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TOXOPLASMA GONDII, AN HISTORICAL ACCOUNT OF ITS CLASSIFICATION

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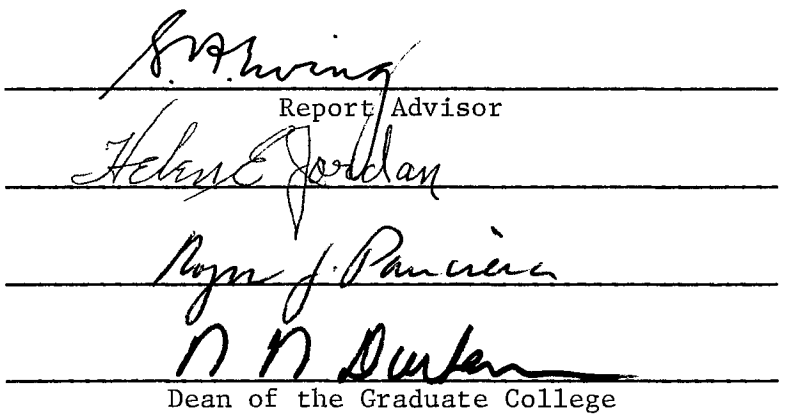

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PREFACE

Toxoplasma gondii, a parasite capable of inducing stillbirths, blindness, central nervous system disease as well as additional systemic manifestations in man and other homeotherms, has been an unclassified protozoon with only fragments of its biology elucidated. Since its recognition in 1908 no natural modes of transmission were known until the late 1930's when carnivorism and *in utero* transmission became recognized. However, carnivorism failed to explain its common occurrence in some populations of vegetarians and herbivores; arthropod vectors could not be incriminated. During the last eight years *Toxoplasma* has been identified as an intestinal coccidian of cats; with oocysts now known to be a stage in its development, contamination of herbage may explain the presence of *Toxoplasma* in animals subsisting on plants. The oocyst of *Toxoplasma* in the cat is identical to that of *Isospora bigemina*, small race, which has been recognized since 1923. In light of these more recent revelations it was felt that an historical accounting of the taxonomy of these two species which have identical oocysts would be most interesting.

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CHAPTER I

INTRODUCTION

Toxoplasma (taxon - bow, arc; plasma - form), a protozoan parasite of man and other warm blooded animals, was described in 1908 by Charles Nicolle and L. Manceaux (1909). First recognized as a lethal pathogen of the gondi, a North African rodent, by 1939 the organism was recognized as a cause of encephalomyelitis in human neonates (Wolf, et al., 1939). Since its discovery nearly 60 years ago the definitive taxonomic classification and complete life cycle have been in doubt. Assumed at first to be a protozoan farther removed from the trypanosomes than Leishmania (Nicolle and Manceaux, 1909), it has been classified subsequently by most authors as either a protozoan or an organism of uncertain classification (Wenyon, 1926b; Feldman, 1968). Electron microscopy revealed similarities to other Sporozoa, such as Plasmodium and Eimeria (Garnham, et al., 1962; Sheffield and Melton, 1968). However, fortuitous transmission studies utilizing the domestic cat revealed that Toxoplasma is a coccidian which undergoes schizogony and gametogeny in the small intestinal epithelium of that host, and undergoes endodyogeny in a wide variety of avian and mammalian hosts (Dubey, et al., 1970). The oocyst of the Toxoplasma strains examined thus far is produced only in the felidae; it is morphologically indistinguishable from the oocyst of Isospora bigemina, small race (Siim, et al., 1969; Frenkel, et al., 1970; Sheffield and Melton, 1970), a form recognized

since 1923 as occurring in the cat (Wenyon, 1923-24). This paper, therefore, will also include a taxonomic account of Isospora bigemina, small race.

The recognition of Toxoplasma as an intestinal coccidian of the cat is a milestone in understanding the biology of coccidia. Our knowledge of accepted host ranges of known intestinal coccidia infecting intermediate hosts in extraintestinal sites is now broadened (Frenkel and Dubey, 1972). In addition, we can speculate that other Sporozoa of uncertain classification such as Besnoitia, M-organism and Sarcocystis are likely to be coccidian life stages. Recently, isolated Sarcocystis cysts fed to cats have resulted in the formation of coccidian oocysts identical with those of Isospora bigemina, large race (Rommel, et al., 1972; Sloss, 1970).

CHAPTER II

HISTORICAL REVIEW

Toxoplasma gondii

From Animal Recovery, 1908, to Human Isolation, 1939

During 1908 Nicolle and Manceaux described a spontaneously occurring disease in three *Ctenodactylus gondi*, rodents from Matamata, South Tunisia of North Africa (Nicolle and Manceaux, 1909). Giemsa-stained splenic imprints revealed both clusters and individual organisms. As clusters they were present in mononuclear cells or as gangues resembling merozoital cysts. The free forms were typically crescent shaped with one extremity more tapered than the other; they measured 5u - 5.5u long by 3u-4u wide and had a 2u-3u diameter nucleus near the center of the organism. Large forms reached 7u by 5u. No flagella or centrosomes were present. Intracellular forms were generally grouped in pairs or series of pairs; hence, division was assumed to occur through bipartition. Dividing forms became rounded or oval with beginning segmentation of the nucleus. They assigned the name *Toxoplasma gondii* to this organism.

Nicolle and Manceaux considered *Toxoplasma* to be further removed from trypanosomes than *Leishmania*. Splendore, in 1908, had also described a similar organism from a rabbit, but considered it to be a

Leishmania (as cited by Nicolle and Manceaux, 1909). Nicolle and Manceaux examined preparations of Splendore and found an organism identical to the one they had described.

Nicolle and Manceaux (1909) successfully transmitted the parasite utilizing intraperitoneal inoculation of a splenic suspension. Five of five gondis and one of twelve cobayas were infected; further passage from the one cobaya to three additional cobayas was unsuccessful. Subcutaneous and intraperitoneal inoculations of the splenic suspension into two macaques and intraperitoneal inoculation into three white rats were not successful.

However, as cited by Wenyon (1926b), Splendore was able to infect rats, guinea pigs, frogs, and rabbits with his Toxoplasma of rabbit origin. Carini, using Splendore's isolate, infected pigeons in 1909. With Toxoplasma canis, Carini infected rabbits in 1911, and Carini and Maciel infected pigeons in 1913. During 1916, Carini and Migliano described T. caviae in guinea pigs and transmitted it to pigeons.

Wenyon (1926b) felt that some of the so-called toxoplasmata morphologically similar to Toxoplasma were merozoites of either hemogregarines or coccidia resulting from schizogony. Toxoplasma gondii, which replicates by binary fission and is readily inoculable from animal to animal, could not, therefore, be classified with either the hemogregarines or coccidia. Because of the lack of host specificity and failure to replicate by schizogony, Wenyon (1926b) declared that Toxoplasma was unclassifiable. (Wenyon erroneously states that Nicolle and Manceaux [1909] concluded Toxoplasma replication was by either longitudinal division or schizogony. Nicolle and Manceaux caution that Toxoplasma within cells may be mistaken for merozoital cysts

[schizonts], but in the case of Toxoplasma, the organisms tend to occur in pairs due to replication by bipartition.)

In 1939 Wenyon again emphasized that the replication of Toxoplasma was invariably by binary fission, which may appear as schizogony due to masses of parasites pressed together in certain preparations. Since schizogony does not occur in Toxoplasma he concluded that the exoerythrocytic schizonts of avian malaria had no relationship to Toxoplasma (Wenyon, 1939).

Impetus to Toxoplasma research came in 1939 when Toxoplasma became recognized as a zoonotic parasite rather than a laboratory curiosity. Wolf, Cowen and Paige isolated Toxoplasma from a human neonate with encephalomyelitis and transmitted it to laboratory animals (Wolf, et al., 1939; 1940). Sabin (1939), utilizing the human isolate, demonstrated biological and immunological identity of Toxoplasma from man and guinea pig.

Serologic Identity, 1939-1971

Sabin (1939) was in agreement with Aragao, 1933 (as cited by Sabin, 1939), that the use of morphology alone to classify suspected Toxoplasma could be misleading and confusing. Multiplication and disease production in a variety of mammals and birds were considered to be prime taxonomic characters. Sabin (1939) claimed the first recognition of Toxoplasma in North America based on transmission criteria since he and Olitsky (1937) demonstrated that Toxoplasma of guinea pig origin produced fatal infections in guinea pigs, mice, rabbits and chickens and non-fatal disease in the rhesus. Sabin (1939) cited earlier works by Mooser, 1939, Markham, 1937, Manwell and Herman, 1935, Herman, 1937,

and Wood and Wood, 1937, who may have demonstrated Toxoplasma, but doubted their findings based on morphology alone or transmission studies between birds since no transmission to mammals was attempted. He credits Wolf, Cowen and Paige (1939) as the first to transmit Toxoplasma from the brain of a child with encephalomyelitis to a broad range of hosts including rabbits, infant mice, guinea pigs, and chicks.

Utilizing the isolate of Wolf, Cowen and Paige, Sabin (1939) demonstrated biologic and immunologic identity to the human isolate and his guinea pig isolate. Pathogenicity of the human isolate was demonstrated for mice, rabbits, and chicks. Serologic identity was demonstrated using necrosis of rabbit skin at the inoculation site of Toxoplasma as an indicator; cross neutralization in sera from recovered rabbits was demonstrated with Toxoplasma of human and guinea pig origin.

In 1942 Reis (as cited by Sabin, 1942) demonstrated the pathogenicity of Toxoplasma of pigeon origin for chicks, mice, guinea pigs and rabbits. Immunologic identity was shown utilizing the rabbit-dermal-neutralization system.

It is now accepted that there is one serotype of T. gondii (Feldman, 1968), although the specificity of immunotyping has been challenged. In 1948 Sabin and Feldman introduced an antibody assay based on the non-staining of Toxoplasma with alkaline methylene blue in the presence of antibody plus human "accessory" factor (Sabin and Feldman, 1948). The specificity of the Sabin-Feldman Dye Test was questioned by Mühlpfordt (1951) who found that guinea pigs, rats, and hamsters inoculated with Sarcocystis of sheep and goat origin became positive to the Sabin-Feldman Dye Test. Furthermore, Awad (1954) reported that rabbits and mice inoculated with Trichomonas vaginalis and

mice infected with Trypanosoma cruzi reacted positively to the cytoplasm-modifying antibody of the Sabin-Feldman Dye Test. Cathie and Cecil (1957) refuted the findings of Mühlpfordt and Awad by demonstrating a heat labile factor in human, guinea pig and sheep sera which gave false positive reactions. In addition, it has recently been demonstrated that the transmission of Sarcocystis of sheep origin to cats may be complicated by the simultaneous transmission of Toxoplasma unless isolated sarcocysts are given alone (Rommel, et al., 1972).

Further substantiation of the specificity of the Dye Test has been shown by Kulasini (1960) and de Andrade and Weiland (1971). Kulasini failed to induce positive reactions in rabbits inoculated with Eimeria steidae, Leishmania enrietti and Crithidia fasciculata, in guinea pigs inoculated with Atoxoplasma sp. and in mice given Trypanosoma cruzi. de Andrade and Weiland observed specific reactions to Toxoplasma antigen with both the indirect fluorescent antibody method and the Dye Test. An antigenic relationship, however, was seen between Eimeria scabra, E. nieschulzi, Isospora felis and T. gondii utilizing agar diffusion precipitation, but no such relationship was established with Sarcocystis tenella.

Morphologic Comparisons, 1909-1968

The taxonomic affinities of Toxoplasma have intrigued investigators since its discovery. Morphology of the organism as a basis for taxonomic relationship was recognized to be inadequate. Yet, with the discovery that toxoplasmosis was a potential zoonosis, investigators began to compare similar-appearing organisms to Toxoplasma. Such comparisons sometimes led to erroneous conclusions, but the work of

Goldman, et al. (1958) utilizing light microscopy of silver stained organisms, and Garnham, et al. (1962) and Sheffield and Melton (1968) utilizing electron microscopy, revealed close relationships to coccidia. The conclusions of the morphologists supported the findings of subsequent transmission studies which culminated in the recognition of Toxoplasma as an intestinal coccidian of the cat.

The first to recognize Toxoplasma as a new organism, Nicolle and Manceaux, considered it to be a protozoan more removed from the trypanosomes than Leishmania (Nicolle and Manceaux, 1909). Splendore simultaneously recovered the parasite from a rabbit, but thought it was a Leishmania sp. (Nicolle and Manceaux, 1909). Wenyon (1926b) considered it a protozoan and doubted the significance of naming different species of Toxoplasma according to the host of origin since they were morphologically similar and transmissible between species.

In her review, Wolfson (1940) considered that up until that time three different organisms seen in birds all had been classified as toxoplasmas based on morphology alone. She admitted that exoerythrocytic plasmodia and Toxoplasma were difficult to distinguish and could also be confused with leukocytic hemogregarines, but the ease of transmission and the ability to kill experimental animals within several days were criteria unique to Toxoplasma. Wenyon (1939) felt there was no relationship between Toxoplasma and avian malaria since Toxoplasma replicated by binary fission whereas schizogony occurred in the exoerythrocytic malarial schizonts.

According to Cross (1947), Wenyon (1926b and 1939) was of the opinion that toxoplasmas were not protozoa, but vegetable organisms related to Histoplasma capsulatum or similar yeast-like organisms.

(The writer has found no mention of this opinion in reviewing the original references listed by Cross.)

Sabin (1942) considered Toxoplasma to be a protozoan rather than a yeast because it was Gram negative and contained a nucleus demonstrated by the Feulgen method. Manwell, et al., 1945, (as cited by Cross, 1947) also concluded it was not a fungus since it could not be grown on Sabouraud medium.

Toxoplasma has been thought by some workers to be identical with Encephalitozoon (Nosema) and Sarcocystis. As cited by Cross (1947), Pinkerton and Weinman, 1940, and Kean and Grocott, 1945, assigned Toxoplasma and Sarcocystis to different genera. This separation was also considered valid by Biocca (1949). According to Cross (1947), Sabin, 1942, Wolf, 1939, and Guimeraes, 1942, considered Encephalitozoon to be identical with Toxoplasma, but Perin, 1943, and Weinman, 1944, did not. Biocca (1949) also considered Toxoplasma and Encephalitozoon to be synonymous. Encephalitozoon (Nosema) is now known to be a microsporidian (Noble and Noble, 1964). A recent paper indicates Sarcocystis, like Toxoplasma gondii, is an intestinal coccidian (Rommel, et al., 1972).

Utilizing electron microscopy, Gustafson, et al. (1954) concluded that Toxoplasma was unique with no morphologic similarities to organisms of recognized taxonomic status. They described a hollow, truncate cone, the conoid, at the pointed end of the organism; the base of the conoid is open to adjacent cytoplasm. Fourteen to eighteen toxonemes are seen as long, roughly cylindrical bodies of dense homogenous material, varying in length but extending as far posterior as the nucleus. That portion of each toxoneme close to the conoid becomes slender and tortuous

as it enters the conoid. The toxonemes were then considered unique to Toxoplasma.

Goldman, et al. (1958) contributed to understanding the taxonomic relationship utilizing light microscopy of silver stained smears. Replication was seen to occur via internal budding wherein two nuclei form anteriorly to the parent-cell nucleus with a subsequent longitudinal membrane developing within the parent-cell cytoplasm. Upon completion of division, two contiguous daughter cells resulted. They concluded that such a method of replication made it doubtful that Toxoplasma could belong to the Trypanosomatidae as did Westphal, 1954, or to the Eugregarinida as did Van Thiel, 1956, (as cited by Goldman, et al., 1958). Besnoitia jellisoni (of uncertain classification) was the only other species, then recognized, with this method of internal budding, termed "endodyogeny."

Gavin, Wanko and Jacobs (1962), in an electron microscopic study of replicating Toxoplasma, substantiated the findings of Goldman, et al. However, they recognized three replicating forms. One, corresponding to that seen by Goldman, et al., resulted in two organisms arising within one parent cell. Beginning replication was recognized by two parabolic profiles appearing in the anterior cytoplasm; eventually the concave surfaces become opposed to the parent nucleus. The parent nucleus partially divides as the parabolic profiles extend posteriorly incorporating the nuclear material. As the daughter cells begin to separate the parent cell contributes the outer cell membrane to what was originally the parabolic profile. Later, the daughter cells are attached posteriorly as a pair. As sequestration continues, a small residuum may remain which originated from the parent-cell cytoplasm.

Asynchronous development was recognized utilizing this method of replication.

Rosettes, consisting of at least four organisms with interconnecting cytoplasm posterior to the level of the nucleus were also recognized. Gavin, et al. conceded that these multinucleated Toxoplasma could have arisen from asynchronous endodyogeny. They considered the multinucleate forms to be schizonts and endodyogeny to be a specialized form of schizogony wherein two daughter cells are formed. The third replicating form seen was thought to have resulted from bipartition; two juxta-opposed organisms oriented at right angles were seen occasionally with no nuclear changes recognized preceding this form. Although the rosettes seen by Gavin, et al. resembled the schizonic stages of Plasmodium gallinaceum and P. lophurae, they concluded that Toxoplasma could be considered a sporozoan but there was not enough evidence to link it with the hemosporidia.

Affinity to the hemosporidia was demonstrated by Garnham, et al. (1962) with the electron microscopic demonstration of a micropyle, a well-defined depression in the cell wall near the organism's middle. The outer cytoplasmic membrane is interrupted at the micropyle which is bound by only the inner cytoplasmic membrane. Originally described in the sporozoites of malarial parasites by Garnham, et al. (1962), a micropyle was also reported to occur in Sarcocystis and the "M-organism" by Ludvik, 1962 (as cited by Garnham, et al., 1962). They concluded that since a micropyle has never been demonstrated in the flagellates, amoebae or ciliates, the organelle's presence justifies the classification of Toxoplasma with the sporozoa.

In their detailed electron microscopic description Sheffield and Melton (1968) concluded that Toxoplasma contained organelles common to coccidia and other sporozoa of uncertain classification. Subpellicar fibrils, 22 in number, are rather constant for Toxoplasma, Besnoitia jellisoni and Eimeria bovis. Micronemes, coiled tubules present in the anterior end in association with the paired organelles, are common to Toxoplasma, Sarcocystis, Eimeria and Besnoitia jellisoni. Toxonemes, or paired organelles, club-like structures of the anterior cytoplasm varying in number from four to eight, are common to Toxoplasma, Haemamoeba (=Plasmodium) gallinacea, Lankesterella, M-organism, Sarcocystis tenella, and Eimeria bovis. The conoid, paired organelles, micronemes, subpellicar fibrils and micropyle are structures all unique to sporozoa.

By 1968 Toxoplasma had been shown to contain several electron microscopic structures in common with known coccidia, i.e., Plasmodium sp. and Eimeria sp.

Transmission, 1909-1970

Experimental transmission of T. gondii to the cat ultimately supported and extended the conclusions of the morphologists. Prior to the recent recognition of an intestinal oocyst stage in the life cycle, only the proliferative tissue stage and encysted tissue stage were known. The rapidly proliferating stage found intra- and extracellularly in acutely ill animals was the first form recognized by Nicolle and Manceaux in 1908; inoculation of tissue suspensions was recognized early as a means of transmitting this stage of the organism. The encysted organism may be present throughout the body, including the

brain and skeletal muscle, in chronically infected asymptomatic hosts.

Although contact transmission was not known to occur, transmission via carnivorism was the first recognized natural mode of transmission. Sabin and Olitsky (1937) could not demonstrate contact transmission among mice until animals ate the carcasses of others recently dead of the disease; accordingly, they postulated carnivorism as a mode of natural transmission. As cited by Jacobs (1956), Adams, 1949, von Thiel, 1949, and Cowen and Wolf, 1950, achieved only occasional transmission by feeding carcasses of acutely infected mice. Eichenwald, 1948, as cited by Jacobs (1956), consistently did it with chronically infected mice and Jacobs (1956) reported more consistent transmission with chronically infected rats. Jacobs felt the encysted form was more resistant to digestive juices than the proliferative form. Schmidtke (1954) failed to infect mice or guinea pigs by feeding brain tissue with large numbers of cysts. Later, Jacobs reported that the proliferative form was killed by peptic juice in a few minutes while the released encysted form survived for two hours; the proliferative form survived 1% trypsin for only three hours, but the liberated encysted form survived six hours (Jacobs, et al., 1960b). Jacobs, et al. (1960a) found the encysted form to be rather common in the meat of slaughtered swine, cattle and sheep. The first outbreak of toxoplasmosis in man resulting from eating poorly cooked beef was reported in 1969 (Kean, et al., 1969).

Wolf, Cowen and Paige (1939, 1940) first conclusively demonstrated toxoplasmic encephalomyelitis in human neonates although, according to Sabin (1942), Jankû, 1923, had described similar organisms in the retina

of an infant with hydrocephalus, and Torres, 1927, in an infant dying with convulsions at two days of age. On the basis of these observations in utero transmission was suspected. Paige, Cowen and Wolf (1942) confirmed their suspicions when an in utero craniotomy was done on an infant with hydrocephalus; the infant was found to have toxoplasmic encephalomyelitis.

The possibility of arthropod vectors was raised by Sabin (1942) and Weinman (1944) since a fatal human case reported by Pinkerton and Henderson in 1941, as cited by Weinman (1944), had a history of removing ticks from his dog and two ticks from his own leg. Woke, et al. (1953) were unable to transmit Toxoplasma with seventeen species of arthropods save for one dubious louse transmission to a rabbit. The failure of arthropod transmission was also supported by Haulik, 1951, utilizing ticks, and Giovonnovi, 1952, utilizing mosquitoes (as cited by Jacobs, 1956).

That contact transmission could occur from diseased animals was considered unlikely by Jacobs (1956) since the proliferative form shed in feces was poorly resistant to the external environment. Until 1956, only one report of direct transmission (Olafson and Monlux, 1942) had been reported; this occurred with a sick puppy in contact with its litter mates. As cited by Jacobs (1956), Jacobs, Melton, and Cook could not duplicate the results reported by Olafson and Monlux. The attempt to transform Toxoplasma in the intestine into a resistant form that could serve as a means of spread had always met with failure (Jacobs, 1956). Siim, et al. (1963) felt that, except for congenital infection, direct transmission of toxoplasmosis by contact had not been demonstrated in man or animals. Hutchison (1965) was the first to

report fecal-oral transmission of Toxoplasma; his report initiated a flurry of activity which, within five years, revealed the new taxonomic status of Toxoplasma as an intestinal coccidian of the cat.

Hutchison (1965) fed a mouse chronically infected with Toxoplasma to a six-month-old cat infected with the ascarid Toxocara cati. Two weeks later the cat feces were subjected to zinc sulfate flotation and washed once with tap water by sedimentation. After remaining in water at room temperature for three months, the sediment was administered orally to six mice, two of which died fourteen days later with toxoplasmosis; the four surviving mice were examined two months post-inoculation and encysted Toxoplasma were present in the brain. A subsequent experiment revealed that the inoculum, kept in tap water at room temperature, remained infective for one year. Of 48 mice fed fecal sediment during the year, 37 survived for the two-month period prior to examination; the brains of all but two of the 37 contained Toxoplasma cysts. Examination of the cat feces revealed (large) oocysts of Isospora sp. and eggs of Toxocara cati. Hutchison erroneously speculated that Toxoplasma could be liberated from food or from intestinal lesions, become associated with an intestinal nematode and, subsequently, pass into the external environment within eggs of the helminth.

In a 1967 paper presented to the Royal Society of Tropical Medicine and Hygiene, Hutchison (1967) gave his reasoning behind selection of the cat for transmission studies with Toxoplasma. According to Hutchison (1967), Jacobs, et al., 1950, Rifaat, et al., 1963, and Frenkel, 1965, had failed to implicate biting arthropods as vectors. Hutchison believed the encysted stage was essential in the life cycle and reasoned that most invertebrates, apart from carrion arthropods and

endoparasites, would not have access to these cysts "locked" in tissues. Furthermore, protozoa requiring an invertebrate vector, such as Plasmodium and Leishmania, when subjected to temperatures below that of their mammalian host (37°C) transform into different, viable stages in their life cycle; however, subjecting Toxoplasma to either 18°C or 29°C results in lowered infectivity. A final argument against insect vectors was that there was no seasonal incidence to toxoplasmosis.

Continuing, he reasoned that intestinal nematodes of carnivores could have access to the cysts as their hosts ate infected carcasses. Since the encysted form could withstand short periods of digestion, the short survival time of this stage would not be detrimental. Ingestion by an invertebrate residing within a homeotherm would not be detrimental to the organism; once within the egg the temperature would drop after leaving the host, but the reduced metabolic activity would conserve food supplies of the protozoan. Similar transmission of the protozoan Histomonas meleagridis within eggs of Heterakis gallinae had been shown by Smith and Graybill in 1920 according to Hutchison (1967).

If a nematode was to be incriminated, it must have a cosmopolitan distribution in accord with the distribution of Toxoplasma. According to Hutchison (1967) Enterobius vermicularis was eliminated since the Navajo Indians had a high incidence of the nematode as reported by Brooke, 1961, but very few positive Dye Test titers as reported by Feldman, 1961. The invertebrate would more likely be one in a domestic animal living close to man; cats and dogs had long been suspected as being reservoirs of infection. Toxocara sp. are among the most common nematodes in cats and dogs. Dogs were discounted because man had more control over his diet, whereas cats continually catch and eat birds and

small mammals from which they could become infected. Toxocara sp. have the ability to infect a wide range of homeotherms. Hutchison decided, therefore, to work with Toxocara cati-infected cats exposed to Toxoplasma.

In his 1967 report Hutchison also gives further evidence supporting the transmission of Toxoplasma within the egg of Toxocara cati. Two cats were utilized, one harboring Toxocara cati and one not. Each was fed Toxoplasma-infected mouse carcasses for five days. After collecting feces from these cats for 30 days, the cat harboring T. cati was given an anthelmintic and the ascarid-free cat was infected with T. cati. Again each cat was fed infected mouse carcasses and their feces collected for thirty days. Results indicated Toxoplasma could be transmitted fecally to mice only when T. cati eggs were present. Further corroboration was provided when three positive fecal inocula were filtered to remove the nematode eggs and the fecal infectivity was lost.

Jacobs and Melton (1966) supported Hutchison's 1965 findings as did Dubey in 1966 (Dubey, 1968b).

The nematode egg transmission theory was short lived, however, because subsequent workers were soon able to fecally transmit Toxoplasma in the absence of nematode eggs. Jacobs, 1967, was the first to report that fecal infectivity could occur in the absence of helminth eggs (Hutchison, et al., 1968). Dubey (1968a), utilizing Toxoplasma serology and fecal examinations of 100 cats, concluded that it was unlikely that T. cati was the sole transmitting agent of Toxoplasma; only 34 of 64 seropositive cats (Dye Test 1:4-1:64) harbored T. cati and 15 cats harboring T. cati were seronegative. Dubey (1968b) also reported infectivity in the feces of a helminth-free cat. Hutchison, et al. (1968)

reported on two cats that had fecal infectivity in the absence of T. cati eggs. It was also noted that embryonated T. cati eggs arrested at the eight-cell stage could still transmit infection even though they were incapable of hatching; they were convinced that unknown forms of Toxoplasma were passed in the feces independently of the ascarid eggs.

Sheffield and Melton (1969) also reported fecal transmission of toxoplasmosis in the absence of helminth eggs. They determined that the feces was transiently infective from days 4 to 11 after feeding the cats infected mice and that for the fecal matter to become infective for mice it must remain outside the host for a minimum of three days at room temperature.

Frenkel, Dubey and Miller (1969), utilizing feces of cats containing T. cati eggs and possessing Toxoplasma fecal infectivity, found that the feces retained infectivity after removing the eggs by filtration. Feces remained infective after passing through a 37u to 44u wire mesh but not after filtration through a mesh having a pore size of 10u to 15u.

Work and Hutchison (1969a) reported a new cystic form of Toxoplasma gondii. Two mice chronically infected with Toxoplasma were fed to four Dye Test positive cats (1:10-1:250) each day for seven days. Feces were collected and pooled every sixth day; each pool was repeatedly filtered and washed and the sediment floated, using saturated sodium chloride and 33% zinc sulfate. At six and twelve days post exposure (but not at eighteen days) flotation revealed small cystic structures of uniform morphology measuring approximately 9u by 14u with a wall of uniform thickness and an interior forming a slightly granular mass. After maintaining the sediment in tap water for three weeks, the interior of the

cysts changed: two separate, granular bodies were visible, but structural details of the bodies were not discernible.

The fecal sediment was passed through a 53u filter to remove any ascarid eggs, and Toxoplasma infectivity was demonstrated conclusively by inoculating four mice intraperitoneally with a single cyst and subsequently recovering Toxoplasma. Groups of six mice were given serial, ten-fold dilutions of 10,000 cysts to .1 cyst orally. Those receiving 10,000 cysts either died or were dying in 8 to 11 days; of four examined serologically, all were Dye Test positive. Of those receiving 1,000 cysts, one died in ten days; the surviving five were Dye Test positive. All six mice given 100 cysts survived 18 days; they, too, were Dye Test positive. Of those receiving 10 cysts, four of six became Dye Test positive. In the two groups given the equivalent of 1 cyst and .1 cyst, all were serologically negative.

In a subsequent report, Work and Hutchison (1969b) infected additional groups of mice with the fecal cyst stage. Three serologically negative cats fed mouse carcasses became Dye Test positive and shed fecal cysts for 6 to 14 days post feeding. No additional characterization of the cyst was given, but it was stated that it was remarkably similar to an isosporan oocyst.

Siim, Hutchison and Work (1969) characterized the fecal cyst as an oocyst similar to that of Isospora bigemina. After incubation for three weeks at room temperature, the cysts were embedded in paraffin, sectioned and stained with H & E; they were seen to contain two sporocysts, each containing four sporozoites.

Rapid support of these findings was forthcoming. Sheffield and Melton (1970) infected monkey kidney tissue culture cells with excysted

sporozoites and demonstrated Toxoplasma by both light and electron microscopy. Groups of mice fed oocysts either became seropositive, developed brain cysts or had Toxoplasma in the lung of those animals dying acutely.

Frenkel, Dubey and Miller (1970) also reported findings supporting the oocyst stage as a form of Toxoplasma. Confirmation of infectivity was accomplished using either serology or the presence of tissue cysts in experimentally fed animals. Varied handling techniques of the fecal cysts were employed; sporulation of the cysts was inhibited or delayed using temperature variants, anaerobic incubation and chemical preservatives. Fecal infectivity varied in direct proportion to inhibition of cyst development. Fecal infectivity was also correlated with the presence of cysts within a filtrate. Measuring 9u to 14u in diameter, the cysts passed through a wire sieve having 25u interstices and through a spherical copolymer column having 18u interstices with no loss of Toxoplasma infectivity in the filtrates. However, passing the cyst suspension through a column with 9u interstices reduced its Toxoplasma infectivity while a column with 8u interstices eliminated it. Sucrose density gradient separation revealed the highest Toxoplasma infectivity and fecal cyst numbers in the .92 Molar fraction (specific gravity 1.11). Continuous particle electrophoresis also demonstrated that infectivity was dependent upon the presence of fecal cysts. Coccidian stages in the intestinal epithelium of kittens fed oocysts were fluorescent-antibody-positive with Toxoplasma-conjugated-antiserum as were the sporocyst walls and sporozoites in sporulated oocysts. Toxoplasma infectivity of the feline fecal oocysts was shown for mice, rats, golden hamsters, guinea pigs, dogs, cats, raccoons, skunks, pigs,

domestic rabbits, opossums, Japanese quail and chickens, but not pigeons. However, only in cats did subsequent oocyst development occur.

Frenkel, Dubey and Miller (1970) postulated that the tissue-encysted form, proliferative form and oocyst were each different developmental stages of Toxoplasma and cited as evidence the different prepatent periods in kittens fed these forms. The tissue-encysted form induced oocysts in 3-5 days, the proliferative form in 7-9 days, and the oocyst in 20-24 days.

Dubey, Miller and Frenkel (1970) concluded that Toxoplasma should be placed in the suborder Eimeriorina or Eimeriina as a member of the family Toxoplasmatidae with the following characteristics:

> . . . schizogony and gametogeny in the gut epithelium of cats; oocysts with two sporocysts, each of which have four sporozoites developing outside the host; trophozoites multiplying by endodyogeny in many types of cells, leading to the production of [tissue] cysts with many merozoites, mainly in the brain and muscle; being facultatively heteroxenous in many mammals and birds in which only an asexual extraintestinal cycle has been observed.

The unsporulated oocyst of Toxoplasma gondii measures 10u X 12u (9u-11u X 11u-13u) and is spherical to subspherical in shape; a micropyle and polar granule are absent. Sporulated oocysts, of a similar shape but distended by the sporocysts, are 11u X 12.5u (10u-11u X 11u-14u); an oocyst residuum is absent. Free sporocysts measure 6u X 8.5u (5.0u-6.5u X 8.0u-9.5u); the sporocyst residuum consists either of compact granules at one end or a few scattered granules. Freed sporozoites measure 2u X 8u (Dubey, et al., 1970).

Overdulve (1970), Werner and Janitschke (1970), and Hutchison, et al. (1971) have supported the conclusions of Frenkel, et al. (1970).

The oocyst of Toxoplasma gondii cannot be differentiated morphologically from Isospora bigemina, small race (Dubey, et al., 1970; Hutchison, et al., 1971), an oocyst first reported in the cat in 1923 (Wenyon, 1923-24) and in the dog in 1925 (Wenyon and Sheather, 1925). Most of the literature dealing with Isospora bigemina, small race, is based on oocyst morphology alone with inadequate descriptions of developmental stages within the dog or cat. Frenkel, et al. (1970) could not induce Toxoplasma oocyst development in the dog. The need to reassess the biology of Isospora bigemina for clarification is obvious in light of recent developments with Toxoplasma. The taxonomic status of Isospora bigemina has been dubious and remains so.

Isospora bigemina, 1874-1970

In his extensive review of the literature on coccidiosis in cats and dogs, Wenyon (1923-24) describes in some detail the findings of earlier workers. According to Wenyon (1923-24), Rivolta, 1874, 1877, and 1878, was the first to recognize the parasitic nature of coccidian forms in the intestinal villi of cats and dogs. Furthermore, Finck, 1854, and Virchow, 1860, had seen similar structures within the villi of cats and dogs, respectively; both considered them to be products of fat absorption.

Rivolta (as cited by Wenyon, 1923-24), describing his findings in dogs in 1878, reported sporulation occurring within the villous lamina propria; in Müller's (potassium dichromate) solution each sporocyst (oviform cell) contained four long corpuscles with rounded ends; the sporocysts measured 8u-12u X 8u. Rivolta named these forms in the dog and cat Cytospermium villorum intestinalium canis.

Leukart, 1879 (as cited by Wenyon, 1923-24), reported seeing all developmental stages of coccidia, including sporulation, in the intestinal epithelium of cats. Leukart's report contained no illustrations or measurements; Wenyon (1923-24) felt it was not possible to accurately interpret his findings.

Ralliet and Lucet in 1891, according to Wenyon (1923-24), accepted the name Coccidium bigeminum (Stiles, 1891) for coccidia resembling those described by Virchow and Rivolta occurring within the intestinal villi of dogs and pole cats. They listed three varieties of Coccidium bigeminum occurring in the dog, cat and pole cat, but no details were given as to their observations in cats. The sporocyst measurements (listed as oocyst measurements by Wenyon [1923-24]) are: C. b. var. canis - 7u-9u X 12u-15u and C. b. var. cati - 7u-9u X 8u-10u (Wenyon, 1926a).

Lühe (1906) placed Coccidium bigeminum into the genus Isospora.

According to Wenyon's 1923 review, earlier authors had reported oocysts with clearly distinguishing characteristics from Isospora bigemina Lühe, 1906, (Stiles, 1891) in the dog and cat as Coccidium bigeminum, Diplospora bigemina and Isospora bigemina. Wasielewski in 1904, according to Wenyon (1923-24), described Diplospora bigemina; gametogeny occurred in the intestinal epithelium of the cat giving rise to large oocysts varying from 19u X 22u to 28u X 40u. Wenyon, agreeing with Reichenow, 1921, noted this was probably a mixed infection with oocysts of the size ranges 19u-22u X 22u-25u and 23u-32u X 35u-40u. Swellengrebel in 1914, according to Wenyon (1923-24), described the oocyst comparable to the larger size described by Wasielewski as Isospora bigemina. Additional examples of the confusion in identifying

coccidia are the papers by Hall (1917), Hall and Wigdor (1918), and Davis and Reich (1924).

Hall (1917) found 2-3% of 67 Michigan dogs passing oocysts measuring 28u-32u X 36u-40u; their large size precluded their being Diplospora bigemina (Coccidium bigeminum) since the oocysts described by Stiles in 1892 reached only half this size. In a subsequent paper Hall and Wigdor (1918) concluded that Hall's Michigan dogs were indeed infected with Diplospora bigemina, despite the discrepancy in size of oocysts. They mention that Ralliet (no reference given) in a review of a paper by Gillibeau, 1917, concluded that Diplospora bigemina from the intestinal mucosa and submucosa was the only known coccidium in the dog. Hall and Wigdor (1918) note that Fantham, 1916, gave the size range of Diplospora bigemina as 19u-28u X 22u-40u which are the exact measurements given by Wasielewski and were later thought by Wenyon (1923-24) to be those of two distinct species. Hall and Wigdor (1918) were puzzled by oocysts in one dog which measured 18u X 20u, and were undecided as to whether the oocysts should be regarded as a strain, variety or species; they thought that there were either several species in the dog or one species developing various strains under certain conditions. If Stiles in 1892 (as cited by Hall and Wigdor, 1918) was correct, they concluded that the oocysts of Diplospora bigemina from European dogs measured about 10u in diameter, the smaller ones from Michigan measured 20u in diameter and the larger ones measured 40u. Cross immunization studies with one dog supported their contention that the small and large Michigan coccidian oocysts belonged to different strains of the same species.

Davis and Reich (1924) reported that the oocysts of Isospora bigemina in the cat and dog lie between 25u-30u X 20u-25u. One fecal

specimen submitted to them from a cat contained oocysts measuring 27u-40u X 35u-47u (33u X 41u). They noted that oocysts of Isospora bigemina appeared in various stages of development, including the sporocyst stage, but none contained sporozoites. (Details on how the fecal specimens were handled prior to examination were not given.)

After reviewing the literature and making original observations on cats and dogs in England, Wenyon (1923-24) determined that there were isosporan oocysts of three size ranges in the dog and cat; small forms reported by various authors measured 12u-16u X 12u, 12u-15u X 7u-9u, 8u-10u X 7u-9u, 8u-12u X 6u-8u, and 13.6u-15.9u X 7.9u. The intermediate size oocysts measured 27u-30.8u X 22u-24u (20u-25u) (Wenyon, 1926a), and the largest measured 23u-32u X 35u-40u. Wenyon recognized three species in the genus Isospora and designated them as follows: I. bigemina (Stiles, 1891); Isospora rivolta Grassi, 1879; and Isospora felis n. sp.

Wenyon (1923-24) described one case in a kitten with the small form developing sporulated oocysts within the intestinal villi; he also mentioned that Sheather of the Royal Veterinary College had found unsporulated oocysts 12u in diameter in a cat's feces. Using Sheather's observation, Wenyon concluded that the small form may also be passed unsporulated in some instances. In a subsequent report Wenyon (1926a) listed the oocyst size, as seen developing in the kitten's intestinal villi, as 8u-10u X 12u-16u with sporocysts measuring 6u-8u X 9.5u-10u. In this particular instance no coccidian oocysts had been recovered in the feces of the kitten which had been utilized in a study with Entamoeba histolytica.

Wenyon and Sheather (1925) described an "acute" phase of Isospora bigemina in a dog which passed only undeveloped oocysts in the feces; they measured 10u-11u X 7.5u-9u, although some measured up to 14u across (Wenyon, 1926a). Fully developed sporocysts measured 7.5u-9u X 5u-7u. The oocysts were of the size usually found in cats. Histologic sections of the intestine revealed gametes and schizonts with only eight merozoites in the epithelium, not within the villi (Wenyon and Sheather, 1925). Wenyon (1926a) refers to these as the small oocysts of I. bigemina.

In addition, the small oocysts were recovered from a dog with an acute and persistent diarrhea of one month's duration (Wenyon, 1926a). As the diarrhea subsided oocysts could no longer be recovered. Wenyon suggested that in extraordinarily heavy infections I. bigemina probably developed in the subepithelial tissue and continued to develop after the epithelial infection waned; trapped in the subepithelial tissue, the oocysts sporulated prior to escape from the host. Wenyon concluded that two types of Isospora bigemina exist: the smaller one, measuring 10u-14u X 7.5u-9u, occurring during acute infections in dogs and cats, and the larger one, measuring 14u-16u X 18u-20u, occurring during the chronic infections in dogs.

Wenyon (1926a) also discussed the possibility that the small I. bigemina oocysts of two size ranges could be two different species. The larger oocysts, which developed in the lamina propria, had thinner walls which were easily distorted or ruptured and often were more closely wrapped around the sporocysts than those of the oocysts which developed in the intestinal epithelium. Wenyon raised the possibility that even though the oocyst of I. rivolta measured 20u-25u instead of

18u-20u and usually developed in the intestinal epithelium, the findings of Wenyon and Sheather (1925) indicated that all developmental stages could occur in the subepithelial tissue. Since an oocyst developing in the subepithelium may be smaller than that developing in the epithelium, Wenyon thought it possible that the sporulated oocyst of the large I. bigemina may be I. rivolta reaching maturity in the subepithelium.

Until recently there has been little work reported to either confirm or deny Wenyon's inferences.

Lee (1934). conducted cross infectivity studies with Isospora bigemina, small race. Using oocysts from dogs, two kittens were infected with oocysts which measured 8u-15u X 7u-11u (8u X 10u) and required 96 hours for sporulation. The kittens developed a bloody diarrhea. In one kitten the prepatent period was six days and oocysts were passed for 22 days; the other had a five day prepatent period and passed oocysts for 20 days. Unlike the Toxoplasma oocyst described by Dubey, et al. (1970), and the Isospora bigemina, small race, oocyst described by Levine and Ivens (1965) and Shah (1970), the sporulated oocyst contained a residual body.

A similar oocyst of silver fox origin, measuring 12u-17u X 9u-11u, was used to infect two kittens (Lee, 1934). From the dog and fox transmission studies Lee concluded that Isospora bigemina of dogs, cats and foxes were cross infective even though no small race of feline origin was utilized.

The occurrence of I. bigemina was reported by Gassner (1940), but the data are confusing. Seventy-four percent of 252 hospitalized dogs were passing coccidian forms; 71% of these were harboring I. bigemina, small race, and 3% I. bigemina, large race. Although his measurements

of the oocysts are in agreement with those of Wenyon (1926a), his findings differ in that few of the dogs passed the unsporulated oocyst of the small race. The data are presented in percentages of incidence of I. felis, I. rivolta, and I. bigemina, small and large races, so that individual numbers of observations are not given. However, it is clear that all samples contained free sporocysts (up to 25 per slide) while only 16% of these contained oocysts. The data on oocysts and sporocyst measurements indicate measurements were taken but neither the number of measurements nor the number of animals with oocysts of I. bigemina is stated. No mention is made of the unsporulated oocysts of I. bigemina, small race, being recovered.

Benbrook and Sloss (1948) depict two types of Isospora bigemina oocysts of dogs, foxes and cats, and state, without references, that the oocysts of the smaller form are not sporulated when found in feces while those of the larger form sporulate before leaving the body and sometimes rupture liberating two sporocysts. Sloss (1970) gives the actual measurements of the two oocysts: small race, 10u-14u X 7u-9u; large race, 18u-20u X 14u-16u. Again without references, hosts are listed as the dog, fox, mink and cat; the illustrations are identical to those published in 1948.

Morgan and Hawkins (1952) list the oocyst of I. bigemina, small race, as varying in size from 10u-14u X 7.5u-9.6u, and the large race varying in size from 18u-20u X 14u-16u. An infected animal passes either one or the other race, but not both; intermediate sizes do occur. Hosts are given as the dog, fox, mink, cat, and closely related carnivores. Unsporulated oocysts are passed in the acute stage of infection and sporulated oocysts or single sporocysts are passed at other times.

It was recognized that the two size ranges of oocysts could represent two species, but they were considered to be one species.

In his extensive catalog of the Eimeriidae, Becker (1956) stated that oocysts of Isospora bigemina appear in stools fully sporulated. Oocysts of the small form were said to measure 10u-16u X 7.5u-10u and the large form 18u-20u X 14u-16u. The cat, dog and certain other carnivores were designated as hosts. No mention was made that the oocysts might be passed in an unsporulated state. The size range given for the small form exceeded that given by Wenyon (1926a) as being the most common (7.5u-9u X 10u-14u), but those of the large form were in agreement with Wenyon's measurements.

Horton-Smith (1958) concurred with Wenyon (1926a) and Morgan and Hawkins (1952) that the oocysts of I. bigemina occurred in two size ranges.

Levine (1961) agreed with Wenyon (1926a) on the sizes of oocysts and added that a micropyle, oocyst polar granule and residuum are absent from the sporulated oocyst of both the large and small race. The reported absence of an oocyst residual body differs from the findings of Lee (1934). Levine stated that sporocysts were ellipsoidal, contained a residuum, but not a Stieda body, and measured 7.5u-9.0u X 5u-7u. Levine did not mention that the oocysts passed in the acute infection are unsporulated whereas those passed in chronic infections are sporulated. He concluded that the organism deserves further investigation since Wenyon and Sheather in 1925 left questions unanswered.

Davies, et al. (1963) added nothing to the description of I. bigemina, but did compile the observations of Wenyon, Lee and Gassner.

They also stated that the two different size oocysts reported may represent different species.

In his extensive monograph on coccidia, Pellerdy (1965) quotes Wenyon's 1926 measurements of Isospora bigemina oocysts in the dog and cat. However, in complete reversal of Wenyon's conclusions, Pellerdy describes the location of the developing oocysts in the intestine differently; the larger oocysts were said to develop in the epithelium and the smaller ones subepithelially where sporulation began inside the host. Pellerdy also mentions that the larger oocysts are shed first, whereas Wenyon (1926a) felt they were a sequela to the acute infection.

Siim, Hutchison and Work (1969) demonstrated that the oocyst form of Toxoplasma reported by Work and Hutchison (1969a) measured approximately 9u X 14u in unstained, wet preparations. After three weeks at room temperature two sporocysts measuring 3u X 7u had developed within the oocysts and each contained four sporozoites, thus showing its affinity to Isospora.

Frenkel (1970) thinks that what has been designated Isospora bigemina, small race, of the dog and cat, should now be changed in light of the recent recognition of the Toxoplasma oocyst. Frenkel would restrict the use of I. bigemina to coccidians in dogs and recommends that sporulated oocysts measuring 10u X 12u in cat feces should be identified tentatively as I. cati. If, on the other hand, the oocysts are capable of infecting other species and induce Toxoplasma antibody, then they must be identified as Toxoplasma gondii. Hutchison, et al. (1971) believe that Isospora bigemina, small race, may be identical to Toxoplasma gondii with sexual stages yet to be shown in hosts other

than the cat. These authors agree with Frenkel that the oocyst should be designated Toxoplasma, rather than Isospora.

CHAPTER III

SUMMARY AND CONCLUSIONS

Until recent clarification, the taxonomic status of Toxoplasma has been uncertain. As a consequence of inadequate knowledge of its biology, the epidemiology and ultimate control of Toxoplasma have been unknown. Carnivorism was recognized by 1937 (Sabin and Olitsky, 1937) as a means of transmission. However, similar infection rates among carnivores and herbivores and meat-eating and nonmeat-eating human beings implied other means of transmission (Feldman, 1968). In utero transmission, recognized first in man in 1939, is considered a rare event (Frenkel, 1970), and arthropod vectors are not known to exist (Woke, et al., 1953). The origin of toxoplasmosis in man's domesticated animals has long been a zoonotic uncertainty (Olafson and Monlux, 1942).

By 1968, taxonomists had demonstrated similarities to other coccidia, namely Plasmodium and Eimeria, as well as affinities to other organisms of uncertain classification such as Sarcocystis and Besnoitia (Sheffield and Melton, 1968). Yet, the biology of Toxoplasma could not have been surmised solely from these similarities.

The fecal transmission of Toxoplasma demonstrated by Hutchison (1965) was the key to revealing its taxonomic identity. Though transmission through Toxoplasma-infected nematode eggs was suspected at first, further investigation revealed an oocyst with Toxoplasma

infectivity morphologically identical to that of Isospora bigemina, small race (Dubey, et al., 1970; Hutchison, et al., 1971). Therefore, fecal contamination of vegetation by cats may explain the earlier enigma of infection in herbivores and nonmeat-eating human beings (Frenkel, et al., 1970). Furthermore, the recognition of Toxoplasma as an intestinal coccidian has fostered reinvestigations of the biology of well known coccidia, such as Isospora felis and I. rivolta, as well as new investigations involving Sarcocystis (Frenkel and Dubey, 1972; Rommel, et al., 1972). These investigations have shown that coccidia which are usually considered to have a narrow host range may have unconventional ranges in a variety of intermediate hosts; oocyst formation occurs only in certain hosts. The criterion that an oocyst need generate additional oocysts after infecting its host can no longer be a valid basis for determining the host range of a given coccidian oocyst form.

The resemblance of Isospora bigemina, small race, oocyst to that of Toxoplasma raises the question - are they the same organism? Gametogeny and oocyst production have been induced only in cats (Dubey, et al., 1972; Hutchison, et al., 1971). Although the details of earlier workers are often not reported, it is possible to compare some of those findings in light of presently understood relationships.

Wenyon (1923-24) was the first to report an unsporulated oocyst (12u in diameter) of Isospora bigemina from a cat. Two years later similar oocysts were observed from a dog (Wenyon and Sheather, 1925). Although Wenyon (1926a) believed that the sporulated oocysts of the cat (8u-10u X 12u-16u) and those of the dog (14u-16u X 18u-20u) could appear in feces as the smaller form or small race of Isospora bigemina,

it is now probable that the sporulated oocyst developing within intestinal villi as seen by Wenyon and earlier workers is identical to the oocyst of Sarcocystis (Rommel, et al., 1972). Wenyon and Sheather (1925) described schizogony and gametogeny of the small race occurring in the gut epithelium of a dog; the location and size of the structures correspond to the development of Toxoplasma in the cat; Hutchison, et al., (1971) postulate that Wenyon and Sheather were describing Toxoplasma.

In cross infection studies conducted by Lee (1934), Isospora bigemina, small race, was transmitted from the dog to the cat; however, cat to dog transmission was not attempted. Unsporulated oocysts measured 8u-15u X 7u-11u (8u X 10u). Unlike the oocyst of Toxoplasma, the sporulated oocysts used by Lee contained an oocyst residuum. Shah (1970) described the sporulated oocysts of I. bigemina, small race, from a cat as being without an oocyst residuum similar to that of Toxoplasma. Levine and Ivens (1965) described similar oocysts from the dog. However, the oocysts were slightly larger and the sporocyst residuum consisted of scattered granules rather than a ball-like mass. If Lee's (1934) description is accurate, then the oocysts described by Shah (1970) and Levine and Ivens (1965) are distinctly different since neither contained an oocyst residuum.

Hutchison, et al. (1971) believe that I. bigemina, small race, oocysts from the dog may be Toxoplasma. They state this even in face of Frenkel, et al.'s (1970) failure to induce oocyst production in Toxoplasma-infected dogs.

Until the report of Frenkel, et al. (1970), no in situ oocyst development comparable to I. bigemina, small race, had been described in

the cat. Leukart, 1879 (Wenyon, 1923-24), describing all developmental stages, including sporulation, as occurring in the feline intestinal epithelium most certainly did not describe Toxoplasma development.

With some skepticism, I. bigemina, small race, of the cat and dog, has been accepted as the same organism since its descriptions in the mid 1920's. Lee's (1934) findings supported this conclusion although there have been no additional transmission studies except Jacob's (1949) brief report on transmission from the silver fox to the cat. From Lee's work it would appear that I. bigemina, small race, is common to cats, dogs, and foxes; however, unlike the I. bigemina oocyst of Shah (1970) and Levine and Ivens (1965), it had an oocyst residuum.

Nevertheless, oocysts have been found in the feces of the cat and dog which cannot be differentiated from Toxoplasma oocysts. Sporulated oocysts of Toxoplasma measure 11u X 12.5u (10u-11u X 11u-14u) and have a sporocyst residuum of compact ball-like granules at one end or a few scattered granules (Dubey and Frenkel, 1972). These oocysts resemble those described from a cat (Shah, 1970) and from dogs (Levine and Ivens, 1965) as I. bigemina, small race. Since Frenkel, et al. (1970) failed to induce Toxoplasma oocyst production in the dog, it is probable that I. bigemina, small race, in the dog is not Toxoplasma. However, oocysts from the cat cannot be morphologically differentiated from Toxoplasma or may be Toxoplasma. (Oocysts similar to those described by Lee [1934] have not been reported from a spontaneous infection of a cat.)

The question of whether the oocysts in cat feces are all Toxoplasma or a species with an oocyst identical to Toxoplasma awaits further investigation. If Toxoplasma oocyst formation can be induced in the dog, then the perplexing problems seen presently in the cat will be extended.

SELECTED BIBLIOGRAPHY

de Andrade, C. M. and G. Weiland.

1971 "Serological Studies on the Antigenic Relationship between Toxoplasma, Sarcosporidia and Coccidia." Berl. Münch Tieranztl Wschr, Vol. 84, 61-64.

Awad, F. I.

1954 "The Diagnosis of Toxoplasmosis: Lack of Specificity of Sabin-Feldman Dye Test." Lancet, Vol. 267, 1055-1056.

Becker, Elery R.

1956 "Catalog of Eimeriidae in Genera Occurring in Vertebrates and Not Requiring Intermediate Hosts." Iowa State College Journal of Science, Vol. 31, No. 1 (August 15), 85-139.

Benbrook, E. A. and M. W. Sloss.

1948 Veterinary Clinical Parasitology. Ames: Iowa State University Press.

Biocca, E.

1949 "Osservazioni Sulla Poizione Sistematic del Toxoplasma." Rivista di Parassit., Vol. 10, No. 2, 73-92.

Cathie, I. A. B. and G. W. Cecil.

1957 "Sarcospores and Toxoplasm Serology: An Investigation of their Alleged Cross-reaction." Lancet, Vol. 272, 816-818.

Cross, J. B.

1947 "A Cytologic Study of Toxoplasma with Special Reference to its Effect on the Host's Cell." J. Inf. Diseases, Vol. 80, 278-296.

Davies, S. F. M., L. P. Joyner, and S. B. Kendall.

1963 Coccidiosis. Edinburgh, Great Britain: Oliver and Boyd, Ltd.

Davis, N. C. and W. W. Reich.

1924 "Notes on Coccidial Oocysts from Domestic Animals in California." J. Parasitol., Vol. 10, No. 3, 137-146.

Dubey, J. P.

1968a "Toxoplasma Infection in English Cats." Vet. Record, Vol. 82, No. 13, 377-379.

1968b "Isolation of Toxoplasma gondii from the Feces of a Helminth Free Cat." J. Protozool., Vol. 15, No. 4, 773-775.

Dubey, J. P., N. L. Miller and J. K. Frenkel.

1970 "The Toxoplasma gondii Oocyst from Cat Feces." J. Exp. Med., Vol. 132, 636-662.

Dubey, J. P. and J. K. Frenkel.

1972 "Cyst-induced Toxoplasmosis in Cats." J. Protozool., Vol. 19, No. 1, 155-177.

Feldman, Harry A.

1968 "Toxoplasmosis." The New England Jour. of Med., Vol. 279, Nos. 25 and 26, 1370-1375 and 1431-1437.

Frenkel, J. K.

1970 "Pursuing Toxoplasma." Jour. Inf. Dis., Vol. 122, No. 6, 553-559.

Frenkel, J. K. and J. P. Dubey.

1972 "Rodents as Vector Hosts for Feline Coccidia, Isospora felis and I. rivolta." J. Inf. Dis., Vol. 125, 69-72.

Frenkel, J. K., J. P. Dubey and N. L. Miller.

1969 "Toxoplasma gondii: Fecal Forms Separated from Eggs of the Nematode Toxocara cati." Science, Vol. 164, 432-433.

1970 "Toxoplasma gondii in Cats: Fecal Stages Identified as Coccidian Oocysts." Science, Vol. 167, 893-896.

Garnham, P. C. C., J. R. Baker and R. G. Bird.

1962 "Fine Structure of Cystic Form of Toxoplasma gondii." British Med. J., Vol. 5271, 83-84.

Gassner, F. X.

1940 "Studies in Canine Coccidiosis." J. A. V. M. A., Vol. 96, No. 755, 225-229.

Gavin, M. A., T. Wanko and L. Jacobs.

1962 "Electron Microscope Studies of Reproductive and Interkinetic Toxoplasma." J. Protozool., Vol. 9, No. 2, 222-234.

Goldman, M., R. K. Carver and A. J. Sulzer.

1958 "Reproduction of Toxoplasma gondii by Internal Budding." J. Parasitol., Vol. 44, 161-171.

Gustafson, P. V., H. D. Agar and D. I. Cramer.

1954 "An Electron Microscope Study of Toxoplasma." Am. J. Trop. Med. Hyg., Vol. 3, 1008-1021.

Hall, M. C.

1917 "Parasites of the Dog in Michigan." J. A. V. M. A., Vol. 51, No. 3, 383-396.

Hall, M. C. and M. Wigdor.

1918 "Canine Coccidiosis with a Note Regarding Other Protozoan Parasites from the Dog." J. A. V. M. A., Vol. 53, No. 1, 64-76.

Horton-Smith, C.

1958 "Coccidiosis in Domestic Mammals." Vet. Res., Vol. 70, 256-262.

Hutchison, W. M.

1965 "Experimental Transmission of Toxoplasma gondii." Nature, Vol. 206, 961-962.

1967 "The Nematode Transmission of Toxoplasma gondii." Transactions of the Royal Soc. of Trop. Med. and Hyg., Vol. 61, No. 1, 80-89.

Hutchison, W. M., J. F. Dunachie, J. Chr. Siim and K. Work.

1970 "Coccidian-like Nature of Toxoplasma gondii." British Med. J., Vol. 1, 142-144.

Hutchison, W. M., J. F. Dunachie and K. Work.

1968 "The Fecal Transmission of Toxoplasma gondii." Acta Path. Microbiol. Scand., Vol. 74, 462-464.

Hutchison, W. M., J. F. Dunachie, K. Work and J. Chr. Siim.

1971 "The Life Cycle of the Coccidian Parasite, Toxoplasma gondii, in the Domestic Cat." Trans. of the Royal Soc. Trop. Med. and Hyg., Vol. 65, No. 3, 380-399.

Jacob, E.

1949 "Parasitologische Notizen." Berl. Münch. tierärztl. Wschr., No. 5, 56-57.

Jacobs, Leon.

1956 "Propogation, Morphology and Biology of Toxoplasma." Annals of the New York Academy of Sciences, Vol. 64, 154-179.

Jacobs, L., J. S. Remington and M. L. Melton.

1960a "A Survey of Meat Samples from Swine, Cattle and Sheep for the Presence of Encysted Toxoplasma." J. Parasitol., Vol. 46, 23-28.

1960b "The Resistance of the Encysted Form of Toxoplasma gondii." J. Parasitol., Vol. 46, 11-21.

Jacobs, Leon and M. L. Melton.

1966 "Toxoplasmosis in Chickens." J. Parasitol., Vol. 52, No. 6, 1158-1162.

Kean, B. H., A. C. Kimball and W. N. Christenson.

1969 "An Epidemic of Acute Toxoplasmosis." J. A. M. A., Vol. 208, No. 6, 1002-1004.

Kulasini, C.

1960 "The Specificity of Sabin-Feldman Dye Test with Reference to Protozoal Infections." J. Clin. Path., Vol. 13, 339-348.

Lee, C. D.

1934 "The Pathology of Coccidiosis in the Dog." J. A. V. M. A., Vol. 85, No. 6, 760-781.

Levine, Norman D.

1961 Protozoan Parasites of Domestic Animals and Man. Minneapolis: Burgess Publishing Co.

Levine, N. D. and V. Ivens.

1965 "Isospora Species in the Dog." J. Parasitol., Vol. 51, No. 5, 859-864.

Lühe, Max.

1906 "Anhang: Coccidien," in the chapter "Die im Blute schmarotzenden Protozoen und ihre nächsten Verwandten" in Handbuch der Tropenkrankheiten, edited by Dr. Carl Mense, Verlog Von Johann Ambrosius Barth, Leipzig, Vol. 3, 264.

Morgan, B. B. and P. A. Hawkins.

1952 Veterinary Protozoology. Minneapolis: Burgess Publishing Co.

Mühlpfordt, H.

1951 "Das Verhalten Sarcosporidien-infizierter Tiere im Sero-Farbtest auf Toxoplasmose nach Sabin-Feldman." Z. Tropenmed u Parasit., Vol. 3, 205-215.

Nicolle, C. and L. Manceaux.

1909 "Sur Un Protozoaire Nouveau du Gondi (Toxoplasma N. Gen.)" Archives De L'Institute Pasteur de Tunis, Vol. 2, 97-103.

Noble, E. R. and G. A. Noble.

1964 The Biology of Animal Parasites, 2nd ed. Philadelphia: Lea and Febiger.

Olafson, P. and W. S. Monlux.

1942 "Toxoplasma Infection in Animals." Cornell Vet., Vol. 32, 176-190.

Overdulve, J. P.

1970 "The Probable Identity of Toxoplasma and Isospora and the Role of the Cat in Transmission of Toxoplasmosis." Tijdschr. Diergeneesk, Vol. 95, 149-155.

Paige, B. H., D. Cowen and A. Wolf.

1942 "Toxoplasmic Encephalomyelitis V: Further Observations of Infantile Toxoplasmosis; Intrauterine Inception of the Disease; Visceral Manifestations." Am. J. Dis. Child., Vol. 63, 474-514.

Pellerdy, L. P.

1965 Coccidia and Coccidiosis. Budapest: Akademiai Kiadi.

Rommel, Von M., A. O. Heydorn and F. Gruber.

1972 "Beiträge zum Lebenszyklus der Sarkosporidien; I. Die Sporozyste von S. tenella in den Fäzes der Katz." Tierärztliche Wochenschrift, Vol. 6, 101-105.

Sabin, A. B.

1939 "Biological and Immunological Identity of Toxoplasma of Animal and Human Origin." Proceedings of the Society of Experimental Biology and Medicine, Vol. 41, 75-80.

1942 "Toxoplasmosis: A Recently Recognized Disease of Human Beings," Advances in Pediatrics (New York, 1942), 1-56.

Sabin, A. B. and P. K. Olitsky.

1937 "Toxoplasma and Obligate Intracellular Parasitism." Science, Vol. 85, No. 2205, 336-338.

Sabin, A. B. and H. A. Feldman.

1948 "Dyes as Microchemical Indicators of a New Immunity Phenomenon Affecting a Protozoan Parasite (Toxoplasma)." Science, Vol. 108, 660-663.

Schmidtke, L.

1954 "Zur oralen Übertragbarkeit der Toxoplasmose." Zbl. Bakt. I; Vol. 160, 470-478.

1956 "Zur Ubertragung der Toxoplasmose durch Verfütterung von parasitenhaltigem Gewebe." Z. Tropenmed. u. Parasit., Vol. 7, 80-86.

Schmidt-Hoensdorf, F. and J. Holz.

1953 "Verschue zur Klärung der serologischen Verwandtschaft zwischen Sarcoplasmen und Toxoplasmen." Berl. Münch. tierärztl. Wschr., Vol. 66, 12-13.

Shah, H. L.

1970 "Isospora Species of the Cat and Attempted Transmission of I. felis Wenyon 1923 from the Cat to the Dog." J. Protozool., Vol. 17, No. 4, 603-609.

Sheffield, H. G. and M. L. Melton.

1968 "The Fine Structure and Reproduction of Toxoplasma gondii." J. Parasitol., Vol. 54, No. 2, 209-226.

1969 "Toxoplasma gondii: Transmission through Feces in Absence of Toxocara cati Eggs." Science, Vol. 164, 431-432.

1970 "Toxoplasma gondii: The Oocyst, Sporozoite and Infection of Cultured Cells." Science, Vol. 167, 892-893.

Siim, J. Chr., U. Biering-Sørensen, and T. Møller.

1963 "Toxoplasmosis in Domestic Animals" in Advances in Veterinary Science, 8, Academic Press, New York. 335-429.

Siim, J. Chr., W. M. Hutchison and K. Work.

1969 "Transmission of Toxoplasma gondii: Further Studies on the Morphology of the Cystic Form in Cat Feces." Acta Path. Microbiol. Scand., Vol. 77, 756-757.

Sloss, M. W.

1970 Veterinary Clinical Parasitology, 4th ed. Ames: Iowa State University Press.

Weinman, D.

1944 "Human Toxoplasma." Puerto Rico J. Pub. Health and Trop. Med., Vol. 20, 125-161.

Wenyon, C. M.

1923-24 "Coccidiosis of Cats and Dogs and the Status of the Isospora of Man." Annals of Trop. Med. and Parasitol., Vol. 17, 231-278.

1926a "Coccidia of the genus Isospora in Cats, Dogs and Man." J. Parasitol., Vol. 18, 253-266.

1926b Protozoology, A Manual for Medical Men, Veterinarians and Zoologists, Vol. II (facsimile 1926 edition). New York: Hefner Publishing Co., 1965.

Wenyon, C. M.

1939 "Various Preparations Illustrating the Method of Reproduction of Species of Toxoplasma." Trans. Roy. Soc. Trop. Med. and Hyg., Vol. 33, 10.

Wenyon, C. M. and L. Sheather.

1925 "Exhibition of Specimens Illustrating Isospora Infections of Dogs." Trans. Roy. Soc. Trop. Med. and Hyg., Vol. 19, 10.

Werner, H. and K. Janitschke.

1970 "The Developmental Phases, Developmental Cycles and Systematic Position of Toxoplasma gondii." Zentbl. Bakt. Parasit. K. di I., Vol. 214, 540-550.

Woke, P. A., L. Jacobs, F. E. Jones and M. L. Melton.

1953 "Experimental Results on Possible Arthropod Transmission of Toxoplasmosis." J. Parasitol., Vol. 39, 523-532.

Wolf, A., D. Cowen and B. H. Paige.

1939 "Human Toxoplasmosis: Occurrence in Infants as an Encephalomyelitis; Verification by Transmission to Animals." Science, Vol. 89, No. 2306, 226-227.

1940 "Toxoplasmic Encephalomyelitis IV: Experimental Transmission of the Infection to Animals from a Human Infant." J. Exp. Med., Vol. 71, 187-214.

Wolfson, F.

1940 "Organisms Described as Avian Toxoplasma." Am. J. Hygiene, Sec. C., Vol. 32, 88-99.

Work, K. and W. M. Hutchison.

1969a "A New Cystic Form of Toxoplasma gondii." Acta Path. Microbiol. Scand., Vol. 75, 191-192.

1969b "The New Cyst of Toxoplasma gondii." Acta Path. Microbiol. Scand., Vol. 77, 414-424.

VITA

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Master of Science

Report: TOXOPLASMA GONDII, AN HISTORICAL ACCOUNT OF ITS CLASSIFICATION

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Title of Study: TOXOPLASMA GONDII, AN HISTORICAL ACCOUNT OF ITS CLASSIFICATION

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Major Field: Veterinary Parasitology and Public Health

Scope and Method of Study: In light of recent revelations concerning the classification of Toxoplasma gondii, a retrospective study of its classification from its discovery in 1908 until 1972 was made. Taxonomic criteria reviewed included morphology, serology, and experimental transmission. The present classification concedes it is an intestinal coccidian of the cat with oocysts indistinguishable from those of Isospora bigemina, small race, of the dog and cat. Therefore, an historical accounting of Isospora bigemina from 1874 to 1972 was also undertaken.

Findings and Conclusions: The present classification of Toxoplasma as an intestinal coccidian of the cat evolved during the short period from 1965 to 1969 using the cat in transmission trials. From its recognition in 1908 until 1968, morphologists had established it to be a protozoan (sporozoan) with affinities for other recognized coccidia, namely Plasmodium and Eimeria. Serologic criteria established that Toxoplasma of man and other animals was indistinguishable and unique among protozoa. The study of Toxoplasma has revealed that intestinal coccidia may have an unconventional host range in a variety of intermediary homeotherms. Oocyst formation can no longer be used as the criterion for establishing the host-range of a given coccidian.

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