Enzootic Toxoplasmosis in Caged Squirrel Monkeys

Saimiri sciureus

G.E. McKissick, H.L. Ratcliffe and A. Koestner

Spontaneous and experimental toxoplasmosis have been reported sporadically as the cause of death in nonhuman primates. These reports indicate that New World monkeys and marmosets (cebidae and hapalidae), are more susceptible to infection by Toxoplasma gondii, or more often develop disease in response to this infection than monkeys and baboons of the Old World (cercopithecidae). This difference is not readily explained, but it parallels a difference in a capacity to adapt to confinement. An enzootic of fatal toxoplasmosis in a laboratory group of squirrel monkeys (Saimiri sciureus, a New World primate) is described.

Review of Literature

Cowen and Wolf (1945) initially reviewed the literature in their report of the first proven case of fatal toxoplasmosis in an immature rhesus monkey, Macaca mulatta. The review included 3 reports of natural toxoplasmosis in 3 simian genera and 6 reports of attempts to experimentally infect 4 simian genera. All of the reports involved Old World primates. Their literature review and attempts to experimentally infect 11 animals representing 3 genera of Old World primates led them to conclude that, "In general, monkeys would appear to be relatively resistant to this infection". Other reports involving Old World primates lend credence to this conclusion. Moreover, a serological survey of 164 Old World monkeys representing 3 genera revealed low antibody titers (≤1:8) of questionable significance in 52 of the animals. These results suggested that exposure in their native habitat to toxoplasma is not common. Explanation of the low incidence of fatal toxoplasmosis in Old World primates on the basis of naturally acquired circulating antibodies, therefore, is tenuous.

In contrast, Ratcliffe and Worth (1951) described the lesions of fatal toxoplasmosis in 2 squirrel monkeys, S. sciureus, and a spider monkey, Ateles...
geoffroyi, both of which are New World primates. In 1954 Rodaniche\textsuperscript{56} published evidence of marked susceptibility of 2 more New World primates including a marmoset, Marikina geoffroyi, and a night monkey, Aotus zonalis. At the same time he\textsuperscript{56} also reported a natural infection of toxoplasmosis in an infant whiteface monkey, Cebus capucinus, native to Panama.

Ruch (1959)\textsuperscript{57} first noted that most spontaneous toxoplasma infections in nonhuman primates had occurred in New World species. On the basis of this observation he suggested a difference between Old World and New World monkeys in regard to degree of susceptibility to \textit{T. gondii}. Annual reports of the causes of mammalian deaths in the collection of the Philadelphia Zoological Society support this suggestion. For example, during the past 16 years spontaneous fatal toxoplasmosis was observed in 9 primates representing 4 New World genera at the Philadelphia Zoo\textsuperscript{58} (1954, 1961, 1962, 1966). No similar death was recorded in Old World primates during the same interval\textsuperscript{58} (1951–1967). Benirschke and Richart\textsuperscript{59} confirmed another fatality of acute spontaneous toxoplasmosis in an immature female marmoset, \textit{Oedipomidas oedipus} from Colombia, South America. The enzootic to be described herein was comprised of 9 fulminating infections which occurred in a laboratory group of 17 squirrel monkeys during 22 months of confinement. \textit{S. sciureus} is native to Central and South America.

Materials and Methods

Three separate shipments of \textit{S. sciureus} totaling 40 monkeys were received in Philadelphia from an animal dealer over an interval of 16 days. Upon arrival they were immediately confined to 3 group-cages and were never in contact with animals in other buildings in which toxoplasmosis had occurred\textsuperscript{58, 88, 87}.

Seventeen of the 40 monkeys remained alive in apparent good health 6 months after arrival and were considered to be acclimated to the laboratory environment. None of 23 deaths which occurred during the 6 months of acclimation could be attributed to toxoplasmosis. Stresses of shipment were considered to have contributed to the demise of 19 animals which died within 3 weeks after arrival. The 17 survivors included 10 males and 7 females of varying degrees of physical and sexual maturity. They were randomly divided into 4 groups, 3 of which contained both sexes, and placed on experiment 7 months after arrival. A total of 9 of the 17 monkeys succumbed to toxoplasmosis while 6 died of other causes over an interval of 28 months.

The experiment was a pilot study designed to evaluate the effects of grouping on cardiovascular disease. Each group of monkeys was placed in a battery of 3 interconnecting cages. Each cage measured 45 cms in height and contained an L-shaped platform located halfway between the top and floor of the unit at the site of the passageway between the cages. The total floor space of the battery was 0.63 m\textsuperscript{2}. Diet A of the Philadelphia Zoological Garden\textsuperscript{51} was supplemented with raw ground horsemeat and fed once daily. The quantity fed was in excess of that consumed within 1 hour. Diet A contains approximately 25\% protein and 5\% fat which is chiefly vegetable in origin. Fresh tap water was supplied \textit{ad libitum} daily from inverted graduated cylinders attached to the exterior of the cages.
Cage floors and platforms were scraped clean daily and a mixture of fresh sawdust and fine wood shavings was scattered on the floors as an absorbent. All cages were cleaned with hot water under hose pressure and scrubbed at least once every 2 weeks.

Deaths of the monkeys were spontaneous with the exception of 1 animal which was killed in a moribund state. Necropsies were performed on each animal as soon as possible following death. Tissue specimens of the heart, kidney, adrenal, liver, and spleen were routinely collected and fixed for histopathologic examination in accordance with the original objective of the experiment. Additional tissues were collected if they appeared abnormal upon gross examination at necropsy or when they were suspected of being functionally abnormal as indicated by ante-mortem signs.

The tissue-fixative was buffered formalin. The fixed tissues were embedded in paraffin and 6μ sections were cut and stained with hematoxylin and eosin. LILLIE'S allochrome stain was used on selected sections. Giemsa stain was applied to fresh impression smears of the lung and spleen.

The diagnosis of toxoplasmosis was based on the following: (1) microscopic morphology of the organism in H & E-stained tissue sections and Giemsa-stained impression smears of lung and spleen, (2) the characteristics and distribution of lesions observed in association with the organism, (3) the laboratory history of the monkeys.

Results

Incidence

During an interval of 22 months, 9 of 17 squirrel monkeys developed and died with fulminant toxoplasmosis (Table I). The largest male of the colony died from the disease 6 weeks after the animals were grouped and 8 months after they arrived at the laboratory. The last death from toxoplasmosis occurred 23 months after the animals were grouped and 5 months before the experiment was terminated.

Within 5 months after the monkeys were grouped, deaths from toxoplasmosis had occurred in 3 of the 4 cage-batteries (Table I). The 2 unexposed males from cage-battery 4 (no toxoplasmosis) were re-grouped at that time into cage-battery 1 (+toxoplasmosis) with the 4 exposed occupants. Six was the maximum number of animals confined to any battery and 4 of the 9 deaths from toxoplasmosis subsequently occurred in cage-battery 1. Intervals between deaths within the same cage-battery ranged from 3 days in battery 1 to more than 1 year in battery 3 (Table I).

In contrast, only 1 diagnosis of fatal toxoplasmosis could be made in battery 2. It was the second death recorded for the entire experi-
Table I. Lesions of toxoplasmosis in 9 caged squirrel monkeys (Saimiri sciureus)

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Cage</th>
<th>Mos. on Exp.</th>
<th>Mos. in Lab.</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lymph Node</th>
<th>Heart</th>
<th>Lung</th>
<th>Adrenal</th>
<th>Brain</th>
<th>Kidney</th>
<th>Gut</th>
<th>Pancreas</th>
<th>Ovary</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>L</td>
<td>*</td>
<td>*</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N</td>
<td>L</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>4</td>
<td>11</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>10</td>
<td>17</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>1</td>
<td>10</td>
<td>17</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>17</td>
<td>24</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>L</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>22</td>
<td>29</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>22</td>
<td>29</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>3</td>
<td>22</td>
<td>29</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Organisms and lesions
+ = Organisms
L = Lesions
N = No visible organism or lesion
mental group and it occurred 46 days after the experiment was initiated. Cagemates of the victim included 2 males and 2 females. Two of the 4 cagemates died later from other causes and 2, 1 of each sex, finally represented the only survivors of the 17 monkeys when the experiment was terminated 28 months after initiation.

Clinical Signs

Clinical signs were variable and nonspecific. In 2 instances the monkeys appeared to be in good health the morning they died. The most common indication of illness was a protracted diarrhea which was first recorded in 1 monkey 43 days prior to death. Other gastrointestinal signs which were occasionally observed included emesis, polydipsia, and anorexia or voracity. A weight loss of 363 g which represented 41% of the total body weight 85 days prior to death occurred in 1 animal. Weight losses in excess of 230 g were recorded for 3 other monkeys.

The usual duration of signs of general illness varied from 1 to 5 days. The sick individual initially isolated itself by huddling in a cage corner. Lethargy was commonly observed as indicated by an indifference to handling. The eyelids of lethargic animals constantly drooped which imparted an appearance of continual drowsiness. The eyes developed a bright glassy appearance and in 1 instance a clear conjunctival discharge was noted. The individuals whose initial signs were characterized by lethargy developed a gradual weakness which progressed to prostration. Dyspnea was noted in 6 of the monkeys and epistaxis was observed in 1 instance.

Two of the monkeys developed dramatic neurological signs. One in particular exhibited circling and grasping of the scalp. It was observed to repeatedly bang its head against the cage wall and then hold its head in its forepaws. The other animal exhibited incoordination as manifested by weaving and staggering. Terminally it moved by dragging itself and it died in a tonic convulsion.

Gross Lesions

Gross lesions, like clinical signs, were variable and nonspecific. For example, the pelage in 2 monkeys was actually luxuriant in con-
Enzootic Toxoplasmosis in Caged Squirrel Monkeys...

trast to various degrees of sparsity in others. Signs of emaciation such as depletion of fat depots were likewise inconstant.

The most common gross lesions were in the lung. They were all related to vascular injury and consisted of diffuse congestion and edema (4 animals) and ecchymoses (3 animals). Hydrothorax was observed in 2 monkeys and a pink foamy exudate was noted in the bronchi, trachea, and over the external nares of others.

Globular cardiac dilatation and hepatic congestion were apparent in 5. Other hepatic lesions included fatty infiltration (5 animals), sparse subcapsular petechiae (1 animal), and profusely scattered gray-white foci, 2 to 3 mm in diameter (1 animal).

Other gross lesions included: lymphadenopathy, especially of the mesenteric nodes, splenomegaly, intestinal ulceration, hemorrhage of intestinal lymphoid tissue, and generalized pallor. Lesions which were attributed to causes other than toxoplasmosis included subcutaneous hematomas of the head due to trauma, renal cicatrices, and excessive accumulations of dental tartar.

Histopathology

The histological distribution of toxoplasma and lesions are tabulated (Table I). Necrosis with and without inflammatory cellular response was the characteristic lesion. Free and cyst forms of toxoplasma were observed in association with the necrotizing process. Both forms of the organism, in addition to intracytoplasmic colonies, were also observed in tissues that were otherwise histologically normal. In the following histopathologic descriptions, an aggregate of organisms with no discernible morphologic characteristic of a parasitized cell including the cellular membrane is referred to as a cyst. Colonies of organisms within the cytoplasm of a cell with an intact nucleus and plasma membrane are referred to as intracytoplasmic forms. Free forms are individual organisms which were not found within a cell.

Lesions and organisms were demonstrated in the livers of all 9 monkeys. Foci of necrosis were randomly distributed and measured up to 1000 μ in longest dimension. Organisms (free forms or cysts) were found most often in the periphery of the lesions (Fig. 1). Lymphocytes, neutrophils, and macrophages occurred in fewer numbers than might be expected from the severity of the necrotizing process. Intracytoplasmic organisms were also demonstrated at sites distant to
necrotic hepatic lesions within Kuppfer cells and parenchymal cells (Fig. 2). Lesions which may have been indirectly attributable to toxoplasmosis included albuminous degeneration and fatty infiltration. Hepatic lesions not attributed to toxoplasmosis included periportal fibrosis, biliary hyperplasia, bile retention, and intraductal flukes.

Organisms and lesions were found in the spleens of all 9 monkeys. Necrosis within splenic follicles (Fig. 3) and red pulp was the most common lesion. Necrotic lesions interrupting the continuity of the splenic trabeculae were numerous. In contrast to other organs, a neutrophilic response was commonly associated with the necrotic lesions, especially in the red pulp. Intracytoplasmic organisms occurred in macrophages, reticuloendothelial cells, and in endothelial cells in which they appeared as elongated aggregates. Cysts were most common within the red pulp where, often, they were not associated with necrosis. Cysts were also found within follicles (Fig. 3) and occasionally in trabeculae. Rupture of cysts appeared to be responsible for extracellular forms appearing singly within and adjacent to necrotic lesions. Lymphoid depletion, reticuloendothelial hyperplasia, thrombosis, congestion, and hemorrhage comprised splenic lesions which were recognized as sequellae to toxoplasmosis.

A severe necrotizing lymphadenitis of the mesenteric and/or thoracic nodes occurred in 6 of the 9 monkeys. Many nodes were virtually destroyed (Fig. 4). Organisms were sparse and were found as cysts after considerable searching through the severely affected tissue. They were more numerous in less severely affected lymph nodes where they were observed in subcapsular sinuses, follicles, medullary tissue, and trabeculae. The organisms were found in normal-appearing tissue as well as necrotic tissue. Intracytoplasmic forms occurred within macrophages and in endothelial cells as elongated aggregates. Neutrophilic response to the organisms and the necrotic process was minimal.

Myocardial lesions occurred in 8 monkeys. The lesions were randomly distributed and consisted of coagulative necrosis, sarcolem-
mal proliferation, and a minimal inflammatory cellular infiltrate. Toxoplasma were demonstrable as cysts or individuals in direct association with the focal necrotic lesions in 7 animals (Fig. 5). Intrasarcoplasmic colonies up to 90 μ were also encountered which did not incite a morphologic reaction (Fig. 6). As in other tissues, organisms were not demonstrable in the plane of section of many lesions and individual toxoplasma were especially difficult to identify in advanced necrotic lesions. Cysts were the most common form of the organism.

Sporadic lesions of the heart which were not attributed to toxoplasmosis included arteriosclerosis in 2 monkeys, lymphocytic perivascularitis in 1, and a focus of lymphocytic epicarditis in another.

The most extensive lesions induced by toxoplasma were those found in the lungs. Generalized edema and fibrinous pneumonia occurred in 8 monkeys. Vascular congestion thickened the alveolar septa and the alveolar spaces were filled with a serofibrinous exudate containing erythrocytes, desquamated alveolar lining cells, hemosiderin-laden macrophages, toxoplasma, and cellular debris. The walls of distended lymphatics within interlobular septa were necrotic (Fig. 7). Foci of necrosis, up to 200 μ in longest dimension and characterized by neutrophilic infiltration, were sparsely scattered throughout the lung. Venous thrombosis was frequently evident. Areas of collateral emphysema often surrounded consolidated portions of the lungs.

Toxoplasma were difficult to demonstrate in histological sections of pulmonary tissue. Cysts were most commonly seen and they were found in alveolar septa (Fig. 7). Intracytoplasmic forms were observed in cells within alveolar spaces and bronchiolar lumens. Organisms were not found in normal-appearing pulmonary tissue. Lillie’s allochrome stain facilitated demonstration of the toxoplasma which appeared red in tissue sections. Giemsa-stained impression smears of the lung revealed individual organisms (Fig. 8).

The most common incidental lesion in the lungs of 7 monkeys was the presence of a large filarial parasite which was tentatively identified as *Filaroides gordius*, the common lungworm of the squirrel monkey.

The adrenals were involved in 8 monkeys. The zona fasciculata was the site of predilection, but toxoplasma cysts were also demonstrated in the glomerulosa and medulla. The lesions were similar to those in the liver, consisting of foci of necrosis which varied from coagulative to caseous in character with little or no inflammatory cellular reponse.
Enzootic Toxoplasmosis in Caged Squirrel Monkeys... 547

Fig. 5. Focus of necrotic myocarditis (interventricular septum) containing toxoplasma aggregates (arrow). Monkey No. 30. H & E, ×290.

Fig. 6. Intracytoplasmic colony of toxoplasma within sarcoplasm of right ventricular myocardial fiber. Monkey No. 32. H & E, ×640.

Fig. 7. Toxoplasma cyst (arrow) in area of fibrinous (F) pneumonia. Necrotic lymphatic wall (L) runs perpendicular length of photomicrograph. Monkey No. 31, H & E, ×500.

Fig. 8. A pair of toxoplasma in an impression smear of lung. No kinetoplast is discernible as compared to Figs. 17 and 18. Monkey No. 24. Giemsa, ×2675.
All forms of the organism were found in direct association with lesions as well as in normal-appearing tissue.

The brains of 3 monkeys (2 of which exhibited neurological signs of disease) contained lesions characterized by microscopic hemorrhages, focal gliosis, and siparse microscopic infarcts (200 μ) in a random distribution (Fig. 10). Hemorrhages were the most common lesions and their greatest measured dimensions (250 μ) followed the plane of the vessels from which they occurred. Intracytoplasmic forms of toxoplasma were observed in endothelial cells of the vessels (Fig. 11) and may have been a predisposing factor to infarction. Other forms of the organism were readily found in affected brains including paired and tetrad clusters (Fig. 12). As in other organs the different forms of toxoplasma were found in tissue which appeared normal as well as abnormal.

Renal lesions could not be unequivocally related to toxoplasmosis. Glomerulosclerosis was most common and acute focal nephritis was observed in 2 monkeys. Other incidental lesions which were seen in individual kidneys included cicatization and microcalculi. Toxoplasma cysts were found within renal cortices in 4 of the 9 animals. The organisms were sparsely located in association with vessels and glomeruli (Fig. 13) and distant to the focal renal lesions.

Lesions and organisms were only occasionally demonstrated in other organs. An ulcerative necrotizing enteritis involved all layers of approximately one-half of the circumference of the jejunum of 1 monkey (Fig. 14). Cysts and intracytoplasmic forms of the organism were found in association with vessels of the serosa, submucosa, and muscularis (Fig. 15). No pancreatic lesion was found in 6 monkeys, although a 20 μ toxoplasma cyst was found adjacent to a capillary in the interacinar connective tissue of 1 individual. Examination of the gonads revealed 1 cyst in the theca externa of 1 ovary where it incited no apparent reaction (Fig. 16). Neither organism nor lesion which

---

**Fig. 9.** Focus of coagulative necrosis within adrenal zona fasciculata. No inflammatory response apparent. Monkey No. 25. H & E, ×296.

**Fig. 10.** Toxoplasma cysts (arrows) within focus of sparse hemorrhage (E), astrogliosis, and malacia (M) in cerebellar white matter. Monkey No. 35. H & E, ×346.

**Fig. 11.** Obstructive intracapillary aggregate of toxoplasma within cerebral white matter near hippocampus. Monkey No. 25. H & E, ×340.

**Fig. 12.** Cysts (C), pair (P), and tetrad (T) of toxoplasma within pons ventral to facial nerve. Monkey No. 25. H & E, ×1375.
could be identified as those of toxoplasmosis was found in sections of salivary glands and thyroids of the squirrel monkeys.

Discussion

A differential diagnosis of trypanosomiasis was considered for the enzootic. The original native habitat of the squirrel monkeys is endemic for *Trypanosoma cruzi* which causes Chagas' disease. Descriptions of this disease in mammals, including man, emphasize similarities of clinical signs and distribution of organisms and lesions induced by the leishmanial forms of *T. cruzi* and those induced by *Toxoplasma gondii*.

Chagas himself reported the first natural infection of the squirrel monkey by *T. cruzi*. Ruch reviewed reports of natural and experimental simian infections, particularly of Old World primates. Actual deaths from *T. cruzi* infections were exceptional. More recent *T. cruzi* infections of New World monkeys including *S. sciureus* have been reported. The significance of these infections in terms of lesion is not well documented particularly for *S. sciureus*. Moreover, Watkins has demonstrated that the distribution of *T. cruzi* and its lesions, at least in mice, is variable, depending upon the strain of the organism. Thus the possibility of latent *T. cruzi* infections existed in the squirrel monkeys described herein.

Identifying characteristics of toxoplasmosis existed in this enzootic. The pulmonary lesions and severe necrotizing effect noted in many of the tissues, particularly of the lymph nodes and gut, are lesions described for toxoplasmosis and not for trypanosomiasis. The basic differential feature was the consistent absence of a kinetoplast within the individual toxoplasma regardless of location (Figs. 1, 2, 3, 4).

---

*Fig. 13.* Aggregate of toxoplasma within renal glomerulus. Proteinaceous transudate (P) within Bowman's space. Monkey No. 30. H & E, x 595.

*Fig. 14.* Ulcerative necrotic enteritis of jejunum. Entire thickness of wall is involved in necrotic process.Outlined area magnified in Fig. 15. Monkey No. 35. H & E, x 29.

*Fig. 15.* Six perivascular aggregates of toxoplasma within necrotic muscularis of jejunum as depicted in Fig. 14. Cyst (C), intracytoplasmic (I), and intraluminal forms (L) are present. Monkey No. 35. H & E, x 550.

*Fig. 16.* Aggregate of toxoplasma within theca externa of ovary. Note interruption of enclosing wall (arrow). Monkey No. 25. H & E, x 1175.
Enzootic Toxoplasmosis in Caged Squirrel Monkeys...
Fig. 17. Intracytoplasmic aggregates of *Trypanosoma cruzi* within myocardial fibers of a rat (experimental infection). Kinetoplasts (K) are distinguishable from nuclei (N) of organisms. H & E, ×1775.

Fig. 18. *Leishmania donovani* within macrophage. Impression smear of human spleen. Kinetoplasts (K) are distinguishable from nuclei (N) of organisms. Giemsa, ×1475.

6, 8, 12, 16). The chromatin kinetoplast is visible histologically both within sections and in smears containing leishmanial forms of trypanosomes (Figs. 17 and 18 which represent experimental and natural trypanosomal infections respectively). The diagnosis of toxoplasmosis in this study, therefore, could be based on morphology of the organism as well as the character and distribution of the lesions.

The pathogenesis of necrosis associated with toxoplasmosis is not completely understood. The induction of the necrotizing effect has been variously attributed to: the status of immunity in the host particularly hypersensitivity\textsuperscript{15, 17, 20}, toxins, including those associated with globulins of exudate produced by toxoplasma\textsuperscript{20, 47}, and the strain of the infecting organism and its rate of intracellular reproduction \textsuperscript{15, 20, 29}. The hormonal status of the individual animal in regard to
adrenal corticosteroids has also been related to the production of lesions of toxoplasmosis.\textsuperscript{16, 17, 21}

None of these factors completely explains the necrotizing effect. For example, Figs. 1 and 2 of the liver of 1 monkey reveal the organism in 3 phases of pathogenesis. Fig. 2 depicts both intracellular multiplication and initiation of cyst formation within intact hepatic parenchyma as compared to the necrotizing process induced in Fig. 1. Liberation of a mass of organisms within the tissue appears to be exerting the necrotizing effect in the latter. Thus it becomes difficult to explain pathogenesis solely on the basis of production of toxin or the immune status of the individual. Certainly the general lack of inflammatory cellular response, as typified in Fig. 9 which depicts focal necrosis of the adrenal cortex, would indicate a lack of cellular antibody which might be associated with lymphocytes and plasma cells.

The organisms which caused this enzootic would most likely represent a single strain based on the relative isolation of these monkeys from the remainder of the zoological collection. Figs. 10, 14, and 15 illustrate lesions from tissues of the same monkey. The organisms appear to be capable of inducing only minimal hemorrhage and gliosis in the cerebellum. Yet in the same animal their presence was directly associated with a severe necrotizing ulcerative enteritis. The lesions produced thus appear to reflect a specific interaction of the tissue infected and the strain of the infecting organism.

Dominance and subordination in regard to behavioral interactions of competition amongst groups of high population density have been proposed as factors influencing hormonal status of individuals comprising the group.\textsuperscript{6, 7, 11, 38 (1961, 1964), 41} The hormonal status is mediated through the pituitary-adrenal axis and is reflected in the dominant animals' general ability to survive, including resistance to infectious diseases. The dominant individual amongst the groups of squirrel monkeys as observed in this particular laboratory situation was the largest male. Yet the largest male of all the monkeys and the smallest male in group 3 were the first and third individuals respectively to succumb to toxoplasmosis. No pattern of deaths was ascertainable in regard to behavioral relationships, physical size, or sex. Since assay for adrenal corticosteroids was not performed one can make no conclusions in relating the hormonal status of the various monkeys to susceptibility to toxoplasmosis.

Incidence and lesions in this enzootic suggest 5 factors to be considered in the pathogenesis of toxoplasmosis in squirrel monkeys. They
are: (1) lack of protective immune response, (2) local concentration of toxin and/or catabolites of reproduction of the organism, (3) individual tissue susceptibility to the organism, (4) capillary thrombosis, and (5) ability of the individual to adapt to its environment.

Fourteen of the 17 monkeys were in close contact with at least 1 fatal case of toxoplasmosis within their respective groups within 4 months after the experiment was initiated. The small spatial allotment per group permits assumption of considerable contact exposure. One would expect an immune response to develop thereafter. Certainly the 22-month duration of the enzootic would suffice for development of immunity. Yet intervals between death from toxoplasmosis within the same groups ranged from 3 days to more than 1 year (Table I). This fact and the general lack of inflammatory response to the lesions and organisms do not support an immune response of a protective or hypersensitive nature.

The question of source and actual mechanism of transmission of toxoplasma in this enzootic can only be answered by circumstantial evidence as it has in others. The monkeys were confined within a controlled-access laboratory from which the public and personnel, other than that of the laboratory, were excluded. Furthermore, the monkeys which succumbed to toxoplasmosis were all confined to 1 room of the top floor of the laboratory to which only caretakers had access during the experiment. The first fatality occurred 8 months after arrival and 1 month after initiation of the experiment. Thus the possibility of infection prior to capture or during transit is possible but equivocal. The most likely sources of infection were caretakers and/or feed.

Cottontop Pinché marmosets (Oedipomidas oedipus), chickens, ducks, and a mouse colony which were used for experimental purposes within the same building remained unaffected throughout the enzootic. These creatures were cared for by the same attendants and often were confined within the same room as the monkeys. Nevertheless, they (including the toxoplasma-susceptible mice) experienced no enzootic of fatal infectious disease and neither microscopic lesion nor organism of toxoplasmosis was observed in several tissues which were examined. Thus, these animals and the attendants can only be considered as the source of the organism with some doubt.

The special diets for the entire zoological collection were prepared in and dispensed from a central kitchen. The raw ground horsemeat supplement which was fed to the monkeys could have harbored the
Enzootic Toxoplasmosis in Caged Squirrel Monkeys...

The organism. The horse, however, remains the exception in regard to infections with natural or experimental toxoplasmosis. There is no report of equine toxoplasmosis.

The possible mechanisms of natural transmission between monkeys of the same group are numerous as suggested by experimental evidence and distribution of the lesions. Monkeys specifically have been infected per os in addition to the restricted space allotment for each group predisposed the individuals to existing infections in spite of the daily cleaning precautions which were followed. Means of dissemination suggested by the lesions include aerosol, fecal, and urinary.

Congenital transmission is the single proven natural means of dissemination of toxoplasma. It did not occur at least during the course of this enzootic. However, the possibility for it is illustrated in Fig. 16 which depicts a cyst within the theca externa of an ovarian follicle. In spite of the incomplete appearance of the enclosing wall there is no discernible surrounding tissue reaction.

The concentration of a toxin and/or catabolites rather than their presence is proffered as the determining factor of the necrotizing effect on tissues. Minimal quantities of toxic materials as might be produced by small numbers of organisms could be dissipated by sufficiently rapid capillary and lymphatic drainage so as to be harmless locally. The same mechanism could be assumed for toxic materials which might leak slowly from newly forming cysts. In Fig. 3 an initial necrotic change appears to be occurring in those cells occupying the central area of the splenic follicle. One can assume that nutrients required for reproduction can enter newly forming cysts if toxic materials can escape. Depending upon the rate of reproduction such structures either rupture mechanically or form a comparatively impenetrable wall which provides protection for the organism as well as the host. Rapid proliferation of the organism leads to rupture of the containing wall. The rapid release of a high concentration of necrotizing toxins is implied in the focal necrotic reactions observed in association with the ruptured structures. Likewise the severe necrotizing reactions seen in the draining lymph nodes and in the lungs which receive the constituents of the general circulation in comparatively high concentrations at a rapid rate support the importance of the concentration of the necrotizing toxins in producing the lesions of toxoplasmosis.
Tissue susceptibility may be variable as suggested by lack of lesions in the kidneys, pancreas, and ovaries of squirrel monkeys in spite of the presence of toxoplasma (Figs. 13, 16). Specific renal lesions of toxoplasmosis have been reported in the dog. The monkey which did not react to the presence of organisms within the ovary, did suffer a severe necrotizing lymphadenitis (Fig. 4). Yet in the same animal, the organisms appear to be multiplying by longitudinal binary fission in extracellular sites within the pons with comparatively little reaction (Fig. 12). Contrast in tissue reactions of other individual animals was evident (Figs. 10, 14, 15). By comparison, no evidence of organisms or lesions was found in the thyroid, salivary glands, or testes of the squirrel monkeys. Thus a concept of specific tissue susceptibility does seem feasible.

The lesions in the brains of microscopic hemorrhages could result from capillary thrombosis as induced by intravascular aggregates of the organisms (Fig. 11). Cyst forms were commonly found in association with vascular channels in other tissues including the intestine in which 3 small aggregates also occurred within the cytoplasm of an endothelial cell (Fig. 15). Vascular lesions such as thrombosis and infarction are logical sequelae to such predispositions.

Finally a species and individual ability to adapt to environment does exist. In its native habitat S. sciureus is an intensely communal animal. In captivity they were the primates of choice for America’s first space-rocket flights. They are commonly sold as pets and are currently being used for neurophysiological and cardiovascular research as they were in this study. Thus individual squirrel monkeys are able to acclimate to captive environments. SANDERSON, however, expresses an opinion that only a few survive of the thousands exported annually from South America. Seventeen of an original total of 40 animals acclimated to a controlled laboratory environment in this study. Nineteen of 23 which were unable to adapt were dead in less than a month after arrival. More than 50% of the 17 which were considered acclimated 6 months after arrival, subsequently succumbed to toxoplasmosis. Four monkeys survived the endemic, 2 of which were finally placed on exhibition in the Small Mammal House at the Philadelphia Zoological Gardens 3 years after arrival. What actually constituted the ability of the 2 survivors to adapt and survive is unknown. ST. CLAIR et al. found that serum cholesterol nearly doubled in concentration for S. sciureus between time of capture and arrival at laboratory destination. In regard to the ability of the species to adapt, cap-
Enzootic Toxoplasmosis in Caged Squirrel Monkeys.

New World monkeys, including saimiri, have a propensity to develop coronary atherosclerosis in addition to their susceptibility to toxoplasmosis as compared to Old World monkeys. At the present time then, disease (infectious or noninfectious) continues to be the final parameter by which the ability to successfully adapt to environment can be measured for the individual or the species.

Summary

An enzootic of toxoplasmosis occurred in caged squirrel monkeys (Saimiri sciureus, a New World primate) which resulted in 9 deaths during an interval of 22 months. Diagnosis was based on morphology of the organism, character and distribution of the lesions, and laboratory history of the monkeys. The character of the lesions was essentially necrotic. The chronological incidence of the disease and distribution of lesions and organisms are tabulated. Trypanosoma cruzi which causes Chaga's disease is differentiated morphologically from toxoplasma. Incidence and lesions of the enzootic suggest 5 factors to be considered in the pathogenesis of toxoplasmosis in squirrel monkeys. They are: (1) lack of protective immunity, (2) local concentration of toxin and/or catabolites of reproduction of the organism, (3) individual tissue susceptibility to the organism, (4) capillary thrombosis, and (5) ability of the individual to adapt to its environment.

Zusammenfassung


Acknowledgments

The photomicrographs in this study reflect the efforts of Mr. Delbert Davis and his colleagues Messrs. Steven Gabriel and James Watkins. The authors are grateful for these efforts.
References


Author's address: Dr. G.R. McKiSSICK, Department of Veterinary Pathology, The Ohio State University, 1925 Coffey Road, Columbus, Ohio 43210 (U.S.A.)