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TOXOPLASMOSIS

Report of a WHO Meeting of Investigators

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WHO MEETING OF INVESTIGATORS ON TOXOPLASMOSIS

Geneva, 25-29 November 1968

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TOXOPLASMOSIS

Report of a WHO Meeting of Investigators

A meeting of investigators on toxoplasmosis was held in Geneva from 25 to 29 November 1968. In opening the meeting, Dr K. Raska pointed out that this infection is widespread in different parts of the world and that knowledge of some aspects of the problem is limited or lacking. The group reviewed recent progress in the taxonomy, physiology, and immunology of *Toxoplasma* and in the epidemiology, diagnosis, and therapy of toxoplasmosis. Clinical aspects were briefly discussed in so far as they are related to these fields, but were not dealt with in detail. Particular attention was paid to immunodiagnosis and epidemiology. The group also drew attention to areas in which further research is necessary.

1. TOXOPLASMA AND RELATED ORGANISMS

The class Toxoplasmoda contains three closely related families — Toxoplasmodae, Besnoitiidae, and Sarcocystidae.¹ Until the life cycles of the different parasites in these families are fully understood, it will be impossible either to classify them systematically or to devise truly scientific measures for the control of the infections that they cause.

These organisms possess so many features in common that it seems probable that the aspects of their development that are at present unknown will also prove to be similar. For this reason, a brief description of the group as a whole is likely to be useful.

Each family contains a single genus, although it is possible that other genera exist within the Sarcocystidae. The family Toxoplasmodae consists of only a single species. The class Toxoplasmoda is probably best placed at present in the subphylum Sporozoa, although it also has obscure affinities to the Coccidiomorpha.

1.1 Distribution in the animal kingdom

Of the three genera, *Toxoplasma* and *Sarcocystis* have the widest distribution. *Toxoplasma* is found in many mammals, including

¹ The characteristics of these families are listed in the Annex.

man and other primates, cattle, sheep, pigs, marsupials, armadillos, hedgehogs, dogs, cats, foxes, various mustelids, and many rodents. Owing to confusion of *Toxoplasma* with *Lankesterella* and exo-erythrocytic forms of malaria parasites, reports of *Toxoplasma* in birds are of doubtful authenticity, except for those dealing with isolations from pigeons, chickens, and a few other species. Parasites found in reptiles and reported as *Toxoplasma* are likely to have been *Lankesterella*, *Schellackia*, or *Besnoitia*.

Besnoitia has the most limited distribution and has been found only in cattle (in both hemispheres); reindeer; caribou; horse and ass (in both hemispheres); the rodent *Peromyscus*; and opossums and lizards (in Central America). Separate species names have been given to these organisms.

Sarcocystis is found in man (although rarely) and other primates (it is common in monkeys), cattle, sheep, goats, pigs, horses, deer, antelopes, domestic and wild rodents, lagomorphs, birds (e.g., wild ducks), reptiles, and fish. It is perhaps significant that the infection is very rare in carnivores.

1.2 Host specificity

Toxoplasma apparently has no host specificity, although multiple passages may be necessary to adapt strains from one animal to other animals.

Strains of *T. gondii* should be designated precisely by means of the name of the host, the locality, the date of their isolation, and the name of the person by whom they were isolated. In the future, it may become possible to describe strains in antigenic and biochemical terms. Strains should be preserved immediately after isolation, since they change their character quickly with passage in laboratory animals (see section 9).

The species of *Besnoitia* are most host-specific, although *B. besnoiti* of cattle has been transmitted to rabbits, *B. jellisoni* of the deer-mouse (*Peromyscus*) to other rodents, and *B. darlingi* of basilisks (*Basiliscus basiliscus* and *B. vittatus*) to various rodents.

Sarcocystis apparently has a stricter host specificity than the other parasites of this class, and the few records of the successful transmission of species such as *S. muris*, *S. tenella*, and *S. miescheriana* to other hosts may be invalid because of the high degree of natural prevalence of *Sarcocystis* in the recipient animals; in fact, the morphology of the parasite in the latter was often found to have changed during these experiments. Another reason for believing that different species exist in this genus is the fact that the morphology of the cyst and of the zoites is characteristic for each host species.

1.3 Location in the host

All members of this class are obligatorily intracellular in nucleated cells, although they may circulate in the blood or lymph as free zoites for a short time.

Toxoplasma inhabits nucleated cells of all types, but particularly those of the reticuloendothelium, muscle, and the central nervous system and its appendages, particularly the retina.

Besnoitia invades reticuloendothelial cells of the subcutaneous tissues, the mesentery, and various organs such as the spleen. The invaded cell is difficult to identify because the early parasite stages are rarely seen.

Sarcocystis is essentially a parasite of skeletal and cardiac muscle fibres. It also occurs in the brain (rarely in the ox, but frequently in sheep in New Zealand). The parasite found in the brains of voles, originally designated *Toxoplasma microti* and since known as the M organism, is almost certainly a species of *Sarcocystis* or an unnamed genus in the family Sarcocystidae.

1.4 Morphology

The size of the zoite is a distinctive feature: *Toxoplasma*, the smallest, has a length of about 5 μ , whereas the length of *Besnoitia* is about 7 μ and that of *Sarcocystis* 9–15 μ , according to the species. The size and shape of the cyst are also characteristic: *Toxoplasma*, the smallest, may attain a length of about 100 μ , whereas the length of *Besnoitia* is 300–400 μ and that of *Sarcocystis* up to 1 cm or more. The cysts of *Toxoplasma* and *Besnoitia* are normally spherical, whereas that of *Sarcocystis* is usually elongate. In muscle, *Toxoplasma* cysts may be distorted into any shape.

The cyst wall differs in each group. *Toxoplasma* cysts have a fine elastic wall which, although quite tough, ruptures under heavy pressure. The origin of the cyst wall is not precisely known, since the relative contributions of the host cell and the parasite to the cyst wall are not understood. It is doubtful if the nucleus of the related host cell ever has been seen.

Besnoitia cysts are intracellular and produce a remarkable transformation of the surface membrane of the host cell, which becomes grossly thickened and laminated, while the nucleus (internal to the thick cyst wall) divides repeatedly and hypertrophies. *Sarcocystis* cysts have less thick walls (relative to the size of the cyst), often with external villi and with internal septae.

Under the electron microscope, the zoites of all these organisms are seen to have a remarkably similar ultrastructure; they possess the

organelles characteristic of the Sporozoa in general, the main difference being the regularity of the rows of so-called "central granules" in *Sarcocystis*.

1.5 Immunology

In the dye test, no cross reactions occur between *Toxoplasma*, *Besnoitia*, and *Sarcocystis*. The fluorescent antibody reactions are completely distinctive among these parasites. The indirect haemagglutination test has suggested a weak antigenic relationship between *Besnoitia* and *Toxoplasma*.

1.6 Behaviour in experimental animals

Virulent strains of *Toxoplasma* multiply rapidly in the proliferative stage in the peritoneal endothelium of inoculated mice; avirulent strains may behave similarly after several rapid passages or in animals to which corticosteroids are administered. Some species of *Besnoitia* can be similarly adapted to mice, but *Sarcocystis* (including the M organism) is rarely infective to mice or any other animals.

1.7 Life cycle

The proliferative and cystic stages of *Toxoplasma* are well known, though perhaps not under completely natural conditions. If they are sporozoan parasites, they should exhibit a sexual phase; it is suspected that this occurs during the passage of the cyst to a new host. Recent work (see section 6.1.1) suggests that the cryptic stage should be sought in the immature stages of nematode worms and in the faeces (after maturation) of infected animals.

It is unlikely that the highly artificial transmission of *Besnoitia* spp. to experimental animals reflects the natural route, which remains completely unknown.

Many attempts have been made to observe the life cycle of *Sarcocystis*, but all have been vitiated by the lack of guaranteed uninfected animals. Reports of observation of developmental phases of the parasite following inoculation or ingestion remain unconvincing. On only one aspect is the evidence better—namely, transmission via the faeces of animals fed on sarcocysts. Experiments in several laboratories with different species of *Sarcocystis* have given positive results in lambs and other animals that had received the "infected" faeces orally.

It is probable that the zoites of the whole group divide only by endodyogeny.

2. PHYSIOLOGY AND BIOCHEMISTRY

Data from the few studies that have been made suggest that there is nothing unique about the respiratory physiology and biochemistry of *Toxoplasma*. There is no information that explains the remarkable facility (and need) of *Toxoplasma* to respire and survive in nucleated cells. It would be most desirable to be able to cultivate *Toxoplasma* in a cell-free medium.

It has been demonstrated that respiration ceases when trophozoites are exposed to pyrimethamine and sulfadiazine. Respiration is not affected by specific antibody, but it ceases immediately if accessory factor is added. Further studies of this type should be undertaken; they might lead to improvements in the chemotherapy of both trophozoite and cyst infections. A better understanding of the chemical composition of *Toxoplasma* would be advantageous in efforts to produce an effective immunizing agent.

3. VIRULENCE AND OTHER FACTORS IN INFECTION AND RESISTANCE

The virulence of a given strain of *Toxoplasma* is usually estimated by means of laboratory experiments with mice. In order to obtain significant measurements, such studies must be quantitative, each group of mice being inoculated with known numbers of viable parasites within a brief period of time, so that the death of parasites in the suspending medium does not obscure the results. The virulence of a strain can be estimated by calculating the number of mice dead per number inoculated (at each dilution) and the survival days of those mice that succumb. Mice that die should be checked to make certain that they were killed by *Toxoplasma*. To some extent, these data can be expressed as LD₅₀ values. However, an additional attempt should be made to determine whether the surviving mice had sero-conversions.

Strains that are virulent for the laboratory mouse are usually virulent for other laboratory species, such as rabbits, guinea-pigs, and pigeons. Highly virulent strains (such as RH) that kill mice in a few days quickly produce fatal infections in rabbits, guinea-pigs and pigeons, depending upon the size of the inoculum. Mice, guinea-pigs, rabbits, and pigeons usually survive inoculation with moderate numbers of parasites of less virulent strains, such as that designated "Beverley"¹ by American workers.

¹ The strain was originally isolated from a rabbit by Beverley in England, and was designated "Rabbit A" strain by him. See Beverley, J.K.A. (1959) *Nature (Lond.)*, **183**, 1348.

Although it is possible to estimate virulence by these means, it must be realized that the phenomenon of virulence is a composite of the invasiveness and rate of reproduction of the parasite and various host factors. Strains cannot be considered virulent merely because they cause the death of inoculated mice on primary isolation, since there is no way to assess parasite concentrations in the original material. As soon as the organisms can be counted, it becomes possible to quantify virulence. This must be done early with a new isolate if changes in virulence are to be determined subsequently.

It has been the experience of some workers, using newly isolated strains from swine and sheep, that differences in virulence depend upon the host. Strains isolated from cases of human disease may be highly virulent for mice, but frequently of low virulence. Strains isolated from human or animal congenital cases may be more virulent because they have undergone passage through two successive members of the same species.

Virulence can be increased by passage through various hosts; continuous passage through laboratory mice is an example. Virulence may rise abruptly or it may increase gradually. A rapid increase of virulence for laboratory animals has been observed following single passages in the multimammate rat (*Mastomys coucha*) and in the marmoset (*Callithrix jacchus*). Relatively stable virulence appears to be the result of continuous passage through chick embryos or tissue cultures.

It is possible that passage from a host having a high body temperature, such as a bird, to one with a lower temperature affects the virulence of the strain. This may be related to interferon induction, which may vary in strains adapted to different temperatures. Such phenomena have been observed in virus infections but not as yet in toxoplasmosis.

As noted above, virulence can be measured in laboratory hosts, but the occurrence of disease in other animals and man depends on the relative resistance of the host and the virulence of the organism for that host. There is not necessarily any direct relationship between these factors in laboratory hosts and in man and other animals. Adult chickens, dogs, sheep and other animals exhibit resistance to disease although they become infected with *Toxoplasma*. Concomitant infections in these hosts must be sought before epizootics of disease in them are ascribed solely to *Toxoplasma*. Among the primates, the rhesus monkey and man appear to be highly resistant to clinical disease. Overt disease in monkeys is unusual even when infection is induced with large numbers of parasites. In human beings, the disease is rare, in comparison with the prevalence of *Toxoplasma* antibodies. The most severe manifestations are observed in congenitally infected infants, due either to immunologic deficiencies in the foetus as compared with the adult or to the second passage phenomenon mentioned above.

Natural resistance in various animals may be related to the presence of a nonspecific, heat-labile anti-*Toxoplasma* factor in their sera, which has been demonstrated *in vitro*. There is no information as to whether the presence of this factor in any way affects the course of infection in such hosts. Natural resistance appears to increase with age, so that there may be an association between aging and the appearance of the serum factor. Natural resistance may be diminished by concomitant infections, such as distemper or hookworm infection in dogs, or by high doses of corticosteroids and other immunosuppressive agents.

The administration of therapeutic amounts of corticosteroids has not been associated with the exacerbation of *Toxoplasma* infections in man. The higher doses administered together with highly potent immunosuppressive drugs in organ transplant cases and in the treatment of malignant disease has resulted in the occurrence of active or reactivated toxoplasmosis in a manner similar to the occurrence of herpes or cytomegalic inclusion virus diseases.

Infection with *T. gondii* confers immunity, as is revealed by the appearance of specific antibodies and by survival following challenge. It is also evident from the histories of women who have borne congenitally infected children; in no case has a subsequent pregnancy in such a woman resulted in a toxoplasmic child. However, immunity acquired from active infection is not always complete. For example, guinea-pigs immunized with an avirulent strain of *T. gondii* survive challenge with a highly virulent strain that kills non-immune animals, but the challenge strain is widely disseminated in their bodies. Consequently, when *Toxoplasma* organisms are isolated from an experimental animal, there is no assurance that they are of the same strain that was used for inoculation.

4. HUMAN INFECTION

Most cases of toxoplasmosis in man are subclinical, but symptomatic toxoplasmosis may occur in any of the following forms.

(1) Congenital toxoplasmosis, which is probably the most serious form of the infection. It is acquired by the foetus during an asymptomatic or mild primary infection of the mother with parasitaemia. The baby may be stillborn or born alive with various combinations of the following signs and symptoms: rash, fever, hepatosplenomegaly, purpura, jaundice, central nervous system lesions, and destroyed areas of the retina, any or all of which may be present at birth or appear weeks to months later. Once tissue has been destroyed (e.g., in the brain or the eye), the effects do not regress. Many babies escape with only minor damage and grow and develop normally; however, it seems that disability occasionally appears some years after birth.

The mother of an infant with congenital toxoplasmosis can be assured that subsequent pregnancies will not result in another such baby. Any woman who has experienced an acute *Toxoplasma* infection with antibodies present before becoming pregnant will not have a congenitally infected offspring.

(2) A benign syndrome of acquired infection, which is characterized by lymphadenopathy. In some parts of the world, *Toxoplasma* has been reported to account for up to 15 % of cases of otherwise unexplained lymphadenopathy.

(3) A more severe disease with fever, rash, malaise, muscular pains, pneumonia, myocarditis, and meningoencephalitis. A patient may have any or all of these symptoms. Fortunately, such cases are unusual.

(4) Uveitis, which occurs frequently in congenital toxoplasmosis, usually bilaterally. It sometimes occurs during a postnatally acquired infection, but its true incidence is unknown.

(5) Abortion, although the evidence that *Toxoplasma* is a significant cause of spontaneous abortion is open to question. If it does induce abortion, the latter occurs as a single event in the life of an individual woman, as a complication of a primary acute infection. It is most important that studies of this problem be continued in a most precise manner.

(6) Diffuse toxoplasmosis, sometimes seen in patients receiving immunosuppressive therapy or suffering from immunopathy. When detected, such individuals should receive chemotherapy independent of the management of their basic problem.

5. THE INFECTION IN DOMESTIC AND WILD ANIMALS

Toxoplasma gondii was first recognized in the gundi (*Ctenodactylus gundi*) and later in rabbits and dogs, many years before its discovery in man. Data from all parts of the world are sufficient to indicate that the organism is geographically widespread, that it occurs in many species of warm-blooded vertebrates, and that it can multiply in any of their nucleated cells. The laboratory-bred mouse is highly susceptible and continues to serve as the animal of choice for the isolation of *Toxoplasma*. No significant host specificity has been demonstrated, nor has any animal, including nonhuman primates, been found to be resistant to *Toxoplasma* in the absence of serum antibodies.

Nonhuman disease closely parallels human toxoplasmosis. Almost every clinical manifestation that has been detected in man has also been observed in animals and birds. Congenital infections have been demonstrated in mice, dogs, swine, cows, sheep, cats, and other mammals. Both

congenital and acquired infections in animals are usually asymptomatic, but they may be manifested by foetal damage and abortion. Less often, animals suffer from acute disseminated fatal infections characterized by fever, anorexia, lethargy, dyspnoea, lymphadenopathy, hepatosplenomegaly, iritis, vomiting, chorioretinitis, cyanosis, and neurological disturbances. Abortions, stillbirths, and premature births occur in a variety of animals. In sheep, mink, and swine and less often in dogs and other domestic animals, the disease results in abortion, stillbirths, and high neonatal mortality rates. Abortions due to toxoplasmosis cause considerable economic losses in sheep in New Zealand and England and congenital or acquired toxoplasmosis causes similar losses in swine in Japan. It is possible that other countries also suffer similar losses.

The pathological manifestations of animal toxoplasmosis duplicate nearly all of the lesions that are found in human infections. Parasitaemia occurs in acute infections in animals but may be low-grade in chronic cases. Cellular destruction is caused by direct penetration of the proliferating organisms during active disease, but cysts with argyrophilic walls are formed as antibodies develop. Secondary lesions result from the rupture of host cells that contain *Toxoplasma* or from unknown factors that allow encysted organisms to escape.

Lesions vary from severe necrosis to granulomatous proliferations of mononuclear cells and lymphocytes. Asymptomatic infections are characterized by cysts with no visible host reaction and may persist during the life of the animal.

Toxoplasmae are demonstrable in nearly all organs of the body, including the brain, diaphragm, myocardium, lymphoid tissue, lungs, ovary, uterus, and placenta. Viable organisms can also be found in the blood, milk, faeces, sputum, vomitus, saliva, vaginal discharges and sometimes in the urine and semen of acutely infected animals with clinical disease. The dissemination of *Toxoplasma* from animals and nematodes (*Toxocara cati* of cats and the swine lungworm *Metastrongylus apri*) harboured by animals, as well as the occurrence of organisms in meat, milk, eggs, and edible organs, suggest possible sources of human infection. (Transmission by nematode eggs and faeces from animals is described in sections 6.1.1, 6.1.2, and 6.1.3.)

The specificity and sensitivity of the dye test (see section 7.2) permits quantitative measurements of *Toxoplasma* antibodies in the sera of warm-blooded vertebrates. A positive dye test indicates experience with *Toxoplasma* and its probable persistence in the animal. More recently, haemagglutination tests have demonstrated widespread infection in animals. The intradermal test has been used to detect infections in swine.

Clinically, pathologically, and epidemiologically, *T. gondii* behaves in a remarkably uniform manner throughout its wide range of host species.

6. EPIDEMIOLOGY

Numerous studies have shown that *Toxoplasma* infections occur in man and other warm-blooded vertebrates throughout the world, with the possible exception of Antarctica, although the frequency of such infections varies considerably from one country to another and within a given country. The expansion of such studies is necessary if a better understanding of the ecology of toxoplasmosis is to be achieved. Such understanding is important not only in its own right, but for the selection of appropriate areas in which to conduct investigations of specific *Toxoplasma* problems. The available data indicate that human-to-human transfer does not occur except from the primarily infected woman to her foetus. There is no evidence that transfer usually occurs from animals and birds to man except by ingestion. Meat and, much less often, eggs can contain *Toxoplasma* cysts so that carnivores and omnivores may acquire infections from these sources; however, the route of infection of herbivores is entirely unknown and requires further investigation.

The value of epidemiological studies is limited by the specificity and sensitivity of the methods used and by the validity of the sample surveyed. The importance of these limitations cannot be over-emphasized. If they are taken into account, the intensive investigation of apparently clustered cases and conditions such as climate, altitude, socio-economic factors, age and sex may lead to the acquisition of important knowledge.

6.1 Transmission through helminths

Recent work on the transmission of *T. gondii* via *Toxocara cati* eggs in the faeces of *Toxoplasma*-infected animals has thrown new light on the epidemiology of toxoplasmosis. The results of these studies, initiated by Hutchison in 1965, and of other, similar experiments are described briefly in the following sections.

6.1.1. Experiments with *Toxocara cati* and other nematodes

In his first experiments, Hutchinson¹ showed that if *Toxocara*-infected cats were fed on 5 successive days with mice whose tissues contained *T. gondii* cysts, the cats subsequently passed the nematode eggs, which were infective to uninfected mice by the oral route. Such eggs, which were passed only 5-30 days after *T. gondii* cysts were fed to the cat, exhibited the following features: (1) they were unable to transmit the infection

¹ Hutchinson, W. (1965) *Nature (Lond.)*, 206, 961; (1967) *Trans. roy. Soc. trop. Med. Hyg.*, 61, 80.

until they became embryonated 14–21 days after having been passed in the cat's faeces; (2) they remained infective during 17 months of storage; (3) they could be collected by zinc sulfate and saline flotation and could be stored in water; and (4) they retained their infectivity after exposure to 5% sodium hypochlorite solution.

Jacobs and Melton¹ confirmed these results. Having found 35 stray cats naturally infected with *Toxocara cati* and with dye-test-positive sera, they collected eggs from the cats' faeces and dissected others from the adult worms, and found that (1) such samples from 2 cats were infective when fed to mice; (2) eggs became infective after storage for 21 days and remained so for 7 months, and exposure to 5% sodium hypochlorite did not destroy their infectivity; and (3) similar experiments with *Toxocara canis* in dogs failed to produce infections in mice.

The above observations were confirmed by Dubey,² who reported the following additional results :

(1) The infectivity of fresh embryonated eggs was destroyed by 1% formalin, but if the eggs were stored for 100 days in water and then exposed to formalin for up to 7 days, infectivity was retained.

(2) If embryonated eggs were treated for 1 hour at 37° C with 5% sodium hypochlorite solution, the outer coats were destroyed; after prolonged washing to remove the sodium hypochlorite, the vitelline membrane enclosing the larva was removed by shaking with sand and water. The freed larvae proved to be infective when mice were inoculated with them subcutaneously.

(3) As few as 4 eggs produced an infection in a mouse but there seemed to be a quantitative relationship between the number of eggs administered and the number of cysts produced in the mouse : 4 eggs gave rise to 48 cysts and 500 eggs to 738 cysts.

(4) At least 12.5% of the eggs were infective.

Rommel et al.,³ using techniques similar to those of Hutchison, transmitted *T. gondii* with *Toxocara cati* eggs, collected 6–22 days after the initial feeding of the cat. These investigators failed to transmit infections with the following combinations of nematodes and hosts : *Toxascaris leonina* in cats; *Haemonchus contortus*, *Ostertagia circumcincta*, *Chabertia ovina*, and *Oesophagostomum venulosum* in sheep; *Ascaris suum*, *Oes. quadrispinulatum*, and *Oes. dentatum* in pigs; *Toxocara*

¹ Jacobs, L. & Melton, M. L. (1966) cited by Jacobs, L. (1967) *Advanc. Parasit.*, **5**, 1.

² Dubey, J. P. (1968), *Vet. Bull.*, **38**, 495.

³ Rommel M. et al. (1968) In : *Proceedings of the Eighth International Congress on Tropical Medicine and Malaria, Teheran.*

canis, *Toxascaris leonina*, *Ancylostoma caninum*, and *Uncinaria stenocephala* in dogs; *Ascaridia galli* and *Heterakis gallinae* in hens; and *Syphacia obvelata* in mice.

Tsunoda¹ obtained positive results with the lungworm, *Metastrongylus apri*, in guinea-pigs infected with this worm and then given *T. gondii*: 5-15 days after infection with the latter, the lungworms were collected from the guinea-pigs' lungs and were thoroughly washed and homogenized. Infections were produced in 24% of the mice that were inoculated with the eggs. Tsunoda also isolated *T. gondii* from a few eggs from the lungs of pigs.

6.1.2 Experiments with other helminths

Ottilio et al.² conducted similar experiments in young rats, each of which was infected with 50 eggs of *Hymenolepis nana fraterna*. When eggs appeared in their faeces (after 1-2 months) the animals were infected with *T. gondii*, orally or by intraperitoneal injection. Subsequently, eggs were collected, washed, treated for 10 minutes with 10% formalin and thimerosal, washed again, and homogenized. Of 15 mice to which the eggs were administered orally, 1 became infected, while 6 of 15 mice that received the eggs intraperitoneally became infected. Moreover, one adult cestode was also found to be infected.

6.1.3 Morphological evidence of infection in helminths

If it is assumed that *Toxoplasma* is a sporozoon, it can be postulated that a sexual phase of development occurs within the invertebrate. No unequivocal evidence of the presence of *T. gondii* in either adult *Toxocara cati* or its eggs has yet been obtained, but this may be because its forms are very different from those seen in vertebrates. The recent demonstration by electron-microscopy of *Histomonas* in the oocytes of *Heterakis* suggests that this technique should be applied to *Toxoplasma*.

6.2 Experiments not involving helminths³

Jacobs & Melton⁴ noted that faeces obtained from 2 cats apparently free from *Toxocara cati* eggs, but infected with *T. gondii* as described above,

¹ Tsunoda, K. (1968) unpublished paper delivered to the 66th General Meeting of the Japanese Society of Veterinary Science.

² Ottilio, J., Machado, L. Said Silva, De Pinho, A. L. & Gomez, F. J. R. (1967) *O Hospital*, 72, 1161.

³ Results published in several journals since the meeting was held have placed the entire theory of transmission by helminths in a new light. With the agreement of the participants in the meeting, this new information is summarized in the Addendum on p. 31 — ED.

⁴ Jacobs, L. & Melton, M. L. (1966) cited by Jacobs, L. (1967) *Advanc. Parasit.*, 5, 1.

also produced infections in mice. Hutchison¹ fed 21 cats free from *Toxocara cati* with *T. gondii*; the faeces of 2 were infectious for mice following filtration through a 35- μ filter and treatment with 5% sodium hypochlorite.

6.3 Significance of recent findings

The following points are of particular significance :

(1) Experimental transmission of *T. gondii* can be effected by (a) eggs of *Toxocara cati* from *T. gondii*-infected cats; (b) faeces of *T. gondii*-infected cats free from *Toxocara cati* eggs; (c) eggs of *Metastrongylus apri* from *T. gondii*-infected animals; and (d) eggs of *Hymenolepis nana* from *T. gondii*-infected animals.

(2) Nematode eggs or host faeces become infectious during a transient period, about 4–30 days after the vertebrate host has been fed *T. gondii*. Only embryonated eggs become infective, about 14 days after being passed in the faeces, and they remain infective for as long as 17 months. Infection in eggs withstands treatment with sodium hypochlorite or formaldehyde solution. Infection in faeces (in which *Toxocara cati* is not present) withstands similar treatment, and the organism passes through a filter of 35- μ pore size.

(3) Well-washed larvae of *Toxocara cati*, freed from shell and membranes, are infective on inoculation.

(4) There is a high rate of infection in the eggs of infected worms, suggesting the possibility that *Toxoplasma* undergoes a reproductive process in the invertebrate.

6.4 Transmission through arthropods

Although many studies have been made of the possibility that *T. gondii* is transmitted by hematophagous arthropods, only those with ticks have yielded positive results. In a laboratory-maintained colony of *Ornithodoros moubata* some naturally *Toxoplasma*-infected ticks were detected.² This batch of ticks had been fed 45 days previously on apparently healthy guinea-pigs. Homogenates of the ground-up ticks caused infections when injected intraperitoneally into mice. Mice also were infected by tick bites. When tested 1 month later, ticks from the same batch were found to be non-infectious.

Other ticks from a *Toxoplasma*-free colony were infected experimentally by feeding on acutely infected mice. These ticks infected other

¹ Hutchison, W. (1969) Unpublished data.

² Castellani-Pastoris, M. (1969) *Parassitologia* (in press).

mice on which they were permitted to feed 13 days later. Passage of *Toxoplasma* through the tick seems to decrease the virulence of the parasite but its original level of virulence is restored by one or two blind passages in mice.

All the feeding experiments described above were performed at room temperature.

7. DIAGNOSTIC METHODS

7.1 Direct demonstration of *Toxoplasma*

Toxoplasma may be sought in tissues or body fluids with ordinary histological stains or by immunofluorescence techniques. With experience, the organism can be detected in unstained preparations. Such studies, especially those using immunofluorescence inhibition, should be controlled whenever possible by isolation trials. For the latter, the original specimens should be retained and stored at 4°C.

Tissues such as lymph nodes, tonsillar tissue, enzyme-digested meat samples, body fluids, and autopsy material are used for mouse inoculation. Healthy, laboratory-reared, white mice (proved to be susceptible to *Toxoplasma*) about 3 weeks of age are inoculated intraperitoneally with 0.5-1.0 ml of a 20% tissue suspension. Alternate mice are used as controls. During the first week, *Toxoplasma* may be demonstrated in the peritoneal exudate, should any appear. After 6 weeks of observation, dye tests are performed on the blood from the tails of surviving mice. If such tests are positive, the brains should be examined for cysts, but if they are negative, further blind passages are unnecessary.

7.2 Dye test

Extensive experience with the dye test has shown it to be a sensitive, specific indicator of *Toxoplasma* antibodies in both man and animals. Reproducible results are regularly obtained, provided — as is true of all serological procedures — certain conditions are observed. Among these conditions are the following :

- (1) Glassware must be scrupulously clean and free from detergent.
- (2) All human sera should be inactivated at 56°C for 30 minutes and animal and bird sera at 60°C for 20 minutes before dilution for testing.
- (3) Accessory factor serum should be free from *Toxoplasma* antibodies and from any thermolabile anti-*Toxoplasma* activity. If stored at a temperature not exceeding -20° C, it will maintain its activity for 2-3 months; if stored at -70°C or in the lyophilized state, its activity is main-

tained for 1 year or more. The activity of stored accessory factor should be re-determined periodically.

(4) Uninfected, healthy, laboratory-reared 3-week-old mice should be used. Each is injected intraperitoneally with 20 000–2000 000 organisms (preferably the RH strain). Their peritoneal exudates should be collected 48–72 hours later when they are fluid and rich in healthy, virulent, extracellular parasites. These will stain sharply and uniformly with alkaline methylene blue, or appear “alive” under the phase-contrast microscope. The concentration of parasites used in the test should be standardized by counting. The reaction is most easily read when 10–20 parasites can be seen in each microscope field.

(5) In performing the test, each serum dilution is mixed with an equal volume of the *Toxoplasma*-accessory factor mixture; the tubes are then placed in a waterbath at 37° C for 1 hour, after which 1 drop of buffered (pH 11) methylene blue solution (freshly prepared from a stock saturated alcohol solution) is added to each tube. If phase-contrast microscopy is used for reading, 1 drop of 4 % formalin should be added in place of the methylene blue. Every test run should include a negative control and a fully titrated positive human serum.

The titre of the serum is that dilution in which 50 % of the parasites are unstained or modified (as revealed by phase contrast). It should be stated whether the initial or final dilution (the latter being preferable) is used to express the titre of the serum. A microtitre modification of the dye test has been described and is especially useful for epidemiological surveys.

In 1967 the WHO Expert Committee on Biological Standardization established a “human serum preparation as the International Standard for Anti-Toxoplasma Serum... and defined the International Unit for Anti-Toxoplasma Serum as the activity contained in 0.090967 mg of the International Standard for Anti-Toxoplasma Serum”.¹

7.3 Complement-fixation test

The complement-fixation test, which is specific and relatively sensitive, is undergoing considerable study at present because several new methods have been developed for the preparation of antigens with different characteristics. Further studies are needed to permit evaluations of the relative usefulness of the antigens prepared by different techniques.

The complement-fixation test can be used to supplement the dye test, but antibodies detected with the “older” soluble antigen appear more slowly than do dye test antibodies and usually disappear in about 2 years.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 384, p. 18.

7.4 Indirect haemagglutination test

The indirect haemagglutination (IHA) test for toxoplasmosis is sensitive and specific except for a reported weak cross reaction with *Besnoitia jellisoni*, which was demonstrated only with sera produced experimentally in rabbits. The IHA test requires an antigen that has been prepared with meticulous care from suspensions of parasites containing no other organisms and that must be stored in the frozen or lyophilized state. In general, good qualitative agreement has been found between the IHA test and the dye test, but the former requires careful standardization in each laboratory that performs it.

In general, IHA titres become positive and rise more slowly than do dye-test titres, in cases of both acute lymphadenopathy and congenital toxoplasmosis. Further studies of the preparation of antigen and of the red cell concentration to be employed are necessary; they may demonstrate that the sensitivity of the test can be increased to reveal antibodies in such cases without decreasing its specificity. Unless such improvement is possible, the IHA test cannot be used as the only test in suspected cases of active congenital or acquired toxoplasmosis. At present it is useful in the diagnosis of chronic infections, such as ocular toxoplasmosis. The IHA test supplements the dye test in the diagnosis of acute disease, owing to the slower rise in IHA antibodies. The IHA test has also been successfully used in sero-epidemiological studies.

7.5 Indirect fluorescence test

Since the indirect fluorescence test (IFT) has been adapted to the diagnosis of a variety of infectious microbial diseases, the requisite instruments are becoming available increasingly in clinical diagnostic laboratories. Consequently, it would be valuable if this technique could be applied to the diagnosis of toxoplasmosis, especially in places where the dye test is impracticable. A number of favourable reports on the sensitivity and specificity of the IFT and on the reproducibility of its results have been published. The reagents for this test are easily obtained and require no local laboratory production, since formalin-fixed antigen is stable on microscope slides or in suspension for at least 3-4 months and fluorescein-labelled anti-human globulin is readily available.

Since the dye test measures antibodies against surface antigens of *Toxoplasma*, it would appear on theoretical grounds that the IFT measures similar antibodies.

The test is still too new for definitive conclusions to be drawn as to the relation of titres to the temporal stages of infection and clinical involvement. These aspects must be evaluated in different laboratories by

comparative studies using standard techniques. Such evaluations should include the establishment of definite end-points in titrations of standard test sera.

7.6 Evaluation of serological tests

It should be emphasized that before new diagnostic tests are used, a detailed comparison should be made between their results and those obtained with established methods. Such tests should also be studied in different stages of the illness in cases of proved human disease as well as in experimental animals. Such evaluations should, if possible, include results obtained with the International Standard for Anti-Toxoplasma Serum.

7.7 General use of serological tests

Of all the serological tests that have been suggested for use in the study of toxoplasmosis, only four — the methylene blue dye test, the complement-fixation test, the indirect haemagglutination test, and the indirect fluorescent antibody test — have received intensive study for diagnostic and sero-epidemiological purposes.

The dye test, originally described by Sabin and Feldman,¹ is the most widely accepted of these procedures. The technical problems it involves have led to the development and evaluation of other procedures.

The complement-fixation test has received limited acceptance for the diagnosis of toxoplasmosis. It is regarded as being of value; however, when soluble antigen is employed, it detects antibody later than does the dye test. Furthermore, complement-fixing antibody tends to disappear, whereas the dye test remains persistently positive. The reactivity of the complement-fixation test seems to be influenced by the type of antigen employed. With improvements, including superior antigens, this technique may well receive wider use.

The indirect haemagglutination test, described by Jacobs and Lunde² in 1957, has been employed for both diagnostic and sero-epidemiological studies. Some of its inherent problems have been overcome by the use of sheep red cells coupled with antigen by diazotization and fixed in an alcoholic solution of formaldehyde. The test appears to be deficient in its ability to detect antibodies early in the acute stage of the infection.

The fluorescent antibody test was initially introduced by Goldman³ in 1957 as an inhibition procedure. This proved to be unsuitable and it

¹ Sabin, A. B. & Feldman, H. A. (1948) *Science*, **108**, 660.

² Jacobs, L. & Lunde, M. N. (1957) *J. Parasit.*, **43**, 308.

³ Goldman, M. (1957) *J. exp. Med.*, **105**, 557.

has been replaced by the indirect fluorescent antibody test. This test offers a good potential in the diagnosis of toxoplasmosis since the results obtained with it appear to be similar to those provided by the dye test.

In addition to these four procedures, various agglutination and flocculation tests have been proposed. In 1959 a direct agglutination test using purified, killed *Toxoplasma* as antigen was described by Fulton and Turk.¹ Siim and Lind in 1960² and Denis in 1967³ reported on acrylic particle flocculation techniques, and Engelbrecht⁴ has described a slide flocculation technique using ultrasonically disrupted toxoplasmae. Although the sensitivities of these reactions are good, their specificity, particularly at higher titres, is not optimum. The techniques are rapid and deserve further investigation to improve their usefulness for diagnosis.

7.8 Skin test

The intradermal test, which evokes a delayed skin reaction, becomes positive a few months after infection by *Toxoplasma* but later than the serological tests. It probably remains positive throughout life. It is useful for the following purposes :

(1) To determine, from the epidemiological point of view, the proportion of a population that has had past experience with *Toxoplasma*. Knowledge of the epidemiology of toxoplasmosis would be increased if the skin test were used in population surveys in remote areas where surveys for the prevalence of other infections are being performed.

(2) As a screening test in chronic ocular disease and in very early pregnancy. In the former condition, a positive intradermal reaction indicates that toxoplasmosis may be considered in the differential diagnosis; a negative result suggests that the ocular lesions are due to another cause, unless other techniques give evidence of recent infection. In early pregnancy, a positive skin test indicates that the pregnant woman was exposed to *Toxoplasma* before pregnancy and is unlikely to bear a child afflicted with congenital toxoplasmosis. Whether other types of congenital damage can occur in the offspring of such women remains to be established.

(3) To determine the role of *Toxoplasma* in the etiology of lymphadenitis. In cases of lymphadenitis of very recent origin, a positive skin test probably demonstrates that the diagnosis is not toxoplasmosis. A negative reaction indicates only that serological tests are required to obtain evidence of recent *Toxoplasma* infection.

¹ Fulton, J. D. & Turk, J. L. (1959) *Lancet*, **2**, 1068.

² Siim, J. & Lind, K. (1960) *Acta path. microbiol. scand.*, **50**, 445.

³ Denis, C. (1967) *Path. et Microbiol. (Basel)*, **30**, 981.

⁴ Engelbrecht, E. (1965) *Path. et Microbiol. (Basel)*, **28**, 907.

(4) As a test for latent toxoplasmosis in swine.

No rises in the titres of serum antibodies have been observed following toxoplasma skin tests in human beings. No activation or modification of an ocular lesion or an infective process has been related to skin-testing.

Skin-test antigens can be prepared from various sources, such as the peritoneal exudates of intraperitoneally infected mice, the chorioallantoic membranes of infected embryonated eggs, or infected tissue cultures. The potency of the antigens from these different sources varies, probably in relation to the number of parasites and the proportion of nonreactive material present. Some workers have used autoclaved (110° C) antigens, which contain few extraneous bacteria or viruses. Others have used chemically treated antigens, in which case it is more important to rule out the presence of extraneous organisms. There may be differences in the stabilities of antigens prepared by different methods; autoclaved antigen is stable for long periods, even at room temperature, if it contains phenol as a preservative.

It is necessary to standardize antigens to obtain comparable results in man. Apart from standardization in guinea-pigs previously sensitized by repeated injections of antigen in an oil adjuvant, this is done by comparing the effects of dilutions of a new antigen preparation with those of a known antigen, in human beings known to be reactive. Suitable control preparations should be used in parallel.

There is a need for criteria for uniform skin-test antigens and for their preparation and safety-testing. Data on the temporal relation of skin reactivity to the course of *Toxoplasma* infection in man and animals are also needed.

8. TREATMENT

Since most cases of human infection with *Toxoplasma* are asymptomatic, they are never treated. The one exception may be an individual who suffers a laboratory accident and requires prophylactic therapy (see section 9.2).

The available evidence indicates that chemotherapeutic agents that have been recommended for the treatment of toxoplasmosis are more active against trophozoites and have little, if any, effect on cysts.

8.1 Congenital toxoplasmosis

The most frequently used combination of drugs is pyrimethamine and sulfadiazine (for the dosage, see section 9.3). Treatment initiated after the recognized onset of disease does not affect the serological reactions.

There is no evidence that the administration of chemotherapeutic agents to neonates with congenital toxoplasmosis in any way affects the course of the disease. At best, it can only be expected to limit further tissue damage. Decisions to initiate such treatment must be made on an individual basis. However, if treatment is undertaken the family should not be encouraged to expect any substantial improvement.

8.2 Acquired toxoplasmosis

Symptomatic cases rarely require more than supportive treatment. Of the wide range of signs and symptoms that may occur in a given patient, the clearest indicators for chemotherapy are meningoencephalitis and/or myocarditis. Such patients often have diffuse skin rashes. When instituted, treatment should be maintained for at least 2 weeks and probably not more than 4 weeks.

8.3 Toxoplasmic uveitis

Since blindness may be one of the complications of toxoplasmic uveitis, patients with this condition require special care. If a patient is believed to have this condition, treatment may be initiated after first obtaining a serum specimen for serological study. In the absence of antibodies, treatment for toxoplasmosis should be discontinued; otherwise, chemotherapy should be continued for at least 2 weeks. Since some of the manifestations of this disease may be due to allergy, steroids may be added to the regimen, especially if there is an active lesion near a macula.

8.4 Immunopathy

Patients receiving immunosuppressive treatment for whatever reason, and those who have a disease that interferes with immunity, should be observed carefully for the development of toxoplasmosis. Should this occur, chemotherapy for toxoplasmosis should be added to the other therapeutic measures.

8.5 Animal toxoplasmosis

There are occasions when it may be desirable to treat animals, especially during an enzootic among domesticated species. It has been suggested that this be done with sulfamonomethoxine and pyrimethamine rather than with sulfadiazine and pyrimethamine. A combination of pyrimethamine and 2-sulfamoyl-4,4'-diaminodiphenylsulfone has been reported to be effective against cysts. Further double-blind treatment studies in animals appear to be indicated.

8.6 Evaluation

The relative infrequency of acute symptomatic toxoplasmosis in man makes it unlikely that double-blind evaluations of the treatment of this condition can be carried out. For obvious reasons, it would be even more difficult to carry out such evaluations in neonates with congenital infections. However, chemotherapy and other treatment measures have been suggested for, and applied to, many clinical conditions that were thought to represent manifestations of chronic *Toxoplasma* infections. It is in these cases, which are relatively numerous and basically not life-threatening, that treatment must be evaluated by precise double-blind methods. In addition to making it possible to draw conclusions regarding the response of these cases to treatment, such studies would also clarify their etiology.

9. LABORATORY MANAGEMENT OF THE PARASITE

9.1 Maintenance

Toxoplasma gondii can be maintained in the laboratory in various ways. Repeated passage in uninfected laboratory-reared mice or in embryonated eggs has been used most often. Another effective method is passage by serial transfers in tissue cultures. Avirulent strains can be maintained in mice without passage as long as the animals survive, the cysts being easily recoverable from the brain. Similarly, virulent strains can be maintained in rats for a year or longer, although it is sometimes difficult to recover organisms from this species. Young rats should be used. All animals should be from stock known to be sero-negative and free from *Toxoplasma*.

The virulence of *Toxoplasma* appears to become enhanced on repeated passages in laboratory mice. Sometimes this occurs precipitately and sometimes gradually. Rapid increase in virulence on passage through certain animals, such as the multimammate rat (*Mastomys coucha*), the marmoset, and the gundi, have been reported. Virulence seems to be maintained at the original level in tissue cultures or embryonated eggs.

Tissue cultures maintained at room temperature permit less frequent renewal of the medium and longer intervals between subinoculations. After proliferation of the parasites, tissue cultures may be kept at 4° C for as long as several months, or they may be kept indefinitely in the frozen state. Intact cysts of *Toxoplasma* in the flesh of infected animals also can be maintained at refrigerator temperature (4–5° C) for several weeks or months.

Deep freezing of the parasites has been accomplished, with the preservation of some viability. This is to some extent a selection process.

The degree of success achieved in preserving parasites by freezing apparently varies with the starting material. For example, in freezing a preparation of peritoneal exudate from infected mice, in which the organisms are principally extracellular, it may be necessary to give more careful attention to the gradual lowering of the temperature or to the addition of glycerol or other reagents than in the freezing of preparations containing intracellular organisms, such as egg chorioallantoic membranes, tissue cultures, or infected animal tissues.

The preservation of parasites by deep freezing is an important subject that merits further study aimed at more precise understanding of the conditions that should be maintained for optimum results.

9.2 Safety precautions

Although the risk of infection is usually low, it can be minimized by the maintenance of ordinary standards of hygiene in the laboratory. In addition, the following precautions should be taken.

(1) Dye test antibodies should be determined in the sera of laboratory personnel before they begin work with *Toxoplasma*. Those who are antibody-positive should be preferred for such work. Pregnant women may be permitted to work in *Toxoplasma* laboratories provided they are sero-positive.

(2) When working with infected animals eyeglasses and gloves should be worn. A glass or heavy plastic screen should be placed between the worker and the animals to avoid contamination by splashing.

(3) Pipettes that are used to transfer parasites should be plugged with cotton.

(4) Disinfectant solutions and detergents should be readily available at each working table.

(5) If an accident should occur in the laboratory, the affected area should be washed immediately and thoroughly with soap and water. If infective material enters the mouth through a pipette, the mouth should be rinsed with a soap solution, and if such material is splashed into the eyes, they should be rinsed immediately with copious amounts of water. A serum sample should be obtained promptly. If the individual is known to have dye test antibodies, nothing further need be done. However, if he is sero-negative or if his antibody level is not known, chemotherapy (see section 9.3) should be instituted and continued for at least 2 weeks. If it is subsequently established that the individual was sero-positive at the time of the accident treatment can be discontinued.

9.3 Chemotherapy of exposed persons

In the circumstances mentioned above, the following drugs should be used in combination : (a) pyrimethamine, 4 doses of 50 mg every 12 hours, followed by 25 mg every 12 hours for 4 days and then 25 mg once daily; and (b) sulfadiazine, 2-4 g initially, then 1 g every 6 hours. Large amounts of fluid should be taken and white and differential blood counts should be obtained twice weekly. The adverse effects of pyrimethamine on the bone marrow may be prevented by the daily ingestion of 2 yeast cakes and 5-15 mg of leucovorin. Follow-up dye tests should be performed on these patients.

A bite from an animal known to be infected with *Toxoplasma* should be treated in the same manner.

10. RECOMMENDATIONS FOR RESEARCH

The recommendations for further research that have been made throughout this report are summarized in the following section, together with certain additional recommendations.

(1) Taxonomic studies of the class Toxoplasmoda should be supported and reviewed periodically in collaboration with the Subgroup on Taxonomy of the International Toxoplasma Subcommittee of the International Association of Microbiological Societies.

(2) Procedures for the maintenance and preservation of strains in animal tissues and tissue culture should be improved so as to prolong shelf life, especially in the frozen state, with least interference with the biological characteristics of the parasite. Studies of the respiration of the parasite should be of help in clarifying these problems as well as those involved in chemotherapy.

(3) Differences in the virulence of different strains should be studied by segregation and cloning of the parasite. The effect of the body temperature of the host should also be studied.

(4) Interferon induction (as observed in virus infections) should be studied in relation to toxoplasmosis.

(5) The dynamics of various serological tests during the evolution of different types of infection should be studied, and the suitability of such tests as diagnostic aids should be further evaluated.

(6) Skin-test antigens should be standardized, and the temporal relationship of skin reactivity to the course of the infection in man and animals should be studied.

(7) Pathogenesis of the congenital disease should be investigated, with follow-up studies in children. Further study should also be devoted to the pathogenesis of eye lesions.

(8) Determinations of antibodies in the amniotic fluid of pregnant women suspected of toxoplasmic disease should be undertaken, and the babies of such women should be followed-up to learn whether the appearance of antibodies in the amniotic fluid was indicative of infection of the foetus.

(9) Antibody levels should be followed after labour, and an attempt should be made to recover parasites in order to learn whether the placenta is infected and whether haematogenic dissemination takes place during labour.

(10) The effect of different oxygen tensions on the parasite and on globulins in the course of infection should be studied.

(11) The influence of concomitant infections (especially with latent viruses, *Mycoplasma*, etc.) on the course of toxoplasmosis in animals should be investigated.

(12) Further studies of lysozymal enzymes and of their relation to virulence should be carried out.

(13) Rodents and other vertebrates from all parts of the world should be examined for the presence of *Toxoplasma* and similar organisms in their brains, lungs, and muscles.

(14) Intracellular and extracellular parasites harboured by naturally infected animals should be examined for forms that might be stages in the life cycle of *T. gondii*.

(15) Cell-mediated immunity to toxoplasmosis in animals should be studied.

(16) Methods of processing foods of animal origin should be studied to determine their ability to destroy *Toxoplasma* and to impede the transfer of *Toxoplasma* from contaminated to non-contaminated foods.

(17) Methods of rendering foods of animal origin free from *Toxoplasma*, such as freezing and thawing and irradiation, should be investigated further and more precisely.

(18) Measures to prevent toxoplasmosis in animals (e.g., vaccination and feed additives) are worthy of intensive study.

(19) Helminth transmission of *Toxoplasma* should be investigated under varied conditions on a wide scale.

11. SERVICES TO RESEARCH

The group recommends the provision of the following reference reagents and services :

(1) A large amount of antigen prepared by a single laboratory should be available for use in collaborative evaluation of the indirect haemagglutination and indirect fluorescent antibody tests.

(2) A bank of serially obtained sera from authenticated cases of acute toxoplasmosis in humans and animals should be established to facilitate the evaluation of new serological procedures.

(3) Consideration should be given by WHO to the possibility of establishing an international reference centre and regional reference centres for providing the following services :

- (a) The identification of strains of *Toxoplasma* and related organisms, and the verification of identifications made by others.
- (b) The preparation and distribution of reference reagents.
- (c) The testing of sera and the provision of other laboratory assistance to epidemiological and clinical investigations.
- (d) The training of research workers.

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Annex

FAMILLIES OF TOXOPLASMIDA AND
DEFINITIONS OF TERMS

1. Families

Toxoplasmidae. Cysts with thin membranes and pseudocysts. Naked zoites possess a vesicular nucleus, a conoid, toxonemes, and a micropyle but no flagella or cilia. Reproduction is by endodyogeny. Locomotion is by subpellicular fibrils.

Besnoitiidae. Cysts with thick, laminated, nucleated walls and pseudocysts. Naked zoites possess a vesicular nucleus, a conoid, toxonemes, and a micropyle, but no flagella or cilia. Reproduction is by endodyogeny. Locomotion is by subpellicular fibrils.

Sarcocystidae. Elongated cysts, often septate and with cytophaneres. Elongated naked zoites possess a vesicular nucleus, a conoid, toxonemes, a zone of central granules, and a micropyle, but no flagella or cilia. Reproduction is by endodyogeny. Locomotion is by subpellicular fibrils.

2. Terms

Trophozoite. The individual organism inside the pseudocyst produced as the result of asexual division (endodyogeny).

Zoite. The individual organism inside the cyst resulting from asexual division (endodyogeny).

Pseudocyst. The collection of trophozoites within the vacuole inside the host cell.

Cyst. A collection of zoites enclosed within a tough, resilient, thin wall.

Endodyogeny. The production of two merozoites within the original surface membrane of the mother organism, commencing with the formation *de novo* of two conoids and the division of its nucleus.

Addendum *

TRANSMISSION OF TOXOPLASMA

As noted in section 6.2, Jacobs & Melton reported that the faeces of 2 cats, although free of *T. cati* eggs, produced *Toxoplasma* infections in mice. Dubey¹ also found the faeces of a seronegative cat that had been fed *Toxoplasma*-infected mice to be infectious for mice. The cat was free of helminths. Hutchison, Dunachie & Work² subsequently reported similar results. In a more recent paper, Work & Hutchison³ described what may be a new cystic form of *Toxoplasma* in the faeces of cats. These "cysts" measure $9 \times 14 \mu$, are ovoid, and remain viable for at least 3 weeks in tap water at room temperature. They are separable from *T. cati* eggs and are infectious for mice. Sheffield & Melton⁴ also produced infections in mice with faeces from *Toxoplasma*-infected cats free of *Toxocara*. Frenkel, Dubey & Miller⁵ have demonstrated that infectious *Toxoplasma* can be separated from *T. cati* eggs in the faeces of cats and that in this form they are resistant to 5% sodium hypochlorite and/or 1% formaldehyde and survive storage at room temperature for at least 3 months.

It now appears clear that in the cat, at least, a very resistant, infectious form of *Toxoplasma* is excreted in the faeces, independently of *T. cati* eggs. It is likely that similar results will be reported for other species, including man.

* See footnote, p. 16.

¹ Dubey, J. P. (1968) *J. Protozool.*, **15**, 773.

² Hutchison, W. M., Dunachie, J. F. & Work, K. (1968) *Acta path. microbiol. scand.*, **74**, 462.

³ Work, K. & Hutchison, W. M. (1969) *Acta path. microbiol. scand.*, **75**, 191.

⁴ Sheffield, H. G. & Melton, M. L. (1969) *Science*, **164**, 431.

⁵ Frenkel, J. K., Dubey, J. P. & Miller, M. L. (1969) *Science*, **164**, 432.

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