A broad survey of information on the epidemiology of toxoplasmosis with an evaluation of data on the transmission of this infection.

The Interrelation of Toxoplasmosis in Swine, Cattle, Dogs, and Man

LEON JACOBS, Ph.D.

THE ORGANISM *Toxoplasma gondii* is an obligate intracellular parasite. Until now, no form has been found which is capable of living for extended periods outside the cells of its numerous hosts. It is capable of invading and multiplying in a wide variety of cell types, such as neurons, microglia, endothelium, reticulum, parenchyma cells of the liver, epithelial cells of the lung and glands, and cardiac and skeletal muscle.

The parasite exists in two forms. The proliferative form, seen during the acute stage of the infection, undergoes rapid intracellular multiplication, and the numerous loosely grouped toxoplasmas thus produced are liberated by rupture and invade new cells. This form of the parasite is motile, showing twisting movements of its attenuated end and gliding movements unaccompanied by any changes in shape or surface visible by ordinary light microscopy. It measures about 3 by 6 microns, has a centrally located nucleus, and glycogen granules of small size. The pseudocyst form probably appears late in the subacute stage of the infection and is the only form which persists in chronic infections. Pseudocysts are generally larger in size than the cells parasitized by proliferating forms. The parasites within them, which are closely packed and more lanceolate, have a subterminal nucleus and larger glycogen granules (*1*). The latter can be considered characteristic of a resting organism.

The wall of the pseudocyst is considered by some workers as the remains of the host cell wall to which are added some products of the parasite. Others regard it as primarily of parasitic origin. Whatever its origin, the wall of the pseudocyst is argyrophilic and elastic, and at least somewhat resistant to mechanical damage. Also, the psudocyst appears to be more resistant to environmental changes than are proliferative forms (*2*).

According to a number of workers, proliferative forms of *Toxoplasma* die rapidly outside the host and in the carcass of dead animals. These forms are destroyed on drying, on changes in osmotic pressure, and on exposure to low heat. Pseudocysts are also unable to withstand drying and are killed by low heat. However, they may survive in dead tissues for up to 2 weeks or longer at refrigerator temperatures, possibly with less attrition than proliferative forms (*3*). The point of greatest difference is in survival during digestion. It is revealed indirectly by feeding experiments.

Tissues of mice dying of acute toxoplasmosis fed to other mice produce relatively few infections (*4*). However, when tissues from chroni-

Dr. Jacobs is head of the Section on Protozoal Diseases, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service. *Based on a paper presented before the Conference of Public Health Veterinarians, November 16, 1956, in Atlantic City, N. J.*

cally infected mice (*5*) or rats (*2*) are fed to mice, more infections result. Acutely infected animals have mainly or only proliferative forms of the parasite, while in chronic infections only pseudocysts are found. It thus appears that pseudocysts are more resistant to digestion.

Epidemiological Data

Toxoplasma gondii is not only indiscriminate as to the cells it parasitizes, but also as to the hosts it infects. It has been isolated in a wide variety of mammals, including primates, artiodactyla, carnivores, rodents, marsupials; in domestic birds such as chickens, pigeons, and ducks; and in wild pigeons and crows. Additional morphological reports, especially on birds, cover some 45 species, but these have not been confirmed. There are also morphological reports of the parasite in reptiles, which have not been confirmed by isolation. In present-day work, it is expected that, in addition to finding the organism, identification will be established by serologic and immunological methods. Other birds such as the canary and grackles, however, and turtles, terrapins, and chameleons have also been given infections in the laboratory.

Toxoplasmosis occurs among animals in both endemic and epidemic form. Surveys in various localities have yielded a number of isolations of the parasite from pigeons, rabbits, rats, dogs, cats, mice, ducks, and chickens. These isolations have been made from healthy animals apparently carrying latent infections. In addition, epidemics of acute toxoplasmosis have occurred on pigeon, chicken, rabbit, and chinchilla farms. Toxoplasmosis has also occurred among wild hares in Denmark, in herds of cattle in Ohio, and in swine in Ohio and Norway (*2*).

Human infections, as far as we know, occur sporadically. Fortunately, we have not seen epidemics of the disease due to this parasite, and even in individual family groups serologic tests reveal that some members have experienced infections while others have not. Human infection has been found all over the world. Apparently there is continued exposure to the parasite throughout life, because there is an increasing proportion of positive serologic reactions with increasing age. Serologic surveys have revealed no differences in prevalence between the sexes, except for one report regarding a Swedish island in the Baltic Sea (personal communication from Dr. Sven Gard of the Sachs' Hospital for Children and the Institute for Virus Research, Stockholm). The sex difference was not found on the mainland of Sweden.

There are differences in geographic prevalence of toxoplasmosis. There is relatively less infection in California than in the east. Comparisons of various cities of the United States and of such widely separated populations as those in Tahiti, Alaska, Iceland, and Haiti show considerable differences (*6*). For instance, Portland, Oreg., has an overall prevalence of about 18 percent, St. Louis 25 percent, New Orleans 31 percent, and Pittsburgh 36 percent. The island of Haiti has a prevalence figure resembling that of Pittsburgh, while Tahiti shows almost 70 percent infected. Icelanders have a low prevalence, and Alaskans are almost entirely free of antibodies. The prevalence is higher in southern than in northern Sweden.

Whether these differences are related to climate is difficult to determine. Accumulated data suggest that toxoplasmosis is more prevalent in warm, moist areas than in cold or hot, dry areas. The coastal areas of Mexico show higher prevalence rates than the region around Mexico City; and the rate among Navajo Indians in Arizona is surprisingly low. There are clues difficult to decipher at present (*2*).

Because toxoplasmosis has been found in practically all domestic animals, a number of postulates have naturally been advanced on the importance of these animals as reservoirs of the infection for man. To cite only a few, Otten, Westphal, and Kajahn reported finding correlations between canine and human toxoplasmosis (*7*). Cole and associates stated that toxoplasmas were found in the blood of an asymptomatic woman whose dog had earlier suffered an undiagnosed disease and at the time of the study showed *Toxoplasma* antibodies (*8*). In a personal communication, Sabin stated that R. H., the unfortunate boy from whose brain was isolated the widely used strain of *Toxo-*

plasma designated by his initials, had had a cat in his household that died of an undiagnosed infection some time before the boy became ill. After the finding of *Toxoplasma* in swine (*9*) and in cattle (*10*), the postulate was advanced that humans acquire the infection from pork and beef.

An outstanding example of infection among humans and animals in relatively close contact was reported by Gibson and Eyles at the meetings of the American Society of Tropical Medicine and Hygiene in November 1956. The investigation started with a fatal case of congenital toxoplasmosis in a woman in Memphis. Tests on her two older children showed high levels of antibodies, but those on her husband proved negative. The family house was close to a garbage dump frequented by numerous stray animals. Examinations of animals caught in the area resulted in isolation of *Toxoplasma* from cats, dogs, mice, pigeons, ducks, and chickens. Thus the infection appears widespread; it seems that there is a sea of *Toxoplasma* infection around us. In order to evaluate the importance of various animals in the spread of the infection to man, it is necessary to relate the characteristics of the infection in many hosts and also to observe the strain differences in the parasite.

Characteristics of the Infection

The acute stage of the infection is initiated after an incubation period which varies in length depending on the size of the inoculum. During the incubation period, there is local proliferation of the parasite at the site of inoculation. This is followed by the generalized spread of the organism through the blood stream and the invasion of susceptible tissue cells all over the body. In these tissues, parasites multiply, with the production of focal areas of necrosis. In animals succumbing to the infection, parasitemia mounts to high levels. Also, in animals with acute toxoplasmosis, parasites are found in the urine and feces of mice and dogs; in the milk of mice, sows, cows, and bitches; in a serous exudate from the conjunctiva of a pigeon; in saliva of mice and rabbits. They have also been demonstrated in one instance in saliva of man (*2*).

The parasites which are released to the outside from such acutely ill animals are proliferative forms. This form of the parasite is very delicate; it does not survive long outside its hosts. This is attested to by the fact that there is little spread of toxoplasmosis among animals even closely confined in the laboratory. Clean mice in the same jar with infected mice do not become infected. There is only one report of such transmission, in which uninfected puppies caged with a littermate dying of toxoplasmosis eventually became infected (*11*). We have been unable to repeat this observation under similar circumstances. Despite the finding of parasites in the lungs and in saliva of rabbits, an attempt in our laboratory to produce infections in these animals by spraying large numbers of proliferative parasites into a confined space holding clean rabbits did not result in transmission. It appears that only under exceptional circumstances of very intimate contact can transmission be effected from sick animals.

The subacute stage of the infection is characterized by the appearance of serum antibodies and by a clearing of the parasitemia. Following this, there is a gradual clearing of the tissues. This has been measured in rats (*12*), in dogs (*13*), in chickens (*2*), and in pigeons (*14*). We also have unpublished data on guinea pigs and cats, and Eyles and his co-workers have additional information on chickens. In general, the liver, spleen, and lung clear of parasites relatively rapidly, the heart somewhat later, and the brain last of all.

The persistence of parasites in pseudocysts is characteristic of the chronic form of the infection. In the brain of mice, rats, and pigeons, parasites have been found for as long as 3 years after infection, and in the brain of dogs, 9 and 10 months after infection. Occasionally other organs are also found positive. In the liver of a dog, for example, we demonstrated parasites 2 years after inoculation. Unfortunately, not enough studies have been made of skeletal muscle to say how long this tissue usually harbors pseudocysts. There is probably considerable variation from host to host in this respect. For example, we found that the skeletal muscle of chickens clears very rapidly following experimental infection. On the other hand, in tests of chronically infected rats currently underway

in our laboratory, some have shown muscle still infective 6 months after inoculation. We have also found muscle positive in a case of human lymphadenopathic toxoplasmosis at least 3 months after onset of the infection, and Kass and associates found the parasites in the muscle of a fatal human case (*15*). Occasional histopathological findings of parasites in human and animal muscle without any inflammatory reaction in surrounding tissues indicate that pseudocysts may persist in the flesh of apparently cured cases. Because of the variation from host to host, each species of host must be studied individually, and some quantitative data must be gathered as to the relative frequency of this occurrence. It is also necessary to study a wide variety of strains of the parasite in these hosts.

Variations in Parasite Strains

Strains of *Toxoplasma* isolated from various species of animals and birds are biologically and immunologically similar, and on this basis only one species of parasite, *T. gondii*, is recognized. However, some variation in strains has been noted. The main criteria for describing strain differences are the virulence of the parasites for laboratory hosts and the characteristics of the disease produced in them.

A comparison of infections produced by a number of virulent strains and by one of relatively low virulence shows that strains highly virulent for mice usually produce the most severe disease in the other laboratory hosts. There is, in general, a lower parasitemia, less tissue invasion, and shorter persistence of parasites in infections with a strain of low virulence. A sudden change in virulence has been reported by Lainson who found that avirulent strains isolated from rabbits were greatly enhanced in virulence by passage through multimammate rats (*16*). Strains isolated from sick animals in nature are frequently highly virulent for experimental hosts. Strains isolated from animals without disease are more likely to produce latent infections in the laboratory. This has been our experience with strains isolated from a dog and from pigeons. Similar reports have been made concerning strains from rabbits, guinea pigs, dogs, cats, and other natural hosts. Since surveys have revealed considerable natural infection in man and animals, and relatively little disease, it seems reasonable to conclude that the parasite as it exists in nature is well adapted to its hosts and that it produces little disease unless it undergoes some spontaneous change or encounters a host which is peculiarly susceptible.

The susceptibility of the host is important. Erichsen and Harboe described an epidemic of toxoplasmosis in a flock of chickens in Norway (*17*). The parasite was isolated and maintained in the laboratory in mice but failed to produce fatal experimental infections in chickens, even when birds were used of the same breed as those on the affected farm. In our laboratory, we have been unable to produce disease in chickens even with large inoculums.

Toxoplasmosis in Dogs

Mello first described spontaneous toxoplasmosis in a dog, with diarrhea, emaciation, anemia, dyspnea, and abdominal tenderness (*18*). Since this report, over 50 cases of the infection in dogs have been reported from all parts of the world (*16*, *19*). Some of these cases were fatal, and others merely were coincidental findings of parasites in histological sections of tissues of dogs dead of other diseases.

There is also serologic evidence that dogs are frequently found infected in nature. Miller and Feldman found dye test antibodies (titers of 1:16 or more) for *Toxoplasma* in 59 percent of 51 dogs at Syracuse, N. Y. (*20*). Siim found 18.5 percent of 54 dogs in Copenhagen had relatively high dye test titers, 1:250 or more (*21*). Otten and associates in Hamburg, Germany, found 35.7 percent of 84 dogs positive by the same test (*7*). Morris and associates examined, by the complement fixation test, 180 dogs from the Middle Atlantic States, and found 25 percent infected (*22*). Lainson, in England, found 42.5 percent of 113 serums from London dogs positive by the complement fixation test (*23*).

Thus, the dog merits attention as a possible reservoir of the infection for man. Miller and Feldman (*20*) and Jacobs and associates (*13*) have pointed out that the widespread occurrence of *Toxoplasma* antibodies among humans and dogs makes it difficult to decide in any particu-

lar case that an infection in a canine pet is related directly to human infection. Nevertheless, a number of instances can be cited from the literature in which "sickness" in a dog occurred in a household where there was a human case of toxoplasmosis. Westphal and Finke report that a woman with a dye test titer of 1:100 had an abortion of an infant with hydrocephalus; the woman's husband and pet dog also had positive tests (*24*). In another case of congenital toxoplasmosis proved by isolation of the parasite, three dogs in the household of the mother had suffered a disease considered clinically, by hindsight, suggestive of toxoplasmosis; the dogs were not tested for antibodies.

In still another case, a sick dog was found in the home of a woman who had borne a hydrocephalic infant. They also reported that a woman with nervous symptoms and a dye test titer of 1:200 had 6 months previously lost a dog which was diagnosed as having distemper. Prior and associates reported 3 cases diagnosed as toxoplasmosis, 2 in women and 1 in a child (*25*). The women both had dogs which were found to have toxoplasmosis. A human case of myocardial toxoplasmosis in England (*26*) proved by isolation of the parasite from the heart (*27*) had been associated with a dog sick with vomiting and diarrhea. Miller and Feldman also cited a sick dog in a household where a case of congenital toxoplasmosis occurred (*20*).

In all of these cases, no evidence was presented that the disease in the animals was definitely toxoplasmosis, except for the report of Prior and associates. Their study presents its own problems in that the dye test titers found in their two adult cases were much lower than those reported by others in proved cases of systemic toxoplasmosis in adults, either mild or acute. Also, the dye test titer in the dog of one of these cases was again surprisingly low relative to the duration of the illness. These reports stimulate further investigation.

Because of the widespread and frequent occurrence of *Toxoplasma* antibodies in both humans and dogs, any correlation between the infection in these two hosts depends on extensive survey techniques. Otten and associates tested 38 people whose dogs had *Toxoplasma* antibodies; they found 23 had dye test titers of 1:25 or higher (*28*). Unfortunately, they had no control group of people in the same age groups and circumstances, and the survey is small. Otten and associates also reported that 5 of 6 veterinarians, 3 of 5 veterinary assistants, and 3 of 3 kennel keepers all had *Toxoplasma* antibodies demonstrable by the dye test. Cole and associates in this country also reported a correlation between *Toxoplasma* antibodies in pets and their masters (*8*). It appears from these studies that there may be a relation between human and canine infections. However, there have been cases of human toxoplasmosis which have not had even remote contact with dogs. This suggests that if the dog is a reservoir of infection for humans, it is not the only such reservoir. Because of their association, dogs and man may acquire toxoplasmosis from the same source or sources.

Experimental canine toxoplasmosis has been studied by a number of investigators with similar results. Nicolle and Conor (*29*), Boez (*30*), and Westphal and Finke (*24*) could not produce disease in mature dogs by the inoculation of virulent parasites by various routes. Laveran and Marullaz produced disease by the intravenous inoculation of parasites (*31*). Chamberlain and associates were able to produce acute toxoplasmosis in 5 bitches 1½ to 2 years of age, by the intravenous inoculation of large numbers of parasites supplemented by additional injections subcutaneously or intraperitoneally, or by oral administration of as many or even larger numbers of organisms (*32*).

Even with these efforts, only two deaths occurred among these animals and one of them remained entirely asymptomatic. In 4 of 8 puppies 45 days of age or less, Jacobs and associates produced acute toxoplasmosis by intravenous inoculation, in 3 instances, of 100,000 parasites of the RH strain, and the fourth puppy succumbed to an intravenous inoculation of 10,000 parasites plus a concomitant infection with 200 hookworm larvae (*13*). Other puppies, including a littermate of the fourth puppy, 5 weeks to 3½ months old, survived intravenous inoculation with 10,000 parasites, or in the case of one 65-day-old dog, 2 million parasites.

Thus, it appears that canine toxoplasmosis is difficult to reproduce in the laboratory, similar

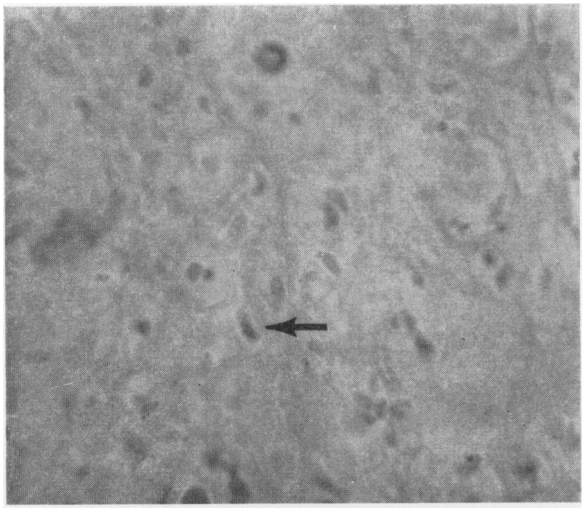

Figure 1. Proliferative forms of Toxoplasma gondii in necrotic liver tissue from a mouse. Arrow indicates one of the organisms.

to the phenomenon mentioned above in regard to chickens. Possibly it is explainable on the basis of sudden changes in virulence described by Lainson (*16*) or by the existence of concomitant viral infections, as noted by Seibold (*33*) and others.

Although puppies with acute toxoplasmosis had parasites in their urine and feces, Chamberlain's bitches had infective milk, and possibly the saliva of dogs can contain toxoplasmas when the lungs are heavily parasitized, these findings are generally limited to animals in the acute stage of the infection, and to those with severe disease symptoms. In our experimental studies on dogs surviving the infection, we could not demonstrate parasites in the feces or urine, even though isolations could be made for at least 2 weeks from the lung, liver, and spleen.

The course of the infection in dogs begins with a period of parasitemia lasting up to 2 weeks, characterized by a generalized spread of the parasite. At about 2 weeks, antibodies appear, the parasitemia ceases, and there appears to be a diminution of parasites in the tissues, as revealed by longer survival time of mice inoculated with them. However, some parasites remain in pseudocysts and can be occasionally isolated from the brain or other organs long after infection. Older dogs were not found to have a parasitemia.

These results are consistent with the usually asymptomatic toxoplasmosis found in dogs in nature. It is hardly likely that natural infections are initiated with tremendous numbers of parasites. Hence, when evidence of past exposure to *Toxoplasma* is found in an apparently healthy animal, there is little reason to suspect that the dog experienced an acute symptomatic infection. Since only acutely ill dogs exude toxoplasmas in their excretions and secretions, and since the proliferative toxoplasmas survive only briefly in the external environment, it seems unlikely that acute disease in dogs is a usual source of human toxoplasmosis. Furthermore, our attempts at producing infections by feeding mice urine and feces of dogs with asymptomatic infections resulted in failure. We therefore lack good evidence of any mechanism by which canine toxoplasmosis can be transmitted to man. Indeed, Feldman and Miller have found that Navajo Indians in Arizona have a very low prevalence of *Toxoplasma* antibodies, while their dogs show a relatively high prevalence (*34*).

One feature of canine toxoplasmosis deserving special mention is the intestinal ulceration frequently reported in spontaneous cases of the infection. This has also been reported in other carnivores, such as cats, foxes, and ferrets. This suggests, first, the need for more studies to determine whether a resistant form of *Toxoplasma* can be shed in the feces from such intestinal lesions, and secondly, whether these animals acquire toxoplasmosis by the ingestion

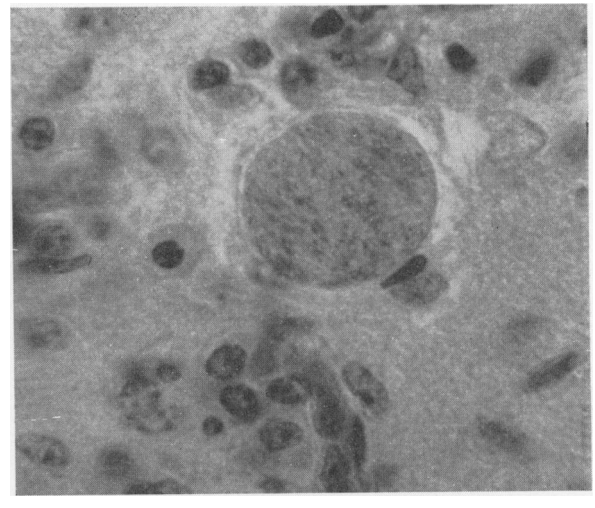

Figure 2. Pseudocyst of Toxoplasma gondii in the brain of a mouse.

of infected flesh. The latter hypothesis has support in the observation of Lainson that 2 of 4 dogs with *Taenia* infections had *Toxoplasma* antibodies, while only 1 of 10 dogs without *Taenia* was serologically positive (*23*). It also has support from already mentioned data on the successful infection of mice by feeding them brains of chronically infected mice and rats, and from some recent studies of the same type using various muscles of rats with latent infections (*35*). Furthermore, we have seen, in at least one dog, a rise in dye test antibodies following the feeding of infected tissue. Therefore, the source of toxoplasmosis in carnivores could conceivably be small animals serving as their prey, or infected meat from larger animals. And, if carnivores can be so infected, what is the situation in regard to omnivores such as man? This brings us to an evaluation of present data on swine and cattle toxoplasmosis.

Toxoplasmosis in Swine and Cattle

Toxoplasmosis was first reported in swine by Farrell and associates in Ohio (*9*). Eleven animals were involved, all of which came from one herd where a recurring undiagnosed disease had existed for many years. Parasites resembling *Toxoplasma* were first observed in pathological sections of a gilt which had died of the disease. Thereafter, similar organisms were seen in 7 additional pigs, 2 of which had died, and the others of which had been destroyed. Suspensions of tissues from eight affected pigs were inoculated into mice. The organs used for these isolation studies were brain, heart, lung, pooled liver and spleen, mesenteric lymph node, and kidney. The pooled liver and spleen inoculum from one pig and the heart of a second pig produced toxoplasmosis in recipient mice. The organisms were identified biologically and immunologically as *Toxoplasma.*

One healthy pig from another farm was inoculated with mouse tissues containing this porcine strain of *Toxoplasma.* At one month, the dye test titer of this animal was 1:160, and toxoplasmas were isolated from its tissues by mouse inoculation. The parasite was also found pathogenic for 2 more healthy pigs, 2 dogs, and 87 mice. In a second report from Ohio, Sanger and Cole stated that they had isolated *Toxoplasma* from some pigs of a naturally infected sow (*36*). Also, the parasite was reported found in the milk of 2 naturally infected sows and 1 experimentally infected gilt, and from the placentas of 2 experimentally infected gilts.

Another outbreak of toxoplasmosis has recently been reported in swine in Norway by Momberg-Jorgensen (*37*). Eleven piglets were involved in this epidemic. In neither this nor the Ohio reports were the hog-feeding practices on the affected farms mentioned.

In addition to these reports, Weinman and Chandler have presented experimental and observational evidence representing an apparently impressive indictment of the pig as a source of human infection (*38*, *39*). In the first study they fed seven young pigs repeatedly with tissues from mice and rats infected with the RH strain of *Toxoplasma.* In two of these pigs, toxoplasmas were later isolated from the brain; in a third the blood was infective to mice on the seventh day after feeding. The other four pigs were all negative in mouse inoculation tests, but there were some increases in dye test titers. Unfortunately, it is not clear from their report whether or not muscle of these pigs was tested for the presence of parasites. However, in another experiment reported in the same paper, they studied an additional seven pigs, all of which they inoculated with millions of parasites (*38*).

One infection of a 15-pound pig ended fatally in 6 days following intracardiac inoculation. In this case, it was reported that toxoplasmas were isolated from ham as well as from brain, heart, lung, liver, and spleen. Three of the other pigs which received these parenteral inoculations were examined for the presence of parasites. In one, the brain was found positive; in another, the blood on the 6th day and the lung on the 42d day; the 3d was negative. Again, no mention was made of whether or not other tissues, such as striated muscle, were tested. Older animals were regarded as less susceptible to infection; the pig found negative after parenteral infection was 4 months old when first used.

In their second paper, Weinman and Chandler report on finding 42 percent positive for

Toxoplasma antibodies among 88 hog serums obtained at a slaughterhouse in New Haven, Conn. (*39*). Furthermore, most of the hogs with antibodies were from one farm, where 16 of 21 pigs examined (over 76 percent) had dye test antibody titers of 1:64 or higher. This farm did not cook the garbage it used as feed, and in addition the premises were overrun by rats.

Weinman and Chandler liken toxoplasmosis to trichinosis and consider it probable that there is transmission from swine to swine, rat to swine, and swine to man. They adduce as additional supportive evidence for this hypothesis certain serologic data on humans. Forty specimens of human trichinosis serums were tested for dye test antibodies; of these, 23 percent were positive at titers of 1:64 or higher, and 18 percent were positive at 1:256 or higher. Because these serums came from all over the United States, they could not assemble an adequate control group of nontrichinosis serums. However, they compare their data with those of Feldman (*6*) for Portland, Oreg., and New Orleans, and with those of Jacobs and associates (*40*) for non-pork-eating orthodox Jews. They conclude that their sample has a higher incidence of high dye test titers than these groups, and presume their sample was of older age and therefore should have had lower titers. There is no justification for such a presumption in regard to the Jewish serums, which were practically all from people in age groups from 45 to over 70 years.

Although this thesis is based on circumstantial evidence, some of which is subject to criticism, this evidence represents more positive information than has been obtained in tests of other postulated mechanisms of transmission, such as by arthropods, contaminative means, or droplet infection. There are, however, certain contrary data which require reconciliation with this hypothesis.

First, the prevalence of *Toxoplasma* antibodies, as already mentioned, varies from city to city within the United States. It is higher in the east than it is on the west coast, and higher in the south than the north. On the other hand, the prevalence of trichinosis in the northeastern States and in California is about the same (*41*), while it is lower in the southern than the northeastern regions. Secondly, the highest prevalences of *Toxoplasma* antibodies have been found in areas such as Tahiti or Guatemala, among populations that only rarely consume meat or do so only when it is well cooked. Third, in a survey of orthodox Jews in the older age groups in New York, 48 percent were positive for *Toxoplasma* antibodies (*40*).

The explanation offered by Weinman and Chandler for such discrepancies is that while pork may be one of the more important modes of transmission of human toxoplasmosis, several other sources may exist. Therefore, the failure to find correlations with pork is not definitive. There is merit in their argument. The high prevalence in Tahiti and Guatemala and in persons who do not eat pork can mean that another method of transmission is involved.

We have, therefore, attempted to determine whether meat other than pork accounts for the high prevalence in persons who do not eat pork. We tested the serums of a group of vegetarians belonging to the Seventh Day Adventist sect, and our findings are reported here for the first time.

Forty-six specimens were obtained, mostly from people over 40 years of age who had not eaten flesh for at least 20 years. The percentage of positive dye tests in this group was 21.6, somewhat lower than might be expected in the general population. However, we do not have a good control series for comparison because of the widely different geographic origins of the individuals involved.

Among the positives, high dye test titers were found. The titer distribution is as follows: 1:16, 1 serum; 1:32, 1 serum; 1:64, 3 serums; 1:256, 4 serums; 1:1,024, 1 serum. The highest titer was obtained in a woman 31 years of age who had never eaten meat in her life. The titers of 1:256 were obtained in people who had not eaten meat for 30 or more years. Thus, even though the percentage of positives in this vegetarian population is small, these high titers point to another means of acquiring *Toxoplasma* infection than by ingesting meat.

The gap in the experimental evidence presented by Weinman and Chandler is their failure to report or find parasitized muscle in their infected pigs. Since the distribution of parasites in tissues varies according to type of host,

we cannot presume that toxoplasmas persist in the skeletal muscle of pigs merely because they have been found in the brain or lung. Furthermore, the finding of infected muscle in an animal dying of acute toxoplasmosis on the sixth day following intracardial inoculation of large numbers of parasites is not adequate evidence for presuming that parasites become distributed throughout the muscle of pigs with asymptomatic infections. Finally, repeated feedings or inoculations of heavily infected material do not duplicate natural conditions. Experimental studies should be prosecuted with smaller inoculums.

In connection with toxoplasmosis in cattle, the report of Sanger and associates concerned four herds in Ohio (*10*). A 4-year-old cow from one herd, which reacted positive to the toxoplasmin skin test, was killed 14 days after she bore a calf. She had no visible illness, and no organisms were recovered by mouse inoculations from heart, brain, liver, spleen, ovary, lymph nodes, adrenals, or skeletal muscle. Microscopic bodies, considered *Toxoplasma*, were, however, reported seen in the uterine wall, spleen, and lung; and 1 of 8 mice inoculated with colostrum from the right front teat developed toxoplasmosis. None of eight mice inoculated with colostrum from the other teats became infected. The calf was killed following birth, and one mouse inoculated from it died of toxoplasmosis; it is not clear from the report which of the organs used, brain, heart, liver, spleen, mesenteric node, or kidney, was the source of the infection. Toxoplasmas were found only in the liver on microscopic examination. In the same herd, 3 cows between 3½ and 5 years of age developed nervous disturbances and died, but no infections could be found in them. Also, of 31 calves born in this herd, 3 were born dead, and 4 developed an obscure illness from which 2 died. No evidence of infection was found in them.

In a second herd, there was a history of continual sickening and death of newborn calves. The symptoms were dyspnea, coughing, sneezing, nasal discharge, and frothing at the mouth, trembling, shaking of the head, dehydration, and occasionally diarrhea with blood and mucus. Forty-five of 78 calves died between the ages of 1 day and 6 months. One 4-week-old calf was killed and its tissues tested by mouse inoculation. Brain, heart, liver, lung, spleen, kidney, pericardial fluid, and cerebrospinal fluid were inoculated separately into mice. Toxoplasmas were recovered in mice inoculated from the lung but not from the other organs. Histologically, toxoplasmas were reported in lesions of the brain, lung, and bronchial lymph node.

In the third herd, one bull developed anorexia, weakness, ataxia, prostration, chewing movements, and bicycling. It died 1 week after onset of illness. Microscopic examination revealed both free and intracellular organisms identified as *Toxoplasma* in the brain.

In the fourth herd, a 7-year-old cow died 2 weeks after parturition. The symptoms were anorexia, diarrhea, depression, fever, and mastitis. *Toxoplasma* was demonstrated microscopically in the lungs, myocardium, pericardium, kidney, gastric lymph node, and stomach; apparently no mouse inoculations were attempted. Some calves in this herd later died of an undiagnosed illness.

In an attempt to determine if *Toxoplasma* alone could be responsible for the symptoms seen in these herds, Sanger and his co-workers inoculated, by various routes, four healthy calves 4 to 90 days old with infective material from mice. Two control calves received a Seitz filtrate of the inoculum. The four test animals developed respiratory and nervous system disease similar to that previously observed. Two died and two recovered. Organisms identical with those seen in spontaneous cases were found microscopically in the lung, brain, liver, and spleen of 3 of the calves, and toxoplasmas were isolated from tissues of 2 of them.

The isolation of toxoplasmas only from the colostrum of a cow, even though the parasites were seen microscopically in other organs, is surprising. It is generally easier to isolate parasites than to detect them in sections. Perhaps this result is explainable on the basis that the inoculations and sections were done from widely separated pieces of the organs; or that strains of *Toxoplasma* in cattle are not well adapted for growth in mice.

This latter postulate could also serve to explain the other isolation failures when organisms could be demonstrated histologically.

However, it is hardly acceptable in the experimental studies where calves were inoculated with materials from infected mice. It is unclear from the paper whether all the isolations were made from the calves which died of experimental infection, or from those which survived.

Obviously, more work must be done on toxoplasmosis in swine and cattle. Experimental data are needed on the distribution of parasites in the muscles of pigs and cattle following inoculation with small numbers of parasites and in the absence of symptoms. Survey data are necessary on the presence of *Toxoplasma* pseudocysts in meat bought at market. Even if meat is involved, we certainly require considerable new information to explain the other means of transmission which must exist.

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Studies conducted in the Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, subsequent to the preparation of this discussion have recently led to the development of a technique for the survey of meat samples to detect *Toxoplasma.* The parasite has been isolated from diaphragm muscle of 11 of 50 pigs slaughtered at a Baltimore abattoir. A similar survey is being conducted on beef (*42*).

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Grants for Health Research Facilities

To encourage expansion of the Nation's health research facilities, the Public Health Service has approved 100 grants for the fiscal year 1958, on the recommendation of the National Advisory Council on Health Research Facilities. The grants, totaling more than $26 million, were awarded to 77 institutions, including hospitals, universities, research institutes, and schools of medicine, dentistry, and public health in 30 States and the District of Columbia.

The awards open the second phase of a 3-year program authorized by the 84th Congress. Each year the program receives $30 million "to assist in the construction of facilities for research in medicine, osteopathy, dentistry, and public health, and in fundamental and applied sciences when related thereto."

A total of 109 research facility grants to institutions in 31 States was previously awarded under the appropriations for fiscal 1957.