-periphery of the right eye in M-4.

Twenty-one days—The fundus lesions appeared to be decreased in both eyes of M-4 (Fig 2B).

Twenty-eight days—The hemorrhage nasal to the disc in the right eye of M-4 had cleared.

Thirty-five days—There was no further change in either M-4 or M-5 until a fresh retinal hemorrhage was noted in the superior temporal quadrant of the right eye of M-4 and a few pigmented cells were seen in the anterior vitreous. The other hemorrhage in the right eye continued to decrease in size. The ocular findings remained the same in M-5.

Six weeks—The inferior temporal lesion in the right eye of M-4 had cleared.

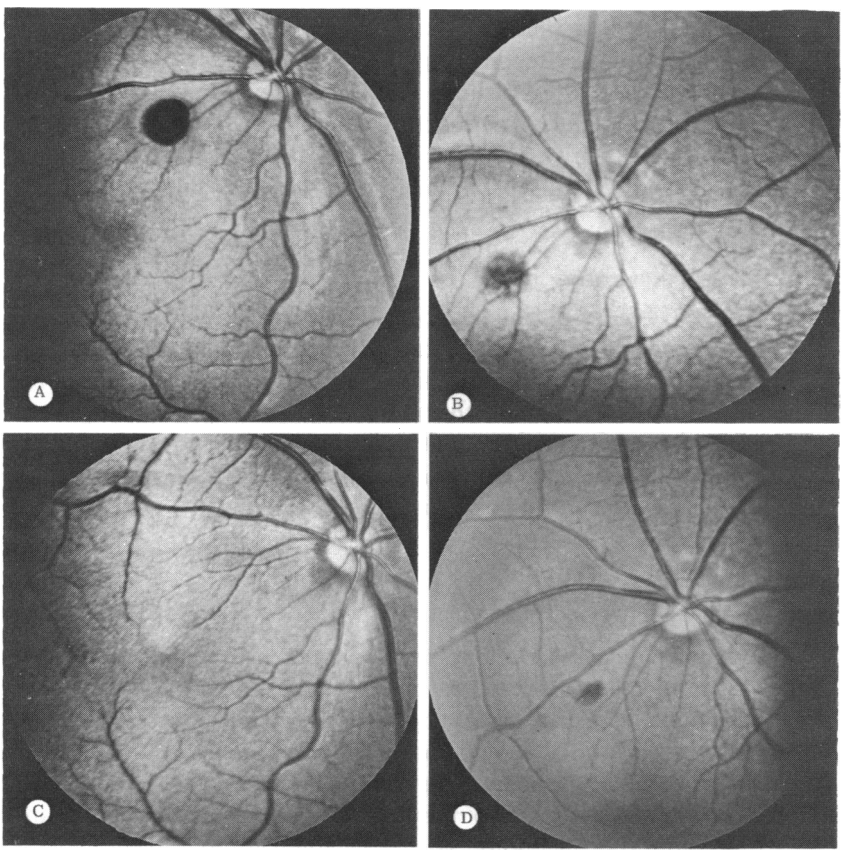

FIGURE 2

A: Retinal hemorrhage, inferior and temporal to the disc 9 days after nasogastric tube infection with embryonated Toxocara canis eggs, M-4, OS ×2. B: Retinal hemorrhage, clearing, 21 days post infection. M-4, OS × 2. C: Retinal hemorrhage, cleared, 49 days after infection, M-4, OS ×2. D: Fresh retinal hemorrhage, inferior and temporal to the disc adjacent to area of previous hemorrhage, 15 weeks post infection and 8 weeks after clearing of previous hemorrhage, M-4, OS ×2.

Seven weeks—The retinal hemorrhage temporal to the disc in the left eye of M-4 was no longer present (Fig 2C). On a subsequent examination, a small pigmented scar was seen in the area where the inferior temporal lesion had been.

Two months—After the initial retinal hemorrhage had cleared in the left eye of M-4, a new small superficial retinal hemorrhage appeared in the same eye immediately adjacent to the location of the previous hemorrhage (Fig 2D). There were no other abnormalities in M-4.

At no time were any larvae seen in either eye of M-4 or M-5 after the systemic infection. Only a few retinal hemorrhages occurred in M-4 and none were seen at any time in either eye of M-5 after NG tube infection. The findings in the right eye of M-5 remained unchanged and the left eye continued to be normal until an injection of larvae into the vitreous was performed 86 days after the original systemic infection. The findings after vitreous injection will be discussed later.

B. CAROTID INFECTION

Monkey #6 (M-6) showed no systemic or neurologic signs or symptoms following the intracarotid injection of larvae. Nine days following infection a possible larvae was seen in the anterior vitreous of the left eye but no other abnormalities were noted. No larvae were observed on subsequent examinations and no ocular abnormalities were noted at any time throughout the 105 days of observation following carotid injection.

C. INTRAVITREAL INFECTION

GENERAL OBSERVATIONS

The following descriptions pertain to monkeys receiving intravitreal injections of larvae. All of the monkeys developed conjunctival edema, especially at the injection site, which cleared in one to two weeks. None of them demonstrated any systemic or neurologic abnormalities and the findings were all localized to the intraocular structures. There were no retinal tears or detachments noted at any time after the injections. However, mild vitreous bleeding was observed in the posterior pole near the disc and macula of the left eye of M-2 immediately after injection but no retinal tears or definite bleeding sites were seen and the blood was thought to probably have originated from the perforation site in the pars plana

and/or was secondary to ocular hypotony. No bleeding occurred with any of the other injections. Tears in the posterior lens capsule due to trauma from the injection needle occurred in the right eye of M-1 and the left eye of M-5. A small opacity, without any obvious tear in the lens capsule, was seen after injection of the left eye in M-7 and may possibly have been due to trauma from the procedure.

There appeared to be minimal intraocular inflammation from the injections and no endophthalmitis occurred. At no time were larvae seen in the cornea, anterior chamber or iris in any of the monkeys.

M-1—OS

Immediate—Examination shortly following vitreal injection of larvae in the left eye of M-1 revealed two larvae in the anterior vitreous. No other changes were seen.

Two days—The eye was normal except for what appeared to be a larva in the anterior vitreous.

Four days—No larvae were seen and there was no vitreous inflammation and the fundus was normal.

The left fundus remained normal until 86 days post-infection when a second vitreal injection of larvae was performed in the left eye. Examination immediately after the procedure revealed 3+ cells and flare in the anterior chamber including RBC's. The vitreous was clear and three or four motile larvae were seen in the anterior portion.

Five days post-second infection OS—The anterior chamber reaction had diminished markedly but a moderate cellular reaction was present throughout the anterior and mid-vitreous. At least one to two dozen larvae were present in the anterior vitreous but none were seen in the retina, vessels or optic nerve. Scattered retinal hemorrhages of varying size, mainly around veins, were present in many areas as well as a hemorrhage extending from the optic disc into the vitreous. The disc was not swollen but the veins were 1-2+ dilated in all areas.

Seven days post-second infection OS—Many larvae were still present in the anterior and posterior vitreous. The disc and some of the other hemorrhages were clearing but several new ones had appeared, some with a white spot in the middle possibly representing a larvae (Fig 3). The vessel dilation had diminished and again larvae were not definitely seen in the tissues or vessels. The monkey was sacrificed at this time.

M-1—OD

Larvae were injected into the vitreous of the right eye of M-1, thirty-two days after the left eye had been injected. Examination shortly following the procedure revealed a large tear in the posterior lens capsule and several dozen very active larvae were seen just behind the lens with some in the lens defect itself. Many larvae moved through the vitreous and several were seen posteriorly near the retina.

Three days—Examination of the right eye revealed 3+ cells and flare and a few keratitic precipitates in the anterior chamber. Many motile larvae were present in the lens primarily in the anterior and posterior subcapsular regions and diffuse mild anterior and pos-

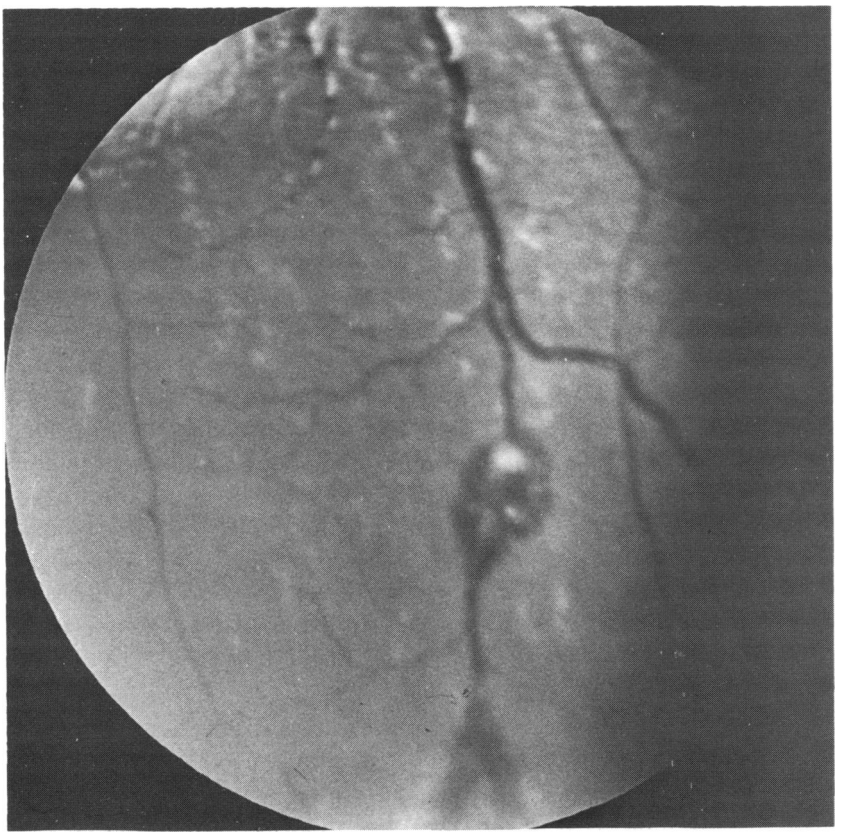

FIGURE 3
Retinal hemorrhage inferior to disc seven days following second vitreal injection of larvae, M-1, OS ×4.

terior subcapsular cataractous changes were present. In some areas with larvae the opacities seemed more dense while in other places the lens was clear even with larvae in those areas. No larvae were seen in the anterior chamber or anterior vitreous and there was essentially no reaction in the anterior vitreous. The disc was hyperemic with a hemorrhage and mild edema and one larva was seen in the posterior vitreous.

Five days—The anterior chamber reaction was much diminished but at least a dozen larvae were still present in the lens with no significant reaction around them. The remainder of the examination was unchanged.

Ten days—The anterior chamber reaction had almost cleared. Many motile larvae were still in the lens especially under the posterior capsule and the cataractous changes appeared stable.

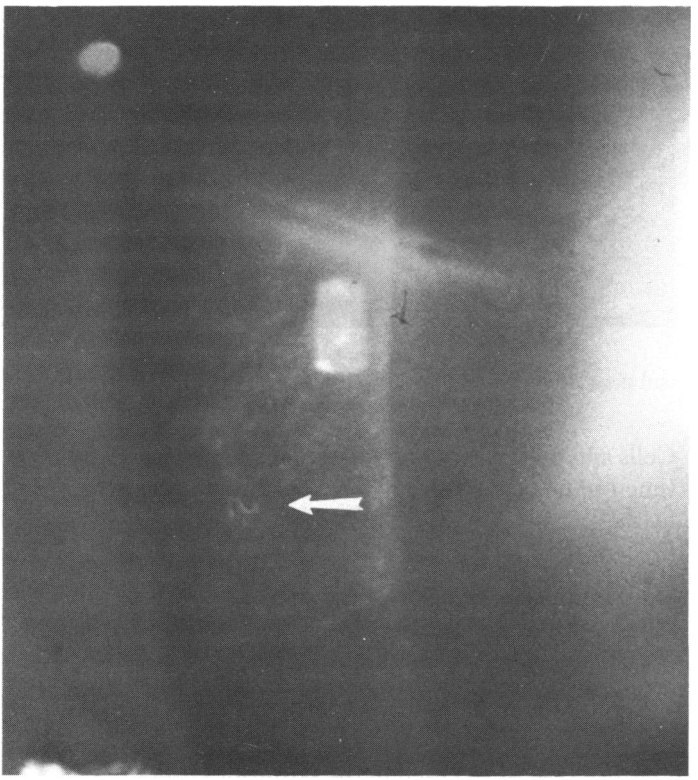

FIGURE 4
Second stage *T canis* larva under anterior lens capsule 14 days following vitreous injection of larvae, M-1, OD.

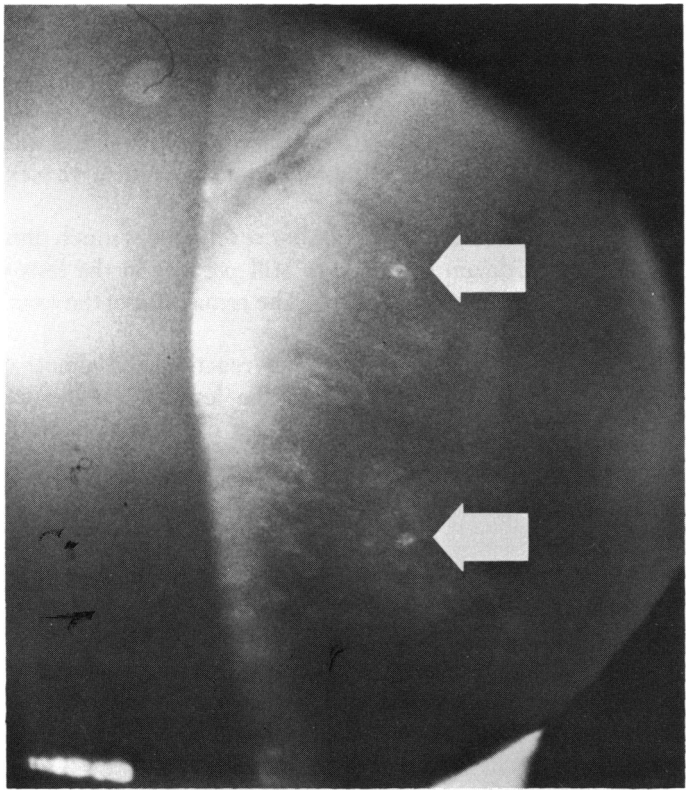

FIGURE 5
Second stage *T canis* larvae (2) under posterior lens capsule 14 days following vitreous injection of larvae, M-1, OD.

Cells and mild flare were noted in the anterior vitreous for the first time but no larvae were noted. Several motile larvae were present in the posterior vitreous and a small yellowish white lesion was present along an inferior vein. The disc hemorrhage had cleared and the hyperemia improved.

Fourteen days—Numerous motile larvae were present under the anterior and posterior lens capsule of the right eye of M-1 (Figs 4 & 5).

Seventeen days—The retinal lesion had cleared with atrophic changes in the retinal pigment epithelium in this area. Several motile larvae were noted in the posterior vitreous as previously but none were seen in any of the tissues. Numerous larvae were still present in the anterior and posterior subcapsular regions of the lens

and their movement was stimulated by the examining light. A few cells were present in the anterior vitreous.

Twenty-one days—A new whitish, slightly elevated small lesion was noted along an inferior temporal vein adjacent to the area of the previous lesion. The cataract progression made examination difficult.

Thirty-one days—Several scattered, new retinal hemorrhages around veins were seen and the disc was hyperemic.

Thirty-eight days—The cataract was very dense preventing visualization of the fundus and only one larva could be seen in the anterior subcapsular region.

Sixty-one days—The anterior segment was quiet and no larvae were seen. A dense cataract prevented visualization of the right fundus.

M-2—OS

Immediate—A vitreous hemorrhage occurred in the left eye of M-2 at the time of vitreous injection which cleared over the next four weeks and a small greyish lesion was present superior and temporal to the macula near the area where a hemorrhage had been seen.

Twenty-one days—The left eye was quiet and no larvae were seen, but cells and a moderate flare were noted.

Twenty-eight days—The anterior chamber was clear and one or two possible larvae were seen in the anterior vitreous.

Thirty-five days—A mild posterior subcapsular cataract was noted in the left eye and the following week a small amount of blood was seen in the anterior peripheral vitreous which gradually cleared.

Ninety-one days—The left eye remained normal and no significant abnormalities were noted. No larvae were ever definitely seen in the vitreous.

M-2—OD

Immediate—Examination of the right eye of M-2 shortly after vitreous injection revealed at least 12 motile larvae in the mid to posterior vitreous, which was otherwise clear, and no gross fundus abnormalities were seen. Larval movement seemed to increase if the examining light was shone on them.

Three days—A mild anterior chamber reaction was noted with a few cells in the anterior vitreous and motile larvae were still present in the posterior vitreous. Slight hyperemia of the disc and dilation of

the veins with a fresh superior temporal perivenous hemorrhage were also noted.

Seven days—The hemorrhage had changed to a whitish area, possibly edema, and some larvae were still present. RBC's were present in the anterior peripheral vitreous.

Seventeen days—No larvae were visible. The superior temporal lesion was clearing and a fresh retinal hemorrhage was noted.

Twenty-four days—The superior temporal lesion had become pigmented and the retinal hemorrhage was clearing. The eye was relatively quiet and remained this way throughout the 59 days of observation.

M-5—OS

Larvae were injected into the vitreous of the left eye of M-5 86 days after oral infection with embryonated ova.

Immediate—Examination immediately after injection revealed a tear in the posterior lens capsule with associated cataract due to trauma from the injection needle. One or two motile larvae were seen in the lens and several in the anterior to mid-vitreous. The fundus was otherwise normal.

Five days—A moderate reaction was present in the anterior chamber and anterior vitreous and one motile larva was seen in the lens cortex with five or six present in the mid vitreous and one to two dozen larvae were noted in the posterior vitreous. Scattered perivenular intraretinal hemorrhages in the nasal fundus were seen.

Seven days—The number of hemorrhages had increased and there seemed to be a decrease in the number of larvae observed. The monkey was sacrificed at this time.

M-7—OS

Immediate—Examination of the left eye revealed four to six larvae in the anterior vitreous. No hemorrhages were seen but a small posterior subcapsular lens opacity was noted.

Three days—Cells and a moderate flare in the anterior chamber and anterior vitreous were present and an early posterior subcapsular cataract was developing. Two motile larvae were seen in the lens under the posterior capsule and the fundus was normal.

Five days—The anterior chamber and anterior vitreous reaction were clearing. Multiple retinal hemorrhages were present, essentially all around veins which were slightly dilated. No larvae were seen.

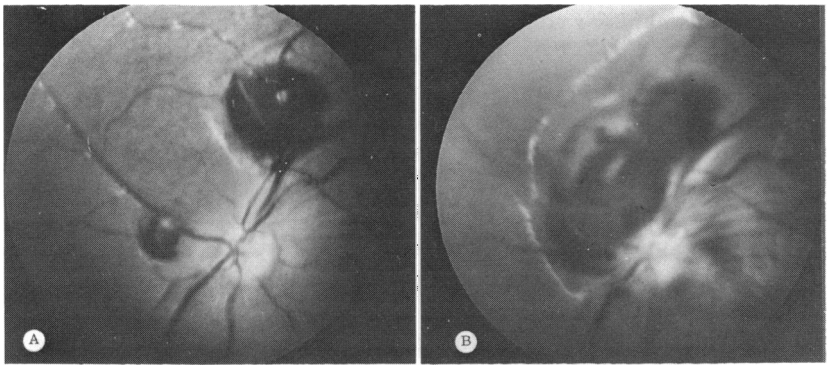

FIGURE 6

A: Retinal hemorrhage 10 days after intravitreal injection of larvae, M-7, OS ×1.8. B: Increased retinal hemorrhages with associated swelling and hyperemia of disc 14 days following injection of larvae into vitreous, M-7, OS ×1.8.

Ten days—The anterior chamber reaction had increased to 3+ cells and flare and there were 2+ cells in the anterior vitreous. The number of retinal hemorrhages had increased and some were becoming yellowish and elevated. The veins were slightly full and the disc mildly hyperemic. No larvae were seen (Fig 6A).

Fourteen days—The anterior chamber reaction was decreased. Retinal hemorrhages around the optic nerve head were increased and extended to the disc which was swollen and hyperemic while those in other areas were decreased (Fig 6B).

Seventeen days—Moderate cellular reaction was noted at the vitreous base near the pars plana. The retinal hemorrhages were clearing and no new lesions were noted. Two days later M-7 became acutely ill, was found mutilated and died within a few hours.

II. LABORATORY STUDIES

Relatively little data is available regarding the normal range of values for many laboratory tests in owl monkeys. Most of the data in this study was obtained by utilizing techniques readily available in most medical centers for testing human blood. The results were compared to the normal values for humans in our hospital laboratory and where possible data was also compared to results found in studies of normal owl monkeys.[89,90]

The tables are complicated and contain much information which requires close scrutiny. This was done to present data from many aspects of the study and it has been simplified as much as possible.

Table II contains composite data on the three monkeys that were systemically infected (M-4 and M-5 orally and M-6 intracarotid). All three showed a dramatic rise in the WBC's and eosinophils, either circulating and/or absolute counts, within nine days after infection and these remained significantly elevated for up to two months in the two animals which were orally infected and returned to pretreatment levels by the third month (M-5). In M-6 (carotid injection) values decreased within one month and then showed a secondary rise at the end of the second month possibly indicating a further larval migration after the initial infection.

Table III illustrates laboratory data on the three monkeys that were infected intraocularly. In M-1 there was a mild rise in eosinophils nine days after infection which returned to preinfection levels after one month. A secondary elevation of the eosinophils occurred over the subsequent two months suggesting that some larvae may have migrated out of the eye to various systemic tissues. There was no initial rise in the WBC's or eosinophils in M-2 but there appeared to be a mild elevation at the end of the second month. Studies on M-7 were incomplete due to the unexpected death.

Eosinophilia of varying degrees was present before infection in all animals and this has been observed previously in Owl and other monkeys in which no definite systemic or parasitic disease could be demonstrated.[90] Similar findings have been noted by Richard B. Swenson, DVM (personal communication 1978) and Willie L. Chapman, DVM, PhD (personal communication 1978). In addition, the degree of eosinophilic response to worms such as *T canis* varies with the species of animal (Willie L. Chapman, DVM, PhD personal communication, 1978). There appeared to probably be an elevation of the preinfection WBC count in M-4, M-5, M-6 and M-7 but when more than one study was done before infection there was some variability and the values fell within the range found in the previously mentioned studies of normal owl monkeys.

The SGOT and SGPT were abnormally elevated in most of the animals prior to infection compared to human values. However, most of these figures fell within the range of values observed in the study of normal owl monkeys. Alkaline phosphatase was abnormally elevated in all monkeys before infection and seemed to decrease after infection. However, data was inadequate to determine whether there was any significant change in these tests after systemic or local ocular infection. Protein electrophoresis was normal except for a mild preinfection elevation of gamma globulin in M-4, M-6 and M-7 although in M-6 this

TABLE II A: LABORATORY RESULTS M-4: SYSTEMIC INFECTION

Date:	7/12	7/28	8/28	9/6	9/29	10/30	1/11
	−47d[1]	−31d	NG tube infect.[2]	+9d	+32d	+63d	+136d
Hgb g/dl	18.1		18.3	16.2	14.8	17.5	18.8
HcT %	53.3		53.5	49.3	45.8	54.2	55.3
WBC	trashy 00.0		14,900	31,300	26,700	18,000	15,000
seg	22		36	18	26	18	20
band	1		0	0	0	0	0
lym	45		42	30	40	35	39
mono	1		0	2	2	1	2
eos	30		22	50	32	44	39
baso	1		0	0	0	2	0
Circ eos (absol)	9,504			17,376	39,969	11,757	5,900
SGOT mU/ml	374						233
SGPT mU/ml	65				20		67
Alk. phos. mU/ml	818						225
Total protein g/dl	9.5						9.4
Alb g/dl	4.3						4.3
Alpha 1 g/dl	1.4						1.4
Alpha 2 g/dl							0.5
beta g/dl	1.0						0.5
gamma g/dl	3.0						2.7
IgG mg/dl			1,335				1,180
IgA mg/dl			<8				<7
IgM mg/dl			131				>256

TABLE II A: LABORATORY RESULTS M-4: SYSTEMIC INFECTION

Date:		7/12		7/28		8/28		9/6		9/29		10/30		1/11
		−47d[1]		−31d		NG tube infect.[2]		+9d		+32d		+63d		+136d
ELISA[7]		T[3]	A[4]	T	A	T	A	T	A	T	A	T	A	
Serum		—[5]	—	1:8	1:8			—	—	1:32	1:8	1:32	—	
Vitreous	OD													
	OS													
Aqueous	OD													
	OS													
IHA[6,8]		1:32	1:128	—	1:128			1:64	1:128			1:1024	1:1024	
BFT[6,9]		—	—	—	—			—	—			1:20	—	
Heterophile[6,10]		<1:8		<1:8				<1:8				—		

[1]Indicates time in days pre (−) and post (+) infection.
[2]Route of infecton; NG = nasogastric tube.
[3]T = Toxocara antibody titer.
[4]A = Ascaris antibody titer.
[5]— = negative.
[6]Tests performed only on serum.
[7]ELISA: titer of 1:16 or > = probably significant; titer of 1:32 or > = significant.
[8]IHA = Indirect hemagglutination test; titer of 1:64 or > = significant.
[9]BFT = Bentonite flocculation test; titer of 1:5 or > = significant.
[10]Heterophile test; any titer esp. a rising titer may be significant.

TABLE II B: LABORATORY RESULTS M-5: SYSTEMIC INFECTION

Date:	7/12	7/28	8/28	9/6	9/29	10/30	11/22	11/29
	−47d[1]	−31d	NG tube infect.[2]	+9d	+32d	+63d	+86d Vit inj OS	NG +93d OS +7d Sacrifi.
Hgb g/dl	14.8		17.4	14.4	15.0	18.1		16.0
HcT %	46.0		52.7	45.3	45.7	56.2		44.9
WBC	23,700		11,300	72,900	51,100	27,400		10,200
seg	28		37	12	35	13		67
band	2		1	4	0	1		1
lym	43		45	28	28	22		19
mono	1		0	3	5	0		3
eos	24		17	53	32	64		10
baso	1		0	0	0	0		0
Circ eos. (absol)	19,456			41,600	28,520			2,112
SGOT mU/ml	136				185			523
SGPT mU/ml	29				30			94
Alk. phos. mU/ml	423				249			146
Total protein g/dl	9.0							8.2
Alb g/dl	4.7							4.7
Alpha 1 g/dl	1.5							0.0
Alpha 2 g/dl								1.0
beta g/dl	0.9							0.8
gamma g/dl	1.9							1.7
IgG mg/dl			960					454
IgA mg/dl			<8					<5
IgM mg/dl			94					70

TABLE II B: LABORATORY RESULTS M-5: SYSTEMIC INFECTION

Date:	7/12		7/28		8/28	9/6		9/29		10/30		11/22		11/29	
	−47d[1]		−31d		NG tube infect.[2]	+9d		+32d		+63d		+86d Vit inj OS		NG +93d OS +7d Sacrifi.	
ELISA[7]	T[3]	A[4]	T	A		T	A	T	A	T	A	T	A	T	A
Serum	—[5]	—	—	—		—	—	1:32	1:2	1:16	—			—	—
OD														—	—
Vitreous															
OS												—	—	—	—
OD														—	—
Aqueous															
OS														—	—
IHA[6,8]	1:16	1:16	1:8	1:8		1:64	1:32	1:256	1:128	1:256	1:128			1:64	1:64
BFT[6,9]	—	—	—	—		—	—	1:10	—	1:5	—			—	—
Heterophile[6,10]	1:16		—			1:16		1:32		—				1:32	

[1]Indicates time in days pre (−) and post (+) infection.
[2]Route of infection; NG = nasogastric tube.
[3]T = Toxocara antibody titer.
[4]A = Ascaris antibody titer.
[5]— = negative.
[6]Tests performed only on serum.
[7]ELISA: titer of 1:16 or > = probably significant; titer of 1:32 or > = significant.
[8]IHA = Indirect hemagglutination test; titer of 1:64 or > = significant.
[9]BFT = Bentonite flocculation test; titer of 1:5 or > = significant.
[10]Heterophile test; any titer esp. a rising titer may be significant.

TABLE II C: LABORATORY RESULTS M-6: SYSTEMIC INFECTION

Date:	7/12	7/28	8/28	9/6	9/29	10/30	1/11
	−47d[1]	−31d	Left carotid inject.[2]	+9d	+32d	+63d	+136d
Hgb g/dl	17.1		15.5	13.8	15.9	15.0	18.0
HcT %	51.3		47.3	42.2	46.7	43.1	51.5
WBC	17,000		8,800	28,700	9,400	21,400	11,100
seg	18		41	22	26	19	22
band	1		0	0	0	0	0
lym	39		37	11	51	30	51
mono	2		1	1	2	1	1
eos	40		21	66	20	49	25
baso	0		0	0	0	0	0
Circ. eos (absol)	20,400			22,880	3,432	7,955	2,800
SGOT mU/ml	154				263		205
SGPT mU/ml	37				37		52
Alk. phos. mU/ml	1,056				332		426
Total protein g/dl	8.6						9.8
Alb g/dl	4.1						5.4
Alpha 1 g/dl	1.1						1.1
Alpha 2 g/dl							0.4
beta g/dl	0.8						0.5
gamma g/dl	2.5						2.4
IgG mg/dl			1,080				1,084
IgA mg/dl			<8				<7
IgM mg/dl			114				125

TABLE II C: LABORATORY RESULTS M-6: SYSTEMIC INFECTION

Date:	7/12	7/28	8/28	9/6	9/29	10/30	1/11
	−47d[1]	−31d	Left carotid inject.[2]	+9d	+32d	+63d	+136d
ELISA[7]	T[3] A[4]	T A	T A	T A	T A	T A	T A
Serum	—[5] —	— —		— —	1:8 1:2	1:2 —	
Vitreous OD							
Vitreous OS							
Aqueous OD							
Aqueous OS							
IHA[6,8]	— —	<1:8 1:16		<1:8 1:32		1:128 1:256	
BFT[6,9]	— —	— —		— —		— —	
Heterophile[6,10]	—	<1:8		<1:8		—	

[1]Indicates time in days pre (−) and post (+) infection.
[2]Route and side of infection; carotid injection.
[3]T = Toxocara antibody titer.
[4]A = Ascaris antibody titer.
[5]— = negative.
[6]Tests performed only on serum.
[7]ELISA: titer of 1:16 or > = probably significant; titer of 1:32 or > = significant.
[8]IHA = Indirect hemagglutination test; titer of 1:64 or > = significant.
[9]BFT = Bentonite flocculation test; titer of 1:5 or > = significant.
[10]Heterophile test; any titer esp. a rising titer may be significant.

TABLE III A: LABORATORY RESULTS M-1: OCULAR INFECTION

Date:	7/12	7/28	8/28	9/6	9/29	10/30	11/22	11/29
	−47d[1]	−31d	Vit inj. OS[2]	+9d	+32d Vit. inj. OD	OD +31d OS +63d	OD +54d OS +86d 2nd Vit. Inj. OS	OD +61d OS#1 +93 OS#2 +7d Sacrif.
Hgb g/dl	18.2		17.6	16.1	17.5	17.3		18.0
HcT %	52.2		52.2	48.1	50.2	54.5		50.0
WBC	11,900		8,900	10,000	8,900	11,200		13,100
seg	21		14	21	30	12		10
band	0		0	1	0	0		0
lym	54		58	35	42	44		28
mono	2		0	0	2	0		3
eos	23		28	43	26	43		59
baso	0		0	0	0	1		0
Circ eos. (absol)	1,936				2,253	6,336		8,192
SGOT mU/ml	123				172			449
SGPT mU/ml	24				37			63
Alk. phos. mU/ml	2,739				2,940			1,012
Total protein g/dl	8.2							7.1
Alb g/dl	3.5							3.3
Alpha 1 g/dl	2.3							0.1
Alpha 2 g/dl								1.1
beta g/dl	0.8							1.0
gamma g/dl	1.6							1.5
IgG mg/dl			427					499
IgA mg/dl			<8					<7
IgM mg/dl			152					134

TABLE III A: LABORATORY RESULTS M-1: OCULAR INFECTION

Date:	7/12		7/28		8/28		9/6		9/29		10/30		11/22		11/29	
	−47d[1]		−31d		Vit inj. OS[2]		+9d		+32d Vit. inj. OD		OD +31d OS +63d		OD +54d OS +86d 2nd Vit. Inj. OS		OD +61d OS#1 +93 OS#2 +7d Sacrif.	
ELISA[7]	T[3]	A[4]	T	A	T	A	T	A	T	A	T	A	T	A	T	A
Serum	—[5]	—	—	—			—	—	—	—	—	—			—	—
OD									—	—					—	—
Vitreous OS					—	—							—	—	—	—
OD															—	—
Aqueous OS															—	—
IHA[6,8]			<1:8	<1:8			<1:8	<1:8			1:32	1:8			<1:8	<1:8
BFT[6,9]			—	—			—	—			1:10	—			—	—
Heterophile[6,10]			—				<1:8				—				<1:8	

[1]Indicates time in days pre (−) and post (+) infection with appropriate eye indicated, OD or OS.
[2]Route of infection and eye infected (ie OD or OS); Vit. inj. = Vitreous injection of larvae.
[3]T =Toxocara antibody titer.
[4]A = Ascaris antibody titer.
[5]— = negative.
[6]Tests performed only on serum.
[7]ELISA: titer of 1:16 or > = probably significant; titer of 1:32 or > = significant.
[8]IHA = Indirect hemagglutination test; titer of 1:64 or > = significant.
[9]BFT = Bentonite flocculation test; titer of 1:5 or > = significant.
[10]Heterophile test: any titer esp. a rising titer may be significant.

TABLE III B: LABORATORY RESULTS M-2: OCULAR INFECTION

Date:	7/12	7/28	8/28	9/6	9/13	9/29	10/13	10/30	1/11
	−47d[1]	−31d	Vit. inj. OS[2]	+9d	+16d	+32d	+46d Vit. inj. OD	OD +17d OS +63d	OD +90d OS +136d
Hgb g/dl	18.9		16.1		14.5	15.8		17.5	18.1
HcT %	56.4		48.4		44.1	48.0		54.2	52.4
WBC	9,400		6,800		3,700	8,300		13,400	9,900
seg	38		36		12	31		24	18
band	0		0		2	0		0	0
lym	45		44		69	45		37	63
mono	4		0		0	4		0	4
eos	13		20		17	20		35	15
baso	0		0		0	0		4	0
Circ. eos. (absol)	5,920				666	3,098		154	1,500
SGOT mU/ml	164					227			67
SGPT mU/ml	61					53			170
Alk. phos. mU/ml	7,502					4,812			3,140
Total protein g/dl	7.2								7.3
Alb g/dl	3.4								
Alpha 1 g/dl	1.5								
Alpha 2 g/dl	0.9								
beta g/dl	0.7								
gamma g/dl	0.7								
IgG mg/dl			256		206				
IgA mg/dl			<8		<8				
IgM mg/dl			33		42				

TABLE III B: LABORATORY RESULTS M-2: OCULAR INFECTION

Date:	7/12		7/28		8/28		9/6		9/13		9/29		10/13		10/30		1/11
	−47d[1]		−31d		Vit. inj. OS[2]		+9d		+16d		+32d		+46d Vit. inj. OD		OD +17d OS +63d		OD +90d OS +136d
ELISA[7]	T[3]	A[4]	T	A	T	A	T	A	T	A	T	A	T	A	T	A	
Serum	—[5]	—	—	—			—	—	—	—	—	—			—	—	
OD													—	—			
Vitreous OS					—	—											
OD																	
Aqueous OS																	
IHA[6,8]	<1:8	<1:8	<1:8	<1:8			<1:8	<1:8	<1:8	<1:8					<1:8	<1:8	
BFT[6,9]	—	—	—	—			—	—	—	—					—	—	
Heterophile[6,10]	<1:8						<1:8		<1:8						—		

[1]Indicates time in days pre (−) and post (+) infection with approp. eye indicated, OD or OS.
[2]Route of infection and eye infected (ie OD or OS); Vit. inj. = Vitreous injection.
[3]T = Toxocara antibody titer.
[4]A = Ascaris antibody titer.
[5]— = negative.
[6]Tests performed only on serum.
[7]ELISA: titer of 1:16 or > = probably significant; titer of 1:32 or > = significant.
[8]IHA = Indirect hemagglutination test; titer of 1:64 or > = significant.
[9]BFT = Bentonite flocculation test; titer of 1:5 or > = significant.
[10]Heterophile test: any titer esp. a rising titer may be significant.

was within the range of values for normal owl monkeys. Immunoglobulins were normal in all 6 monkeys.

The ELISA as well as the IHA, BFT and heterophile tests were negative in all samples of serum from the three animals (M-1, M-2,

TABLE III C: LABORATORY RESULTS M-7: OCULAR INFECTION

Date:	7/12	7/28	9/29	10/18
	−79d[1]	−63d		+19d
			Vit. inj. OS[2]	Expired
Hgb g/dl	15.3		14.0	
HcT %	45.9		40.9	
WBC	21,200		20,100	
seg	25		50	
band	0		0	
lym	36		18	
mono	3		6	
eos	36		26	
baso	0		0	
Circ. eos. (absol)	51,328		6,494	
SGOT mU/ml	119		109	
SGPT mU/ml	29		25	
Alk. phos. mU/ml	5,236		2,865	
Total protein g/dl	9.4			
Alb g/dl	2.7			
Alpha 1 g/dl, Alpha 2 g/dl	1.8			
beta g/dl	1.2			
gamma g/dl	3.7			
IgG mg/dl				
IgA mg/dl				
IgM mg/dl				

ELISA[7]		T[3]	A[4]	T	A	T	A	T	A
Serum		—[5]	—	—	—	—	—	—	—
Vitreous	OD							—	—
	OS					—	—	—	—
Aqueous	OD							—	—
	OS							—	—
IHA[6,8]		1:16	1:64	<1:8	<1:8			<1:8	<1:8
BFT[6,9]		—	—	—	—			—	—
Heterophile[6,10]		<1:8		<1:8				—	

[1]Indicates time in days pre (−) and post (+) infection with approp. eye indicated, OD or OS.
[2]Route of infection and eye infected (ie OD or OS); Vit. inj. = vitreous injection.
[3]T = Toxocara antibody titer.
[4]A = Ascaris antibody titer.
[5]— = negative.
[6]Tests performed only on serum.
[7]ELISA: titer of 1:16 or > = probably significant; titer of 1:32 > = significant.
[8]IHA = Indirect hemagglutination test; titier of 1:64 or > = significant.
[9]BFT = Bentonite flocculation test; titer of 1:5 or > = significant.
[10]Heterophile test; any titer esp. a rising titer may be significant.

M-7) infected intravitreally except for the IHA ascaris test on M-7 which was positive on one preinfection study and negative in a subsequent preinfection test.

The serum ELISA test was negative in M-6 which received the intracarotid injection but the IHA test for Toxocara and Ascaris was positive two months after infection.

In M-4 the serum ELISA test for Toxocara became positive one month after oral infection and remained positive at the same level one month later. The IHA for Toxocara was negative and for Ascaris was positive before infection and the titer for both rose significantly by two months after infection. The BFT for Toxocara and Ascaris was negative before infection but the Toxocara titer was positive two months after infection.

In M-5 the serum ELISA test for Toxocara became positive one month after oral infection and was probably positive two months after innoculation. The IHA for Toxocara became positive nine days after infection and for Ascaris became positive 32 days after infection and both remained positive till the animal was sacrified 93 days after infection. The BFT was negative before infection but became positive one month after infection and remained positive for an additional month after that.

Aqueous and vitreous specimens taken at various times pre- and postinfection in M-1, M-2, M-5 and M-7 were all negative to the ELISA test.

Stool examinations for ova and parasites as well as blood smears for parasites were all negative. The blood group and type was B positive in all six monkeys. Anti A and anti B antibody studies were done but the data was inadequate to draw definitive conclusions.

III. PATHOLOGICAL FINDINGS

A. SYSTEMIC PATHLOGY

M-1

Postmortem examination revealed a few dark round hemorrhages in the lungs (possibly related to the post mortem examination procedure) as well as a few pale areas that may have represented atelectasis. The brain, liver, kidney, heart and skeletal muscles appeared normal. The bowel was also normal and a few pinworms were found in the cecum.

Histologic examination of the lung showed a patchy, nonspecific interstitial pneumonia of moderate intensity. No larvae or

granulomas were seen. The heart, kidney, bowel, liver and brain were all normal.

M-5

At autopsy there were some small round whitish lesions scattered throughout the upper and lower lobes of the left lung which questionably were due to parasitic infection. A few whitish, small, round lesions were present in the liver and similar lesions were seen in both kidneys. The brain, heart, skeletal muscle and bowel appeared normal.

Histopathological study of the lung revealed a mild to moderate patchy eosinophilic pneumonia with no granulomas or larvae. In the liver scattered granulomas consistent with parasitic infection were noted but no larvae were seen (Fig 7). In the kidney scattered accumulations of chronic inflammatory cells, with a few eosinophils, were present in the subcapsular region but no definite granulomas were seen. A patchy interstitial nephritis was also noted. The heart, skeletal muscle, bowel and brain were all normal.

M-7

At the time of autopsy portions of the ears, eyes, lips, ankles and

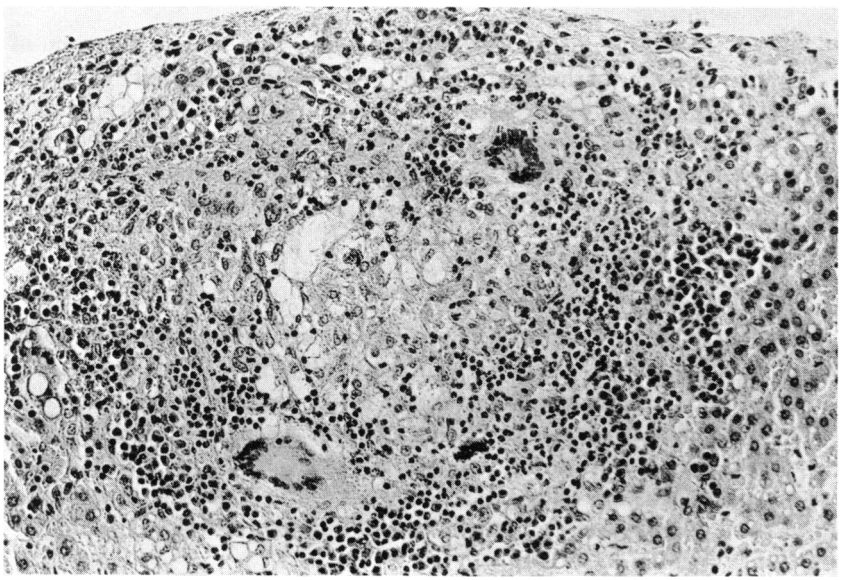

FIGURE 7
Granuloma in liver, M-5, H&E, ×121.

fingers were missing and appeared to have been chewed away. Several small, round dark hemorrhages were present in the lungs and liver. The brain, heart, spleen, kidneys, adrenals and pancreas all appeared normal. The bowel was also normal but numerous pinworms were present in the region of the cecum.

Microscopic examination of the lungs revealed acute interstitial pneumonia of probable viral origin, which was the most likely cause of death. Mild fatty changes were seen in the liver but no granulomas were noted. The brain was normal.

B. OCULAR PATHOLOGY

1. GROSS

No abnormalities of the globe or extraocular structures were noted at the time of enucleation in any of the eyes.

On sectioning the right and left globes of M-1 several small 1-2mm round or elongated-irregular, fairly smooth, elevated yellowish-white lesions were seen on various areas of the ciliary body. Little or no surrounding reaction was noted (Fig 8). A few scattered hemorrhages were seen. The retina was artifactitiously detached following vitreous aspiration and the residual vitreous appeared clear.

No gross intraocular abnormalities were seen at the time of sectioning of the right globe of M-5. However, the left eye of M-5 showed scattered hemorrhages and a few possible early lesions on the ciliary processes. The retina was artifactitiously detached following aspiration of vitreous and the residual vitreous was clear.

2. HISTOPATHOLOGY

M-1—OD

On multiple sections at least two ascarid larvae consistent with *T canis* were seen in the substance of the lens (Figs 9A, 9B). The lack of inflammatory reaction in the lens around the larvae is rather striking. Cataractous changes were present in the lens, especially on the side opposite the larvae, in the region where the lens was damaged at the time of larval injection. A few small retinal hemorrhages were noted but no other significant abnormalities were seen.

M-1—OS

A significant inflammatory reaction consisting primarily of eo-

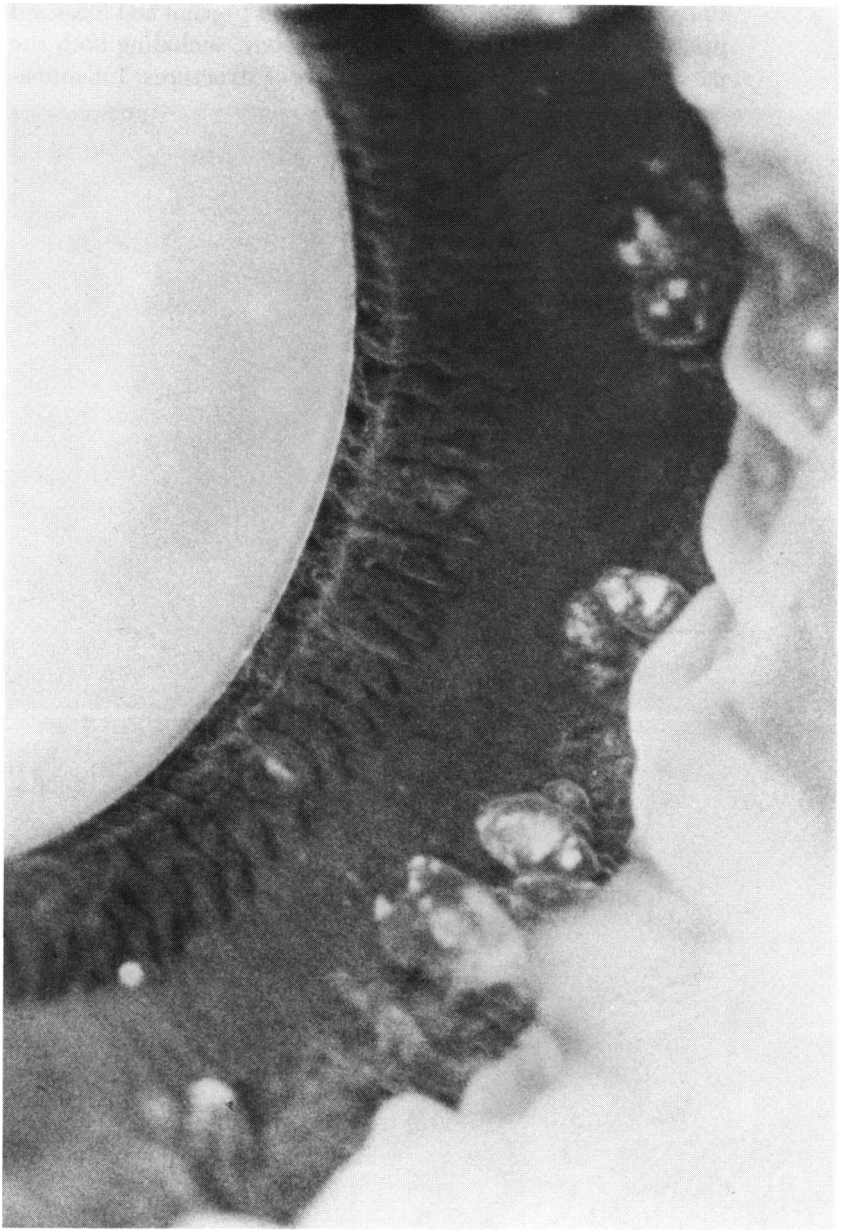

FIGURE 8
Ciliary body lesions, gross pathology, M-1, OS, × 10.

sinophils and some mononuclear cells was present and localized primarily to the region of the ciliary body, including both the pars plicata and pars plana, and adjacent structures. Inflamma-

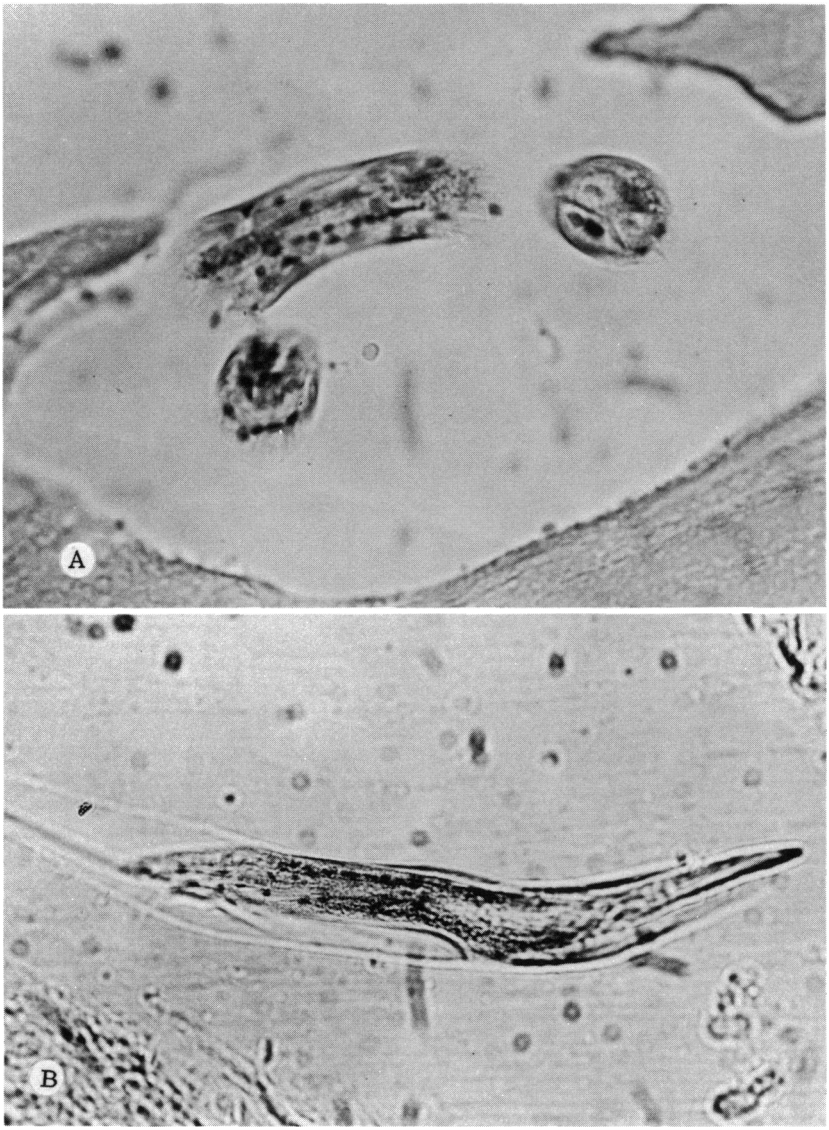

FIGURE 9

A: Sections of *T canis* larvae in lens, M-1, OD, H&E, ×532. B: *T canis* larvae in lens, M-1, OD, H&E, ×332.

tory foci of varying sizes were present under the retinal pigment epithelium in many sections and were most frequently seen near the junction of the pars plana and pars plicata (Fig 10). Many eosinophils were present in these masses and some of them had the appearance of "eosinophilic abscesses". Histiocytes and large multinucleated cells were also seen in some of the lesions and in those areas the masses represented eosinophilic

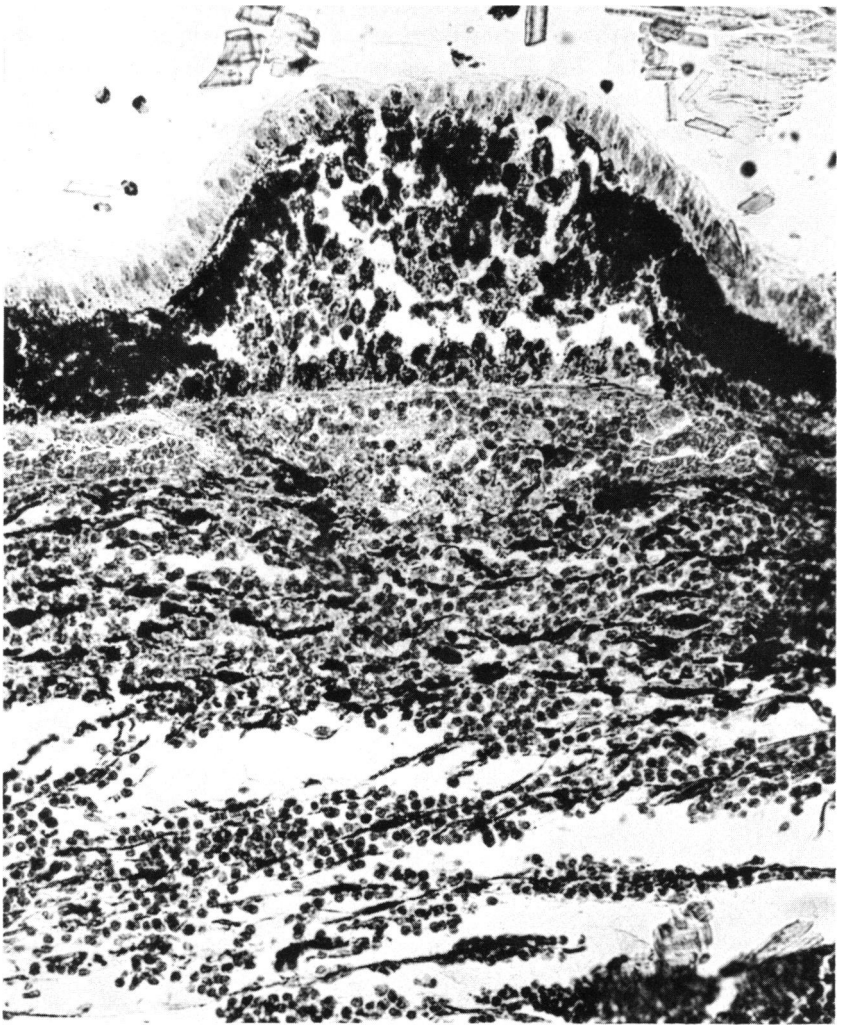

FIGURE 10
Inflammatory mass in ciliary body and under RPE, M-1, OS, H&E, ×175.

granulomas. Some of the masses were composed primarily of necrotic cells and debris suggesting a severe reaction. Eosinophils were seen in the overlying vitreous some of which may have been an artifact of tissue sectioning.

Foci of eosinophils were seen within the ciliary body itself as well as in the adjacent sclera and in some structures perivascular cuffing by eosinophils was noted. The choroid appeared congested but was otherwise normal. Scattered hemorrhages were present in the retina and there was also a hemorrhage in the optic nerve head (Fig 11). The eosinophilic and granulomatous responses are typical of the type of change seen in eyes as the result of invasion with a larva such as *T canis*. However, larvae were not seen in any of the sections.

M-5—OD

No significant abnormalities were noted in any of the sections.

M-5—OS

Lesions similar to those noted in the left eye of M-1 were seen. They contained numerous eosinophils as well as some histiocytes and represent eosinophilic granulomas (Fig 12). Some of them

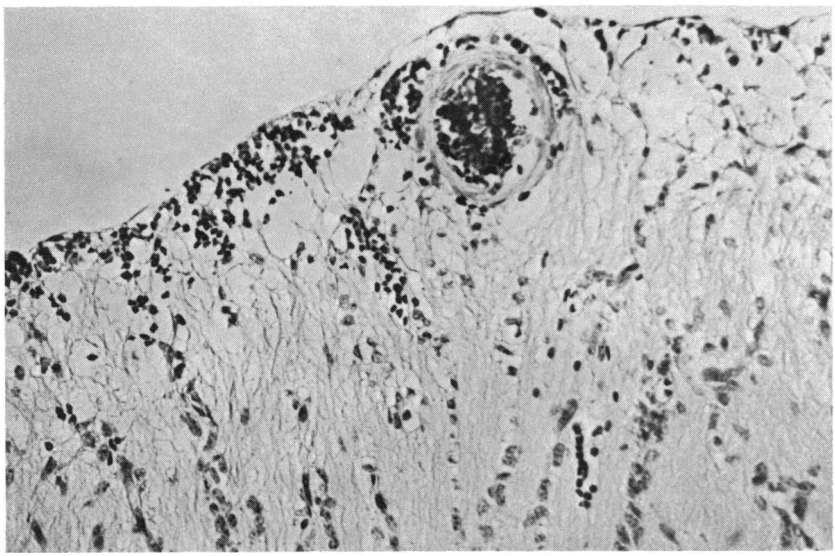

FIGURE 11
Optic nerve head hemorhage, M-1, OS, H&E, ×182.

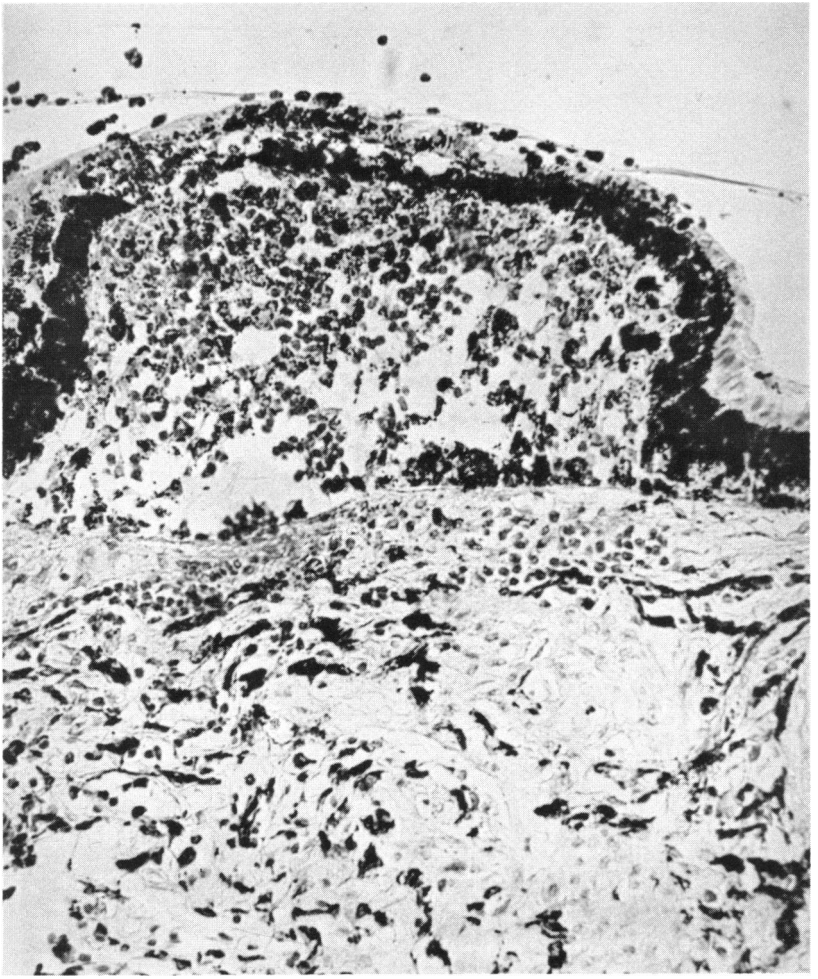

FIGURE 12
Eosinophilic granuloma, ciliary body, M-5, OS H&E, ×175.

had undergone necrosis as in M-1 and in areas there were eosinophils in the overlying vitreous. The lesions, as in the other eye, were located primarily in the ciliary body, especially at the junction of the pars plana and pars plicata and many formed masses under the retinal pigment epithelium. Reaction was also seen around some of the ciliary processes. In one area there was an eosinophilic response in the sclera near the equator and scattered eosinophils were present between this area and lesions

FIGURE 13
Eosinophils in vitreous over ciliary body, M-7, OS, H&E, ×175.

in the ciliary body, suggesting that a larva may have migrated through this region.

In another area near the equator there was an elevated mass under the retinal pigment epithelium similar to those seen in the ciliary body, with a hemorrhage in the adjacent retina and eosin-

ophils in the overlying vitreous. Scattered retinal hemorrhages were also found in other areas. The reaction observed was almost identical to that seen in the left eye of M-1.

M-7—OD

No pathological changes were noted.

M-7—OS

Diffuse scattered retinal hemorrhages were seen. Numerous

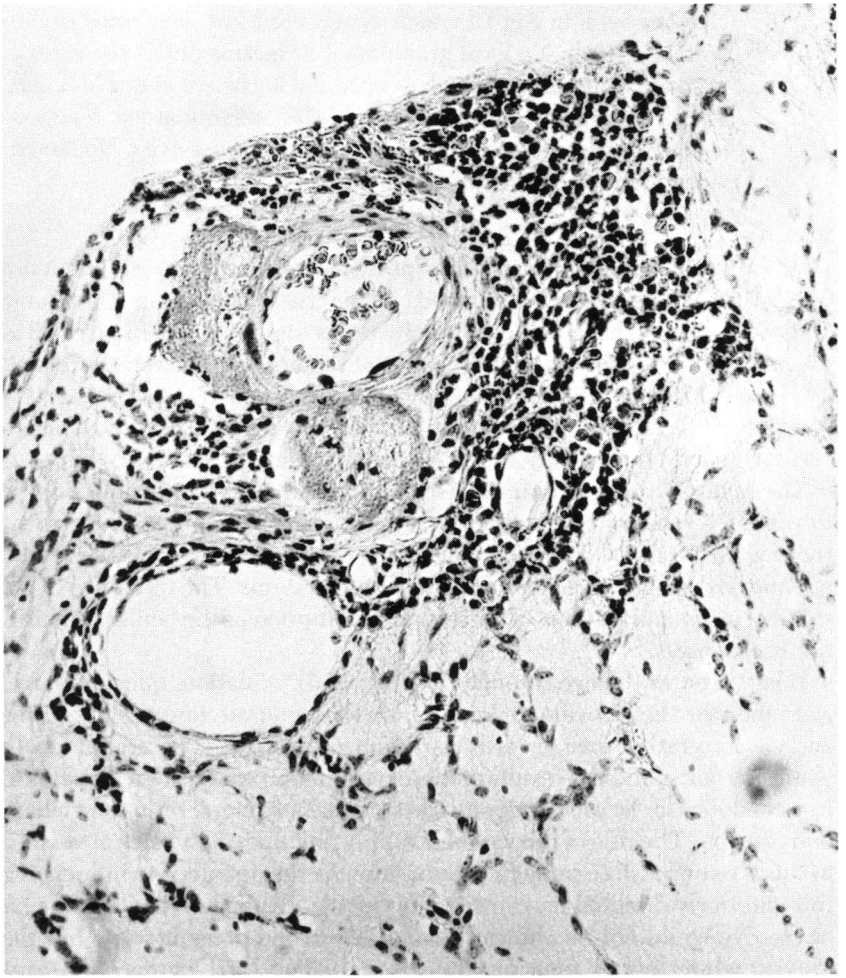

FIGURE 14
Inflammation of optic nerve head and edema of vessel wall, M-7, OS, H&E, ×175.

eosinophils were present in the ciliary body, whereas the iris contained essentially all plasma cells and lymphocytes. Many eosinophils were present in the vitreous overlying the ciliary body which may have been due in part to sectioning but this type of reaction was not noted in other areas (Fig 13).

An inflammatory process was noted in the optic nerve head consisting mainly of eosinophils and mononuclear inflammatory cells. Edema of a vessel wall as well as a possible vasculitis were present (Fig 14). These changes corresponded with fundus changes seen in Fig 12 which were noted five days prior to the animal's death. No focal granulomas or lesions under the retinal pigment epithelium, which were noted in the left eye of M-1 and M-5, were seen and in general, the inflammatory reaction seemed less acute and severe compared to those eyes. No larvae were found in this eye.

DISCUSSION

Human infection with *T canis* has protean manifestations and is more widespread than generally realized. Many basic questions concerning clinical aspects of the disease, especially the ocular involvement, as well as diagnostic laboratory testing and treatment remain unanswered at present. The natural history and clinical course of the ocular disease is not fully understood since most clinical and pathological studies, due to their nature, primarily report only the late manifestations.

The primary route of entry of Toxocara into the eye is presumed to be through the vascular system,[1,13,42] and if this primary assumption is correct there is the further delineation to be made of whether this is via the retinal or choroidal vessels or a combination of both systems. The influence of the route of entry and the size of vessels on distribution of the ocular lesions is not understood.[13,41-43]

If larvae enter the eye through the choroidal circulation, questions arise as to their further movement such as, do they migrate towards the retina and vitreous rather than towards the sclera and if so why? Based upon both clinical reports, and the results of this experimental study, there appears to be a tendency for larvae to migrate towards the peripheral retina and ciliary body region. The role of the vascular supply (volume and/or size of vessels) in this area or possibly some trophic or other factors involved in influencing this anteriorly directed movement is unknown. Unfortunately, this region of the eye could not be clinically examined in the present study but the greyish white lesions seen on the external ciliary body surface on gross pathologic examination, and the granulomas and other inflammatory le-

sions seen in this area on histologic examination, suggest a predisposition for *T canis* larvae to involve these tissues, at least in this experimental model. In some of the specimens there appeared to be migration through or adjacent to, well vascularized tissues such as the choroid, leading to or from the ciliary body region. Unfortunately, there were no areas in which penetration of a vessel by a larva could be seen in histologic sections, which would absolutely verify this concept. The marked inflammatory reponse in and around richly vascularized tissue, compared to the lack of response clinically and pathologically in the avascular lens, suggests that certain factors associated with blood vessels are important determinants in the degree and type of tissue response. This study has not clarified whether toxic, immune, mechanical or other factors or a combination of these are responsible for the observed reaction, rather the study is an initial step in delineating the ocular response to Toxocara when delivered orally or by carotid or intravitreal injection.

The author is unaware of any clinicopathologic animal studies, especially in primates, describing the short and long term ocular manifestations of Toxocara infection and to the best of my knowledge there have been no pathologically proven cases to validate the accuracy of newer serologic tests.

This study, utilizing both normal and abnormal routes of infection, was undertaken in an attempt to produce ocular disease so that the clinical course with its different manifestations could be followed, various diagnostic tests evaluated and the pathophysiology of the disease studied.

Only a few retinal lesions were seen in the eyes of the two monkeys infected with ova by nasogastric tube although they developed positive serological responses and an eosinophilia indicative of a successful infection. The retinal hemorrhages, located in the posterior pole or mid-periphery, occurred by at least the ninth day after infection and seemed to be around arteries although this could not be adequately documented. It is not known if the retinal hemorrhages represent migration of larvae in the ocular tissues, although other studies in mice have shown they can arrive in the eye and produce hemorrhages as early as two to three days after oral infection.[83,84]

The retinal hemorrhages in the eyes infected by intravitreal injection of larvae were, in contrast, located primarily around veins which were often somewhat dilated. This reaction could be due to larvae exiting the eye through the veins with hemorrhages occurring at the site of entry into the vessels. If this is correct, it would support the suggestion of other authors that retinal hemorrhages are associated with migration of larvae through vessel walls.[20,41,42]

A second vitreous injection of larvae in the left eye of M-1 (initial infection by intravitreal injection) and a vitreous injection of larvae in the left eye of M-5 (initial infection by nasogastric tube), both made 3 months after the original infections, were performed to determine if prior local or systemic infection would alter the immediate ocular reaction. These two monkeys were sacrificed one week after the last vitreous injection when the number and severity of fundus lesions, such as retinal hemorrhages, dilated veins and swelling of the optic nerve head, were increasing and larvae appeared to be disappearing from the eye. It was hoped this would provide an optimum time to observe larvae migrating through ocular tissues and vessels as well as to evaluate the tissue response to the infection. Most of the clinical observations were supported by histopathologic findings. The only eye in which larvae were demonstrated pathologically was in the right eye of M-1 where at least two were seen in the lens. No reaction was seen around the larvae in the lens in the right eye of M-1, which had received intravitreal larvae in the left eye 32 days earlier, and little reaction was seen elsewhere in this eye except for retinal hemorrhages.

The number and severity of retinal hemorrhages in the left eye of M-1 (intravitreal) and M-5 (oral) which had been previously infected and in the left eye of M-7 which had no prior infection seemed about equal in intensity and became most marked around five to seven days after intravitreal infection of all three eyes and were decreased by two weeks following infection in M-7. This suggests that prior sensitization by ocular or systemic infection does not significantly alter the clinical picture but that the responses may be related to the size of the infecting dose and migration pattern of the larvae. The pathologic changes, however, were more severe and diffuse in the two eyes which had been previously infected suggesting an increased capability of the tissues in these eyes to respond to a subsequent infection.[84] In contrast to this is the rather benign clinical course and almost total lack of significant pathologic changes except for scattered retinal hemorrhages and a cataract (probably traumatic) in the right eye of M-1 which received an intravitreal injection on only one occasion. However, none of the eyes, regardless of the route of infection, demonstrated an abnormal serologic response in the vitreous or aqueous as evaluated by the ELISA test.

These findings do not indicate how larvae penetrate into the eye or the mechanism of their distribution in the globe. At least one report has suggested that larvae entering through the choroidal circulation produce granulomas in the posterior pole and those entering via the retinal vessels produce peripheral uveitis.[14] It has been stated that larvae will migrate until they reach a vessel with a diameter too small to permit passage and at

that point either die or bore through the vessel wall, and that this may partially explain their ocular distribution since entrapment within a vessel would be a key occurrence to trigger larval penetration into the eye.[39,41-43] It is not known how widely or for what length of time larvae migrate after leaving the intravascular compartment and whether or not they may later reenter a vessel and leave the eye.[83]

The apparent preference for the region of the ciliary body is not understood. It is possible that if the vitreous injections of larvae had been closer to the disc lesions in and around the ciliary body might not have developed. A possible relationship to the excellent blood supply and size of vessels in this area may exist as well as undetermined trophic factors. Ashton[13] commented that larvae are more likely to be swept to the retinal periphery rather than deviated into branch arterioles and this might account for the frequency of peripheral lesions. Another possibility might be the movement of larvae to an area with minimal exposure to light. This is indirectly suggested by the apparent stimulating, or possibly irritating, effect of light on larvae which causes a marked increase in their activity when exposed to a bright light source. This was frequently observed during the course of this experiment as well as in one clinical case seen by the author.

The reaction of avascular tissue to larvae appears to be mild as suggested by the three case reports of larvae located in the cornea, in which some scar tissue formation and a "little nummular keratitis" was noted.[25,28] In the one case of a larva in the lens,[66] a posterior subcapsular cataract was noted but these changes were observed 16 months before the larva was seen and, in addition, the patient had inflammation of the vitreous and received diathermy treatment to the peripheral retina, both of which may have contributed to the cataract. In addition, the larva migrated under both the anterior and posterior capsule of the lens but there were apparently no anterior subcapsular cataract changes.

In the present study numerous (as many as several dozen) motile larvae were observed in the lens, especially under the anterior and posterior capsules, for a prolonged period of time lasting up to at least 38 days and minimal or no reaction occurred around the majority of them. It is realized that inadvertent rupture of the lens capsule may have given the larvae access to the lens and it is important to note the lack of any significant reaction. Most of the cataractous changes seen in the three monkeys with intralenticular larvae were felt to be secondary to the initial trauma from the injection needle. The minimal clinical reaction in the lens is supported by the lack of inflammatory reaction found upon pathologic study of the lens from the right eye of M-1 in which larvae were demonstrated. In addition, only a mild clinical reaction was noted to as many as one to two dozen larvae

which were observed in various areas of the vitreous for as long as 21 days. On the other hand, the more severe tissue reactions appear to be located near vascular tissue especially that physical trauma from the migrating larvae or irritation from excretory products might not play a major role in the observed pathologic changes but other factors such as immunological mechanisms may be most important.

This study has not clarified the relationship between the size of the infecting dose and the severity of the systemic disease or incidence and severity of the ocular infection. Some experimental evidence points to such a relationship[83] but clinical observations of eye disease many years after VLM in patients with few signs or symptoms indicates this may not be entirely correct.[37,38,43] Other experimental work, in addition to observations in this study, suggest that larvae may reach the eye rapidly after infection,[83,84] and larval migration in man probably lasts only a few weeks with minimal migration once the larvae have become enmeshed in tissues throughout the body. However, clinical observations in a few cases have demonstrated that larvae, at least in the eye, can occasionally migrate for prolonged periods of time.[25,29] The stimuli for continued migration or reactivation after a period of quiescence in dogs are not known but may be hormonal or immunologic as exemplified in the bitch during pregnancy.[42,47] It has been well demonstrated that larvae can remain viable and infective in various animal tissues for prolonged periods of time[40] and this may also occur in man but has not been demonstrated. It would be interesting to perform follow-up studies on patients with known or suspected VLM to see if there is reactivation of their clinical disease or changes in laboratory studies during pregnancy or other diseases states and whether the children of any previously infected mothers show clinical or laboratory evidence of the disease.

Another interesting aspect of the problem is the observation that patients with VLM seldom get ocular infections, and conversely patients with eye manifestations seldom have signs or symptoms of systemic disease.[43,44] It has been stated that the incidence of ocular involvement or predilection for the eye may be more apparent than real. It takes only one larva to produce significant ocular signs and symptoms whereas many larvae are needed in other organs to initiate and cause clinical disease.[41,44] Another perplexing problem is why so few ocular lesions of the type seen with *T canis* are observed in the natural host, which is the dog.

The eosinophilic granulomas in the ciliary body region of the left eyes of M-1 and M-5, which received intravitreal injections, are striking in their similarity of appearance and location. Many lesions of this type were seen in the ciliary body and beneath the adjacent retinal pigment epithelium

and these may be the counterpart of the clinical lesions seen in man in this location. Numerous foci of eosinophils, some possibly representing "abscesses," are also present and are probably due to migration of larvae through the involved areas. The cells, mainly eosinophils, in the vitreous overlying the areas of inflammation in the ciliary body may be due to artifact from tissue sectioning, although they are not noted in other areas of the same eye except where associated with underlying inflammation. It is certainly possible that these represent a true vitreous reaction and may correspond to the vitreous cells seen in some human cases as well as certain monkeys in this study. Contrasted to this are the lack of abnormalities in the right eye of M-5 which received a systemic infection by nasogastric tube but no intraocular injection.

As previously commented, the stimulus for larval migration is not known. Apparent migration out of the eye has been demonstrated in two experimental studies and was histologically proven in one. The late elevation of eosinophils in M-1 and M-2 of the present study is suggestive that extraocular larval migration may have occurred but this finding could have been related to sensitization from the repeat larval injections.

There are relatively few histologically proven cases of ocular infection with *T canis* where the larvae have actually been identified.[13-15] In most instances a diagnosis is made on presumptive pathologic evidence, because of difficulty in finding the worm, or on the basis of the clinical history and examination and associated laboratory findings. Most eyes with a presumed diagnosis of *T canis* infection are not being enucleated which makes specific diagnosis and clinicopathologic correlation more difficult. It is therefore apparent that better information about the clinical course of the disease, especially the early stages about which little is known, is needed to better correlate with the laboratory studies thereby permitting earlier specific diagnosis and treatment.

Non-specific laboratory tests which have frequently been used in the past can be confusing. In this study they were not particularly helpful and there was difficulty in interpretation of the eosinophil counts because of technical problems. Further confirmation of the specificity and sensitivity of newer serologic tests such as the ELISA, using larval and secretory antigen, is needed as the few reports of results from these tests have been in cases with solely a presumptive diagnosis. In this study, none of the cases with only intraocular infection demonstrated a positive response to the ELISA, IHA or BFT tests in the serum. In the two monkeys systemically infected by nasograstric tube there was a positive response to the ELISA test and BFT using Toxocara antigen and to Toxocara and Ascaris antigen with the IHA test all done using serum. M-6 which received an intracarotid

injection was negative to ELISA testing of the serum but had a positive response to the serum IHA test with Toxocara and Ascaris antigen.

ELISA testing of vitreous and/or aqueous specimens from M-1, M-2, M-5, and M-7, which includes animals infected systemically and intraocularly, was negative in all samples. It is possible that the low titers or lack of response in the present study may be related to difficulties with specificity of the test in the Owl monkey as well as the duration and severity of the disease. The findings in this study help to support the value of serum evaluation with the ELISA, IHA and BFT tests in the early stages of the disease but do not support the value of testing intraocular fluids to diagnose ocular infection with *T canis*.

There is no known effective treatment for tissue stages of the disease and it is not known whether or not currently available drugs, such as thiabendazole, penetrate the intraocular structures.[20] Further study of current as well as new drugs is needed to find effective agents. This, together with methods for more specific and earlier diagnosis of *T canis* infection in man, will permit earlier treatment before serious damage has occurred, especially to intraocular structures.

It is apparent that much remains to be learned about all aspects of human infection with *T canis*. Further experimental studies are needed to help understand the clinical course, variability in location and type of tissue response, the manner in which the organisms get into and possibly out of the eye, and types of immune reaction which affect the response to serological and other testing and the effect of drugs on various stages of the organism and in different tissues. Once these factors are known, earlier diagnosis and more effective treatment can be made available for this disease which is a significant cause of ocular damage with resultant loss of vision in many cases.

The author recognizes there are pitfalls in this study but an attempt has been made to present an experimental approach to ocular infection with *T canis*. It is realized that many additional areas need to be studied and future experiments are planned to evaluate different aspects of the disease.

SUMMARY

An experimental study of nematode endophthalmitis due to *T canis* and review of the literature has been presented. Six owl monkeys were infected either by nasogastric tube using embryonated *T canis* eggs or by carotid or intravitreal injection of second stage larvae. The clinical manifestations, especially ocular, were observed and various diagnostic tests performed. Only minimal or no intraocular changes were seen after systemic infection

but significant abnormalities such as retinal hemorrhages and venous dilation were noted after intravitreal infection. Motile larvae were observed in the lenses of three eyes and in the vitreous of five eyes and, probably a sixth, after intravitreal injection. The intensity and timing of the intraocular reaction seemed to correlate with the infecting dose and apparent disappearance of larvae from the eye. Pathologic confirmation of larvae in the lens was obtained in one eye. A marked inflammatory reaction occurred in eyes receiving intraocular infection but none was seen in eyes with only systemic infection. Various laboratory and serologic studies were performed, including the ELISA test, which were used to evaluate systemic as well as intraocular responses to infection with *T canis*. The two monkeys infected by nasogastric tube gave a positive ELISA response in the serum but intraocular fluids gave a negative response in all monkeys including those infected systemically and/or intraocularly. Problems in the understanding of clinical aspects of the disease, laboratory diagnosis and treatment are discussed. The need for future experimental studies is emphasized.

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