

# TOXOPLASMOSIS IN SOUTH AFRICA

## I. THE PRESENT CONCEPT OF TOXOPLASMOSIS

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Toxoplasmosis has become established as an important parasitic disease of man and other warm-blooded vertebrates. Of world-wide distribution, it produces effects ranging from asymptomatic invasion to fatal infection. In contrast to surveys of incidence and case reports emanating from other countries, only a few isolated cases have been described in South Africa.<sup>1-4</sup> This may be associated with lack of clinical recognition, inadequate facilities for laboratory confirmation of infection, or an actual low rate of incidence. The purpose of the author is to try to aid the elucidation of these features by a brief survey of the present concept of the disease and the eventual establishment of adequate diagnostic facilities in South Africa and, in due course, to utilize these laboratory methods in a serological survey to gain an indication of the rate of incidence in this country.

### I. THE PRESENT CONCEPT OF TOXOPLASMOSIS

Though reviews of the literature have accompanied cases reported in South Africa, an attempt is made in this article to acquaint the practitioner with the developments of more recent years in an outline of currently accepted views.

#### DISTRIBUTION

*Geographic.* Broadly, human infection appears related to geographic and climatic factors. Available information indicates a rather greater frequency in warm humid climates than in warm dry climates, whilst Icelanders show a low incidence and Alaskans are almost free of antibodies. Unfortunately, no large-scale population survey has been undertaken in tropical Africa; it would do much to substantiate or refute this opinion. Human cases have been reported from nearly all the countries of Europe, namely, England, Norway, Sweden, Finland, Holland, Belgium, France, Germany, Austria, Poland, Yugoslavia, Czechoslovakia, Hungary, Switzerland, Portugal and Italy. In Africa few cases are on record but reports have come from the Congo, Nigeria and the Union of South Africa. In the Americas cases have been described from the USA and the countries of South America; in the East from India, New Zealand, Tasmania, Australia, the Philippines, and recently 5 cases from western Japan. The distribution amongst lower animals presents an equally wide geographical dispersion.

*Species.* The species distribution of the infection is unusually wide, for the causative parasite is without host specificity among warm-blooded vertebrates. Naturally occurring toxoplasmic infection has been proven in man, dogs, cats, wild rats, the gondi (a North African rodent), rabbits, hares, mice, rats, bandicoots, guinea-pigs, cattle, swine, sheep, silver foxes, squirrels, chinchillas, voles, kangaroos, wombats and wallabies. Amongst primates

infection has been described in the baboon and chimpanzee. Many species of bird have been shown to harbour the parasite including pigeons, ducks, fowls, grouse and sparrows.

#### THE PARASITE

*Toxoplasma gondii* was first described in 1909, by Nicolle and Manceaux,<sup>5</sup> who observed the intracellular forms of the parasite in smears taken from the spleen and other organs of a North African rodent, *Ctenodactylus gondii*.

#### Classification

Satisfactory classification of *Toxoplasma gondii* has remained problematical. Originally, Nicolle and Manceaux<sup>5</sup> considered the parasite to resemble leishmania but recognized the absence of a kinetoplast. The characteristic lack of host specificity tends to set this organism widely apart from other protozoa, especially the sporozoa, though van Thiel<sup>6</sup> has suggested a striking similarity to the gregarines.

Thus, at present, *Toxoplasma gondii* may be considered a protozoan of doubtful systemic position, grouped with *Pneumocystis carinii* and sarcosystis.

#### Morphology

The classic morphology is best observed in the extracellular forms found in peritoneal exudate obtained from mice which have been experimentally infected by previous intraperitoneal inoculation with toxoplasma. Such exudate contains an average 300-400 million organisms per c.c. They fill extravasated leucocytes lying in the exudate, and great numbers of parasites lie free in the fluid, floating singly, clustered together, or recently liberated and packed around ruptured cells.

The organism is large, 3-7  $\mu$  long and 2-4  $\mu$  broad, crescentic in shape, and resembling a diminutive banana in outline, the one extremity being more pointed than the other, and the nucleus lying towards the blunt pole. A kinetoplast is not demonstrable. Staining with Wright's or Giemsa's stain reveals a light-blue cytoplasm and a red nucleus. Electron microscope studies of toxoplasma in thin sections of tissue culture by Meyer and Mendonca<sup>7</sup> indicate the absence of organs of locomotion. A thin periplast appears to surround the organism and becomes thickened at the two poles. At one pole the thickening resembles a ring to which long darkly staining internal structures are attached. At the opposite pole a distinct opening is apparent. Similar studies<sup>8</sup> of whole mounts and sections of toxoplasma indicate a large conoid at the anterior extremity measuring 1.3  $\mu$ , which can be invaginated into a collar and probably utilized to bore into the host cell. It is described as being accompanied by 14 fine fibrils extending fan-wise posteriorly. Phase-contrast microscopy of peritoneal exudate of infected mice<sup>9</sup> shows a similar pointed spicula at the anterior extremity used to penetrate host cells.

In tissue sections, fixation renders the toxoplasma shrunken and smaller. When acted upon by antibody they tend to become ovoid and less clearly defined. When a wet preparation is allowed to stand, the parasites enlarge and become more bulky, the cytoplasm often showing a vacuole and appearing granular.

The micro-organisms most likely to be confused with toxo-

plasma on a morphological basis are encephalitozoon, sarcocystis, leishmania, histoplasma and *Trypanosoma cruzi*.<sup>9</sup>

### Physiology

Like viruses and rickettsiae, the organism is essentially an obligate intracellular parasite. It has thus never been cultivated in artificial media in the absence of living cells. The cellular situation of toxoplasms is always intracytoplasmic and no observation has been made of nuclear invasion. The lack of host specificity is further manifest at cellular level; the parasite will invade and multiply within any cell of the body except the red cell of the blood.

Reproduction was considered to be by longitudinal binary fission occurring repeatedly within the invaded cell. Weinman<sup>9</sup> describes a division rate of once every 6 hours during the first 24 hours. Recent investigation by Goldman *et al.*<sup>11</sup> clearly indicates that reproduction is in fact unusual in mode and by a process of internal budding they aptly term endodyogeny, in which the parent cell develops 2 daughter cells within it and finally disintegrates to liberate 2 toxoplasmic offspring. The invaded cell becomes packed with parasites, and paired parasites are frequently observed both within cells and without. Schizogony does not occur. Eventual rupture of the host cell liberates the contained parasites, which enter new cells, either phagocytic or non-phagocytic, to repeat their development.

Routine performance of frequent dye tests, from December 1956 to the present time, has necessitated continued passage of the parasite at intervals of a few days in mice and in simian kidney tissue cultures, to maintain the supply of viable parasites required for the tests. Associated with the passing there is repeated microscopic observation of live parasites in infected mouse exudates and in tissue cultures. During this period I have not once observed the penetration and entry of a toxoplasm into a cell, nor have I seen reports of the actual moment of cell penetration.

Motility is exhibited by free extracellular organisms. It is best observed by the microscopic examination of wet cover-slip preparations on a warmed slide of infected mouse peritoneal exudate harvested and maintained at 37°C. Of a number of parasites clustered, apparently motionless, either clumped together or around a newly ruptured parasitized host cell, one parasite may drift away from time to time, pointed extremity foremost, with a slow momentum gradually falling away, to a distance of some 5 times its own length. After a momentary pause it tends to slowly return to its parent cluster. This action may be repeated several times. Eventually the parasite fails to return and slowly moves away as a lone voyager in the fluid. These free parasites show a definite rocking movement, which is barely perceptible, and a slow gliding movement easily confused with drift associated with currents beneath the cover-slip. Rolling of the crescentic parasite about its long axis, which may or may not be produced by the parasite itself, often suggests a rotary questing movement of the pointed extremity.

Preliminary observations on the metabolism of *Toxoplasma gondii* by Fulton and Spooner<sup>10</sup> have indicated the rate of consumption of oxygen and production of carbon dioxide. They have further studied the rate of utilization of glucose, the major substrate for respiration, and shown that respiration is inhibited by cyanide. Summers<sup>11</sup> has indicated that vitamin B6 is necessary for the growth of the organism.

In contrast to the proliferative form of *Toxoplasma gondii* thus far described, a pseudocystic phase of the parasite occurs in subclinical and chronic infections. In infections reaching these stages the toxoplasms may multiply within a number of cells which, however, fail to liberate the parasites by rupture, the nuclei becoming flattened and granular, and the cytoplasmic residuum reduced to a fine peripheral remnant forming a 'cyst wall'. The parasites themselves apparently have no part in the formation of this membrane. The pseudocyst may contain great numbers of parasites, which are maintained and protected in this situation from antibody and drug action. The experimental crushing of a pseudocyst will liberate the contained toxoplasma cells, each viable and capable of initiating a chain of cellular invasion in any suitable animal host, even after prolonged periods of dormancy within the pseudocyst. Pseudocysts may be observed in sections of any organ, but are particularly frequent in the brain. This phase of the parasite is undoubtedly of great epidemiological significance.

The proliferative form appears to have no unusual resistance to the external environment outside its host, to physical agents such as low heat or changes in osmotic pressure, or to the common antiseptics. Simitch *et al.*<sup>12</sup> report that toxoplasma survives no more than 10 minutes in water, but in saline survival may be prolonged to 24 hours, and that they will survive in smears made on glass slides and exposed to desiccation for 3 hours at room temperature and 1 hour at 37°C. Jacobs, Jones and Melton<sup>13</sup> consider that handling of the parasite during the process of animal passage should be limited to 1 hour because, even in such a short time, deleterious effects on the toxoplasms may result from various suspending media used, with considerable loss of viability. They indicate that under the best circumstances survival in saline is limited to 2-3 hours, but that serum-saline is a superior suspending medium, the parasites being viable after 4-5 hours. My experience with the parasite is the same.<sup>14</sup> Lyophilization is unsuccessful. Prolonged storage at -70°C is impossible unless the temperature is slowly brought down to this level and the organisms suspended in glycerol. In tissues of animals dead of the infection, survival of the proliferative stage of the parasite must be of relatively limited duration. Simitch *et al.*<sup>12</sup> report that toxoplasms remained alive for over 2 days at room temperature and over 4 days at 4°C in carcasses of *Souslik* (a squirrel species) dead of toxoplasmosis.

The pseudocyst form is more resistant and in infected tissue is undoubtedly mainly responsible for the recovery of viable parasites from such material after quite considerable periods have elapsed. This is especially so in chronic infections where the toxoplasms are present in the pseudocystic phase. Weinman and Chandler<sup>14</sup> accept that toxoplasma can survive for at least 10 days in pork refrigerated at temperatures of 2-5°C.

These are indications of the properties of resistance of the proliferative and pseudocystic forms. It is possible that they happen to be the only phase of a life cycle with which we are cognisant. Forms more resistant to the external environment may exist and should they exist, and be discovered, the unknown mode of transmission of this challenging parasite may be unveiled.

Weinman<sup>9</sup> has described a potent factor associated with the organism and termed it toxotoxin. It is produced *in vivo* and occurs in the ascitic fluid of intraperitoneally infected animals. Campbell, Mackay and Vantsis,<sup>15</sup> working on canine toxoplasmosis and the isolation of toxoplasma from a dog, confirmed the presence of this very toxic substance in the peritoneal fluids of infected mice and of an infected dog. However, other workers have failed to find evidence of any such substance and Cook and Jacobs<sup>12</sup> were unable to demonstrate the production of this factor in toxoplasma-infected tissue cultures.

### Variation

The RH strain of *Toxoplasma gondii* was isolated by Sabin from the brain of a fatal case of encephalitis occurring in a 6-year-old boy in 1939. It bears the initials of this luckless child and was considered to have originated from a cat in the household which died from an unknown cause before the child became ill. This strain is maintained and traditionally used by all laboratories performing the dye test. To retain comparability of dye-test results from different countries it has been necessary to use an identical strain of parasite—the RH strain, a regrettable necessity since it is of high virulence.

The CH strain was isolated by Dr. H. R. Siebold of the US Department of Agriculture from chinchillas dying of a toxoplasmic epidemic on a farm in New Jersey.

Strain 113 originated in the laboratory of Dr. Leon Jacobs in the USA. It is of particular interest as a strain of decreased virulence resulting from passage of the RH strain through the larval and nymphal stages of the tick *Rhipicephalus sanguineus*.

Since these earlier isolations toxoplasms have been isolated from a wide variety of mammals from many parts of the world. The major difference between all these strains is a variation in virulence, depending on the host of origin. The essential feature is that all known strains of toxoplasm

are morphologically identical and, with practically no doubt at all, of a single species—*Toxoplasma gondii*.

#### Culture and Maintenance

This sensitive organism, requiring live-cell maintenance, may be cultured in the laboratory animal, in tissue culture, and in the developing chick embryo. It may further be maintained by prolonged storage at  $-70^{\circ}\text{C}$  when suspended in glycerol, or in chronically infected rats.

**Laboratory Animals.** White mice are most satisfactory for this purpose. They are very susceptible to infection with toxoplasmas and any route of inoculation is successful—intracerebral, intraperitoneal or subcutaneous injection, intranasal instillation, deposition on intact mucous membranes and feeding on infected tissue containing pseudocyst forms. In practice, intraperitoneal inoculation is easy and is the method generally used for maintenance of strains. The plentiful peritoneal exudate formed gives a rich harvest of toxoplasma, the fluid being used for the regular 4-5 day passage of the strain from one batch of mice to another, and for the performance of the dye test. With the RH strain in laboratory use, the fluid is mainly devoid of parasites for the first day after inoculation; a few become manifest 24 hours later, rapidly increasing to very great numbers up to 48 hours, and thereafter declining in number. The strain is uniformly fatal to mice some 5-8 days after inoculation.

**Tissue Culture.**<sup>16-18, 42</sup> The growth of *Toxoplasma gondii* is well and rapidly adapted to mammalian cell cultures, there being progressive invasion of cells, which are disrupted to release free organisms into the fluid nutrient medium. After long periods of tissue culture the parasite retains its virulence as shown by mouse inoculation. Tissue cultures inoculated with toxoplasma and incubated at  $37^{\circ}\text{C}$  show the highest number of parasites 5-7 days later. The method provides a satisfactory source of antigen for the complement-fixation test, and infected simian kidney tissue cultures are used for this purpose in our laboratories. We have maintained the parasite by routine mouse passage and simultaneously in simian kidney tissue culture to ensure that any mishap in one or other method of culture will be less likely to result in loss of the strain. In the author's experience tissue culture has not proved to be a satisfactory source of toxoplasma for the dye test because, prolific though development may be, it does not produce enough organisms and too many non-viable forms are present.

**Developing Chick Embryo.**<sup>19</sup> The introduction of toxoplasma into the chorio-allantoic, amniotic or yolk sacs of the 6-12-day-old developing chick embryo will almost invariably result in embryonic death 8 days later. Large yellow nodules are found on the allantoic membrane and scattered lesions in the haemorrhagic embryo. Toxoplasmas are numerous in these sites. Antigen for the complement-fixation test was originally, and is most usually, prepared from infected embryonated eggs.

**Prolonged Storage at  $-70^{\circ}\text{C}$ .**<sup>20-21</sup> This procedure has been a notable advance toward obviating the time-consuming and rather dangerous necessity of repeated animal inoculation in order to maintain a given strain. A suspension of the parasite, containing 5-10% glycerol, is refrigerated and then frozen in a bath of dry ice in alcohol at  $-15^{\circ}\text{C}$ , and the temperature is then slowly dropped to  $-70^{\circ}\text{C}$ . The parasites are maintained at this temperature in the deep freeze. The salient factors of success in this method are the slow dropping of the temperature and the use of glycerol. Parasites may be stored very successfully for 6 months under these conditions, when an inoculation made from the thawed suspension into laboratory mice will produce virulent infection. Chandler and Weinman<sup>20</sup> reported the successful storage of toxoplasma for 184 days by this method. Eyles, Coleman and Cavanaugh<sup>21</sup> emphasize that, at present, the best methods preserve only a small percentage of the toxoplasma exposed to freezing, but have demonstrated survival up to 209 days. In one of our experiments a suspension which had been stored by this method for 270 days was thawed. Microscopic examination revealed morphologically intact toxoplasma but intraperitoneal inoculation of mice with the material failed to produce infection. These mice were challenged 6 weeks later with a small inoculation of virulent organisms, in the hope that the apparently non-virulent toxoplasma would have stimulated the production of protective antibody. All the mice rapidly succumbed. Ordinary freezing or the use of glycerine instead of glycerol is unsuccessful. Roger and Roger<sup>22</sup> have tried slow freezing in a saccharosed isotonic

solution, followed by rapid thawing by immersion of the containing ampoule in boiling water. They showed that infectivity for mice though decreased, was thus maintained for 1 month.

**Chronically Infected Rats.** Parasites may be maintained in pseudocysts in the brains of chronically infected rats. Rats are relatively resistant and are given non-lethal inocula of toxoplasma. When required, an emulsion of infected rat brain is used as an inoculum for experimental animals. Van Thiel<sup>23</sup> working with albino rats and 4 Netherlands strains of toxoplasma maintained 1 strain for a year in this manner and the other 3 strains for a month. The method has little advantage with the advent of prolonged storage at  $-70^{\circ}\text{C}$ .

#### HUMAN INFECTION

There is no significant difference in the apparent incidence of this infection between the human sexes.

Age groups have shown repeatedly a classic distribution of incidence in serological surveys, the number of positive reactions being low in the first decade of life and increasing with age. A characteristic example is the findings of Feldman<sup>24</sup> who, using the dye test and a positive reaction in a titre of 1:16 as significant, conducted a population survey in Pittsburg, Pennsylvania. His findings are summarized in the following table.

Age in years	Percentage Positives
0—4.. .. .	5
5—9.. .. .	10
10—29.. .. .	25—30
30—39.. .. .	45
40—60.. .. .	65—70

The incidence of human infection is exemplified by publications such as that of Feldman and Miller<sup>25</sup> on the serological study of toxoplasmosis prevalence, who, in addition to 24 animal populations, investigated 10 geographically different human populations. Positive dye-test percentages in human groups varied from 0% in Eskimos to 68% in Tahitians. The only published serological indication available to date of possible human prevalence in South Africa is the results obtained by Schneider, Goddard and Heinz<sup>26</sup> who used the toxoplasmin skin test in a survey of Africans in Johannesburg and demonstrated the usual increase of positive reactions with increasing age and a total incidence of 31%.

It is now very evident that there is no reason to doubt the specificity of serological diagnostic tests in current use, a matter to be discussed below. Accepting these findings it might be concluded that toxoplasmosis is one of the commonest infections of man. This conclusion has long been considered unjustifiable, but it may in fact be within the bounds of truth. It must, however, be understood that there may be much infection with little clinically manifest disease; toxoplasmosis would appear to be a long-established infection of warm-blooded vertebrates with a good host-parasite relationship.

Toxoplasmosis has been known as a disease of man since 1939 and is now being recognized with greatly increasing frequency. Human infection may conveniently be elaborated from the following classification: (1) Congenital, (2) acquired—(a) sub-clinical, (b) glandular, (c) ocular, (d) encephalitic, (e) exanthematous.

#### Congenital

Congenital infection was the first form of the disease to be described and hundreds of cases have been diagnosed in various parts of the world. Evidence shows that a foetus becomes infected only if the mother acquires her first toxo-

plasmic infection during the associated pregnancy. The infection in the mother is generally entirely subclinical. The author has not been able to find any report of a woman giving birth to more than one toxoplasmic infant. Wright<sup>27</sup> states that in the entire world literature there is no authenticated instance of a mother giving birth to a toxoplasmic child in any subsequent pregnancy, and that there is no record of a woman with antibody at the start of pregnancy producing an infected child. Millions of women with toxoplasmic antibody give birth to children without toxoplasmosis.<sup>28</sup> Feldman,<sup>29</sup> in a study of 103 congenital cases, reiterates that at present it appears that congenital infection is only a rare manifestation of a commonplace disease.

If the foetus becomes infected during the early stages of a pregnancy, the central nervous system and the eyes show the greatest evidence of parasitic activity, as antibody elaboration causes the parasite to regress from the viscera to the partial protection from antibody action afforded in these two situations. Infection during the latter part of pregnancy affords insufficient time for the elaboration of sufficient antibody antagonism to the parasite, with resultant symptoms indicative of extraneural visceral parasitic activity in addition to that in the nervous system. Thus clinical manifestation may be very varied and the original classical tetrad of symptoms described, namely, hydrocephaly or microcephaly, intracranial calcification, convulsions, and chorioretinitis, is demonstrated in a small proportion of cases only. This is supported by 150 cases studied by Eichenwald,<sup>30</sup> whose findings suggest that toxoplasmosis should be considered as a possible diagnosis of almost any obscure illness during the neonatal period.

Broadly, then, before parasitic regression to the brain, any or all of the following may be demonstrable: Fever, jaundice, a maculopapular rash, splenomegaly, hepatomegaly, pneumonitis, myocarditis, and hydrocephaly or less frequently microcephaly. When the infection has occurred earlier in pregnancy the clinical picture described tends to merge into the more classical symptomatology of convulsions, hydrocephaly or more rarely microcephaly, chorioretinopathy and radiological evidence of cerebral calcification. In survival to infancy psychomotor retardation is usually clearly present. Mild cases of foetal infection may occur.<sup>31</sup> Chronic signs of infection in apparently normal infants may show as reactivation of chorioretinopathy later in life due to activity of surviving ocular pseudocystic parasitic forms.

Eichenwald,<sup>30</sup> in his study of 150 congenital cases, followed most of them from birth to 5 years of age, and found a mortality of 12%, whilst 90% of survivors showed mental retardation, 75% developed convulsions, spasticity and palsies, and about half of them had severely impaired sight. The condition may closely simulate haemolytic disease of the newborn. Attention is drawn to this point by Bain *et al.*<sup>32</sup> in reporting 3 illustrative cases. Foetal death with abortion and stillbirths may be associated with the intra-uterine parasitic invasion and toxoplasma has been isolated from products of conception in such cases.

Cerebrospinal fluid and blood pictures are not specific and are rarely of assistance in diagnosis. In the cerebrospinal fluid xanthochromia may be present, protein possibly increased, sugar levels often decreased, a pleocytosis not infrequently present, and erythrocytes sometimes noted. In the blood eosinophilia may or may not be present.

It is apparent that the varied symptomatology makes a

clinical diagnosis inordinately difficult. Essentially, as in all forms of the disease, clinical suspicion requires laboratory confirmation for a final diagnostic evaluation.

#### Acquired

Congenital toxoplasmosis, being the first type described, has received the major share of recognition, but the significance of the acquired disease forms is increasingly apparent.

*Subclinical.* The high incidence of antibodies in the general population, coupled with general inability to elicit any clear-cut history of toxoplasmic illness from members of the general population presenting antibodies, or from mothers of toxoplasmic infants, suggests that subclinical infection is the most frequent form of invasion in man. However, it is not improbable that clinical features may be manifest which, thus far, are not thought to be associated with the aetiological agent.

*Glandular.*<sup>33-36</sup> The lymphoglandular form is a frequent manifestation of acquired infection. There is no special age or sex incidence. It may run a febrile or an afebrile course. If afebrile the patients often accidentally discover the enlarged glands themselves, though not suffering any other signs of illness at the time. It is likely that many unnoticed infections of the so-called subclinical type are, in fact, cases where lymphadenopathy is the only presenting sign but escapes detection. During the early stages of the infection there is an initial parasitaemia. In acute febrile cases the fever generally persists about 2-3 weeks. The associated lymphadenopathy frequently persists for 6 months and longer. The whole course of the illness is protracted, but usually benign, with complete recovery without complications. Severe fatigue is a very marked feature of most cases and lasts for many months during the convalescent phase. The enlarged lymph glands, after initial tenderness, are painless, firm and discrete, with no unusual attachment to surrounding structures. Involvement may be of one area only, such as the neck, or relatively generalized. The spleen is seldom palpable. A prominent finding in the blood in most cases is a relative lymphocytosis, with atypical large lymphocytes resembling those found in glandular fever. The Paul-Bunnell test is negative. Thus, any case presenting with the physical signs of glandular fever and supportive evidence in the blood findings, but with a negative Paul-Bunnell test, must arouse suspicion of toxoplasmosis. In such cases sero-reactions for toxoplasmosis should be tested. Positive reactions in rising or very high titre will confirm the suspected diagnosis and an attempt should be made to isolate the parasite from lymph-node biopsy.

*Ocular.* Attention was drawn to the existence of acquired toxoplasmic ophthalmitis by reports such as those of Wilder,<sup>37</sup> who studied a series of 53 enucleated human eyes presenting granulomatous uveitis. She identified toxoplasma morphologically in sections, and this was substantiated by a serological follow-up of her patients. The parasite was then successfully isolated from enucleated eyes; Jacobs *et al.*,<sup>38</sup> identified toxoplasma cultured from the macerated inner layers of an adult human eye enucleated for painful blindness due to chorioretinitis of 8½ years' duration, and Pillat and Thalhammer<sup>39</sup> from a case of iridocyclitis which was the sole manifestation of acquired toxoplasmosis. Jacobs *et al.*<sup>40</sup> found that 35% of 298 cases of granulomatous uveitis were apparently toxoplasmic in origin. Choroiditis has been

described in association with the glandular form of the disease. However, the majority of toxoplasmic infections pass unrecognized and are benign; small residual choroido-retinal lesions are noticed because symptoms will readily be produced in this situation. The eye condition may arise as a delayed manifestation of infection acquired long before.

*Encephalitic.*<sup>3, 27</sup> This form of the disease is described as acute with a sudden onset often following a period of some months of feeling vaguely unwell. Manifestations are pyrexia, stupor, papilloedema and unconsciousness. Concomitant signs of lymphadenopathy may be present. A fatal outcome is usual. The cerebrospinal fluid is described as under increased pressure with a pleocytosis of lymphocytes and polymorphonuclear leucocytes. Wright states that only 7 cases are described in the medical literature. Nevertheless, serological evidence has indicated that 3 cases of benign meningo-encephalitis observed in Johannesburg during the course of this study were due to toxoplasmic infection. A brief review of a preliminary study of the dye test and of these cases will be published shortly.

*Exanthematous.*<sup>33</sup> The infection closely resembles typhus fever clinically. It is acute, with sudden onset accompanied by chills, fever and a maculo-papular rash which does not involve the scalp, palms and soles. Pneumonic symptoms are frequent and myocarditis and meningo-encephalitis may be present. It usually ends fatally. This form of the disease also appears to be rare.

In general, clinical presentation may differ within wide limits depending on the anatomical site most involved. Pulmonary forms closely resembling pulmonary tuberculosis are reported. It may present as a space-occupying lesion of the brain. Accidental laboratory infections<sup>43</sup> have been known to confirm the clinical findings.

Toxoplasmic infection as an aetiological factor in conditions such as schizophrenia and epilepsy should always be considered.

*Pathology of Human Infection.*<sup>3, 44-46</sup> In the glandular form lymph-node biopsy shows characteristic features. The normal structure of the gland is present but there is cortical hyperplasia with obvious follicles which show pale cells with large darkly-staining nuclei. Abnormal nuclei are not evident but mitotic figures are frequent. Macrophages are plentiful and contain blackish granules, which are also found outside the cells. Intact toxoplasma are very rarely seen in these sections.

Pseudocysts packed with toxoplasma are often seen in sections of the brain and eye and may be found in subjects coming to autopsy for some unrelated cause. A striking feature is the absence of local reaction around these large structures.

The acquired type with cerebral involvement shows a necrotizing demyelinating encephalitis with gelatinous softening. Free toxoplasma or pseudocysts may be seen on section of the brain or other organs.

In congenital cases the brain shows internal hydrocephalus (rarely microcephaly) with associated aqueduct stenosis and arachnoiditis with cellular infiltration of plasma cells, mononuclears and lymphocytes. The free form of the parasite is often seen and pseudocysts are usually present. In other organs similar reactions may be featured.

*Diagnosis.* The variability of the symptoms makes certain

clinical diagnosis improbable, and laboratory confirmation is essential.

Laboratory methods of diagnosis are (1) isolation of the parasite, (2) the Sabin-Feldman dye test, (3) the complement-fixation test, and (4) the skin test.

### 1. Isolation of the Parasite

The most certain way of proving the infection is to demonstrate the presence of the parasite, but in the past this has been difficult to achieve. However, with improved understanding of the physiology of toxoplasma very much better results are being obtained. A successful method is by animal inoculation; white mice are most satisfactory and have the added advantage that laboratory strains do not appear to be subject to natural toxoplasmic infection. The essentials to possible success are as follows:

(a) Rapid transference of material from the patient to the experimental animal. The organism cannot survive long outside the host, and every moment lost is important. The material is ground and suspended in sterile serum saline and inoculated into mice treated with cortisone. Several subsequent passages are performed before the run is discarded as unsuccessful. If toxoplasma-like forms are isolated cross-immunological studies will finally prove the species should confusion arise from the presence of other similar organisms such as encephalitozoon encountered in the mice.

(b) The use of the proper body fluids or tissues from the patient:

- (i) Material from a lymph node gives a good possibility of successful isolation especially in acquired infections.
- (ii) The cerebrospinal fluid is most satisfactory in active congenital cases but tends to give very poor results in acquired infections.
- (iii) The blood may give evidence of parasitaemia on inoculation into mice, but probably only within the first week or two after infection. This procedure is therefore seldom of use to the clinician who, except in very rare instances such as laboratory infections caused, for example, by the prick of an infected needle, will only see the patient after symptoms have developed and parasitaemia regressed.
- (iv) Tissue obtained at autopsy, especially from the brain, heart, spleen, or lymph nodes, is valuable but again rapidity of transfer is the essence of success.

The demonstration of toxoplasma-like structures in stained films or in histological sections is most significant but the diagnosis is better confirmed by isolation of the parasite or serological investigation of the patient.

### 2. Sabin-Feldman Dye Test<sup>47-52</sup>

The reaction as described by Sabin and Feldman in 1948, though slightly modified by some workers, has firmly withstood the test of a decade and remains the corner stone of diagnostic procedure. The high incidence of antibodies in the general population, coupled with little active disease, indicated either an excellent host-parasite relationship or that the serological procedure was not reliably specific. It was reported that sarcosporidiosis, South American trypanosomiasis and trichomoniasis could all give falsely positive reactions with the dye test for toxoplasmosis. Sarcosporidiosis is excessively rare in man, and Chaga's disease, caused by *Trypanosoma cruzi*, occurs in a single geographical region. *Trichomonas vaginalis*, however, being so ubiquitous a parasite, would be of definite significance. However, more recent investigation by various workers has shown that,

apart from extremely rare cases of human sarcosporidiosis, the dye test is highly specific.

Basically, the test is conducted by heating the serum to be investigated for the presence of toxoplasma antibody to 56°C for 30 minutes to destroy any non-specific antitoxoplasmic factor, which may be present in some sera. Serial fourfold dilutions of the serum are then prepared in saline and to each is added a fixed proportion of toxoplasma suspension. This suspension consists of viable toxoplasma in a freshly harvested highly-infected mouse exudate with a small amount of heparin to avoid clotting to which is added the essential accessory factor, considered to be associated with the properdin system, and used in the form of a non-immune human serum, which must be entirely free of toxoplasmic antibody.

The various serum dilutions with the added toxoplasma suspension are now placed in a waterbath at 37°C for 1 hour. If antibody is present in the serum it will, during this time, and in the presence of accessory factor, have an effect on the cytoplasm of the toxoplasma. This cytoplasm-modifying effect is made more easily recognizable by the addition of alcoholic methylene blue precisely buffered to pH 11. Therefore, after removal from the water-bath, fixed volumes of freshly prepared dye are added to each of the dilutions, which are then stored in the refrigerator for a short while. If adequate antibody is present in a tube the cytoplasm of most of the extracellular parasites in that tube will be so modified that it will not stain with the dye. Intracellular parasites cannot be reached by antibody, and so are stained in all cases. If antibody is not present the cytoplasm of the toxoplasma takes up the dye in a normal fashion. Any dilution of the serum is said to be positive if more than 50% of extracellular parasites are unstained when examined under the high dry power of the microscope.

With each batch of tests, known positive and negative control sera are investigated concurrently and a control is prepared to check the presence of sufficient viable parasites in the toxoplasma suspension. The lowest titre considered to be of diagnostic significance is 1 : 16.

### 3. Complement-fixation Test

The specificity of the complement-fixation test for toxoplasmosis is without doubt. Antigen for the reaction is usually prepared from the harvest obtained from the chorio-allantoic membrane of the infected chick embryo, which gives a good yield without the difficulty of anti-complementary properties associated with antigen culled from infected mouse peritoneal exudate. In these laboratories antigen prepared from infected simian kidney-tissue cultures has been used extensively. Technically the test has the very great advantage that live toxoplasma are not used and that the antigen is stable and can be stored for long periods in the frozen or lyophilized state.

#### *Interpretation of Results of Dye and Complement-fixation Tests*

The clinical diagnosis of toxoplasmosis cannot be entertained without supportive serological findings in one or both of these tests. For purposes of diagnosis these two reactions must always be used in conjunction. The test of basic importance is the dye test, which must be used for all routine purposes. The complement-fixation test is auxiliary to the dye test and assists in detecting acute and more recently acquired infections. Complement-fixing antibody appears later and disappears earlier than dye test antibody; thus a negative complement-fixation test does not rule out toxoplasmosis, and the test cannot be used alone for the diagnosis of toxoplasmosis—a fact to be emphasized.

(a) *Acquired Infections.* In both clinically manifest and entirely subclinical infections, the dye test becomes positive 1-3 weeks after infection. The titre rapidly rises within a few weeks to 1 : 256 or even 1 : 16,384 and higher. Serial samples of blood must therefore be submitted to demonstrate a

markedly rising titre, which is of the greatest significance. Further, a negative result followed by the development of a positive reaction and a rising titre in subsequent specimens is diagnostic. Titres in active infections usually reach high levels. The presence of a large percentage of positive reactions in the general population must be borne in mind and a positive dye test, especially in low and static titre, in no way indicates that a patient's present symptoms can of necessity be attributed to toxoplasmosis; but they may result from past infection.

Complement-fixing antibody usually develops more slowly and the reaction becomes positive about the 28th day after infection. The complement-fixation test is therefore negative at the time when the infection is most active and then becomes positive in rising titre.

Thus in acute infections of recent onset dye-test antibody is strongly present and the complement-fixation test negative during the early stages of the infection. The complement-fixation test then becomes positive and, together with the dye test, remains so in increasing titre during the following weeks. Both may persist in relatively high titre for a year or more. Complement-fixing antibody practically disappears after 6 years whilst the dye-test antibody slowly falls to persist in low titre, probably for life.

In infection acquired many years previously the dye test will be positive and the complement-fixation test negative. The dye test remains positive in low titre and the complement-fixation test remains negative.

(b) *Congenital Infection.* Maternal toxoplasmic antibody will pass the placental barrier. If the mother was infected before the associated pregnancy the antibody developed will be passed to the foetus but, as the parasites are adequately controlled in the mother by her antibody development, the baby will not be infected. The infant will, however, have a positive dye test due to the presence of maternal antibody. Such a positive finding in the newborn does not indicate congenital infection, and in these cases the baby's dye-test titre will slowly fall and be negative by about the 4th month after birth. The mother's dye test will be positive in a titre depending on the time elapsed since her infection, which period can be more accurately indicated by concomitant results obtained with the complement-fixation test. Even though the mother should acquire her primary infection during her pregnancy the foetus will not necessarily be infected. If, as a result of such infection, congenital toxoplasmosis of the baby ensues, the mother will have dye and complement-fixation tests positive in high titre and the baby a very strongly positive dye test and a complement-fixation test perhaps at first negative and then becoming positive in rising titre. These findings would indicate recent and active infection of the infant following on maternal infection acquired during pregnancy.

### 4. Skin Test

The reaction is a dermal sensitivity test and a positive reaction of the delayed tuberculin type develops in persons with circulating specific antibody. The antigen injected is the same used for the complement-fixation test. The test has the following disadvantages:

(a) The degree of local reaction does not indicate the amount of serum antibody.

(b) Some persons with positive dye tests will give a negative

skin test, and therefore a negative reaction does not indicate a definite absence of infection.

(c) There is considerable delay between infection and the development of a positive skin test.

The skin test consequently has no place in the diagnosis of an individual case, and is preferably reserved as a crude tool for investigating the incidence of past infection in population surveys.

Animal and roller-tube protection tests have little place now in serological diagnosis, but work on a possible haemagglutination test for toxoplasmosis<sup>53</sup> shows much promise.

#### Treatment<sup>57</sup>

Many substances have been investigated as possible therapeutic agents and discarded as being of little or no value. However, the antimalarial compound pyrimethamine (daraprim) in combination with one of the sulpha drugs, preferably sulphadiazine has given very good results indeed, the pyrimethamine and sulphadiazine having a marked synergistic effect one on the other. In addition to clinical and experimental experience in man and animal, the degree of protection afforded *in vitro* in tissue cultures of the parasite has been clearly demonstrated by Cook and Jacobs,<sup>54</sup> who have also shown that cells in such cultures can store pyrimethamine.<sup>55</sup> From the nature of the physiology of the parasite it may be assumed that the proliferative forms will be most susceptible to drug action whilst the pseudocystic phase is protected in variable degree, not only from antibody action, but from drugs as well. This, in fact, is the case. The acute stages of the disease show much greater response than more chronic infections, though chronic eye manifestations show a considerable degree of improvement or, at least, a usual halting of further deterioration, when treated with pyrimethamine and sulphadiazine.

In a considerable proportion of acute cases treatment with this drug combination results in cure and in other acute cases the effect is variable, the degree of benefit depending on the promptitude with which treatment is begun after infection.

Little improvement can be expected in congenital cases of any severity, since trauma occasioned by the parasite has reached an advanced stage of irreversibility. In less severe involvement, if symptoms do not regress, at least the development of later symptoms may be controlled.

A pregnant woman who develops positive serological reactions during the course of pregnancy should be offered the opportunity of treatment for possible protection of her baby. Her medical attendant must weigh his decision whether to treat her or not, considering the toxicity of pyrimethamine and the available knowledge of toxoplasmosis. However, it is desirable that pregnant women should have toxoplasmic serological investigations performed at the commencement of each trimester of pregnancy.

A dosage schedule recommended by several workers for an adult patient consists of a loading dose of 6 to 8 g. of sulphadiazine and 75 mg. of pyrimethamine, followed by a maintenance dose of 4 to 6 g. of sulphadiazine and 25 mg. of pyrimethamine daily for 14 days. An important disadvantage of therapy is the toxicity of pyrimethamine which, in adequate and prolonged dosage, may have serious untoward effects such as thrombocytopenia, leucopenia, megaloblastic anaemia

or agranulocytosis, and close observation of haematological findings has to be maintained throughout treatment. The administration of 5 mg. of folic acid daily is reported to diminish this toxicity and possibly allow the continuation of treatment without the withdrawal of pyrimethamine.<sup>57</sup>

#### Epidemiology

The epidemiological aspect of toxoplasmosis has been investigated in many parts of the world but, apart from direct infection in laboratory accidents, the method of transmission of the parasite remains unknown. Toxoplasmas have been isolated from a wide variety of animals and birds, including nearly all the domestic species, and in very many cases from apparently healthy hosts with no signs of active disease. The disease occurs in both enzootic and epizootic forms, as for example in the hares of Denmark, but so far no epidemics have occurred in man. It would appear that man is one of many usual vertebrate hosts for *Toxoplasma gondii*.

Repeated endeavours to demonstrate arthropod transmission as a significant mode of spread are completely unconvincing. It is unusual for healthy animals closely confined over long periods with successive batches of infected animals to show any signs of infection. For a better understanding of the unsolved epidemiological problem it is significant that the parasites are widely distributed through the bodies of infected hosts, and have been found in the parotid glands, sputum, intestinal contents, kidneys and milk of infected mice, in the saliva of rabbits before death, in the urine and intestines of dogs, lining intestinal ulcers in cats, in the eye exudate of infected dead pigeons, alive in pork which had been frozen for 10 days, in the saliva of a human case, and in the macrophages from the bronchioles and alveoli of a man with acute toxoplasmic pneumonitis. A history of the habit of ingesting raw eggs is on occasion associated with the onset of the disease in man. These are indications that the modes of transmission might be numerous.

It is possible that intimate contact with animals acutely ill of the disease may allow transfer of the parasite in secretions and excreta. As the proliferative form is probably destroyed to a large extent by the acid barrier of the stomach, entry is probably effected through the conjunctiva, mucous membranes or skin, especially where there is a breach of continuity of the surface. Infection might also occur during the preparation of carcasses of infected hares or other animals for the table. Pseudocyst forms, being much more resistant, are more likely to pass successfully the acid barrier of the stomach, and experimentally, and very likely in nature, are more prone to give rise to infection by ingestion than the sensitive free proliferative form of the parasite.

The question is too vast to discuss at length but, in conclusion, a further indication of the nature of the problem is afforded by a case reported by Gibson and Eyles.<sup>57</sup> They investigated a newborn Negro infant dead of congenital toxoplasmosis in Memphis, Tennessee. Serological tests suggested that other members of the baby's family had recently been exposed to a common source of infection. Various stray animals were captured in the immediate environment and frequenting a garbage dump near the house of the family. The authors actually succeeded in isolating *Toxoplasma gondii* from 7 of 35 cats, 2 of 3 ducks, 3 of 7 chickens, 1 of 16 pigeons, 1 dog collected 4 blocks away, and 7 of 121 mice (5 of them amongst 20 caught in the family's house).

## SUMMARY

1. A broad outline is given of the distribution, classification, morphology, physiology, variation and culture of the parasite *Toxoplasma gondii*.

2. Human infection and its forms of presentation, diagnosis and treatment are discussed.

3. Attention is drawn to the incomplete understanding of the epidemiological features of toxoplasmosis.

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