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In earlier published works (Galuzo and Krivkova, 1968) we tried to show the role of avirulent forms of Toxoplasma in the circulation of the species in nature. Our conclusion from the previous research led to the following: "The basic factor determining the circulation of Toxoplasma in the natural nidus is the avirulence of the population of the species... and the alimentary route of transmission of the infectious agent."

In the present work we introduce supplementary information in support and development of that position. New data are given on the behavior of avirulent Toxoplasma which get into the recipient host by alimentary means, allowing the species to broaden its circulation in nature by including grass-eating animals (hoofed and rodent species) into its circle of recipients.

In addition, new and supplementary forms of Toxoplasma development are revealed, which in turn shed light on the matter of determining the species' place in the system of protozoa.

The Alimentary Infection Path

Our own research in past years has shown that avirulent Toxoplasma, being in the encysted form, are usually dispersed thruout all the organs and tissues of the body--from the brain to the blood vessels, from the lymph nodes to the heart muscles. This peculiarity in the behavior of the parasite within the donor's body furnishes it with a wide range of routes for transmission from donor to recipient. By feeding the flesh and other tissues of infected animals or whole small animals--Toxoplasma carriers--it is simple to infect healthy ones.

The results we achieved agree with those established earlier by Jacobs (1957), showing that infection per os from chronically sick animals is always more successful than from acutely sick animals. The mechanism of this phenomenon was subsequently set down by Jacobs, Remington and Melton (1960) and by Remington et al. (1961). They showed that Toxoplasma cysts survive significantly longer than the trophozoites in animal tissues, and that the merozoites in Toxoplasma cysts are not destroyed by the peptic fluid, whereas the trophozoites die in this medium within a few minutes.

These observations make it possible to assume that the presence of avirulent forms of Toxoplasma and an alimentary mechanism for their circulation in nature are the parasite's natural means of adaptation.

However, even this is not the whole story. It is true that animals can and do get sick from eating other animals or their products--meat, milk, eggs. But how do the grass-eaters--the hoofed animals and the rodents--become infected?

Fecal Forms of Toxoplasma

V. M. Hutchison (1965) has priority in discovering fecal forms of Toxoplasma. He found that Toxoplasma can be transmitted with the feces of cats which became infected by eating mice chronically infected with Toxoplasma. He also (1967) advanced the hypothesis that Toxoplasma is transmitted from the donor to the recipient thru the egg of the nematode Toxocara cati (for details on this see Galuzo and Bezukladnikova, 1970). Further research (Jacobs, 1967; Hutchison et al., 1968; Dubey, 1968; Frenkel, Dubey and Miller, 1969 et al.) has shown that infection can occur even in the absence of nematodes in cat feces.

Microscopic studies on the feces of infected cats permitted D. Siim and others (Siim, Hutchison and Work, 1970) and Frenkel and others (Frenkel and Dubey, 1970) to observe in the feces a large number of cyst-shaped structures which resembled the oocysts of coccidia. Moreover, they also saw the development of these structures outside the host; it resembled the development of the coccidium Isospora.

Our research, conducted over the last few years, is represented by data from the following experiments.

Experiments with hamsters. The strains kept in our laboratory, the avirulent PDP, VFG and LTJ strains, were isolated respectively from sparrows, a fox and a hare and were kept alive in white mice.

In one experiment the brain of a white mouse infected with strain PDP was fed to 2 golden hamsters; in the second the same method was used to infect another 2 hamsters with VFG; in a third LTJ was used to infect a 3rd pair of hamsters. No Toxoplasma oocysts were found in any of the careful daily studies of feces that were carried out.

In the last 2 experiments Toxoplasma cysts were observed in the brains after the animals were killed on the 12th and 23rd days.

Similar experiments were 3 pups, 6 rabbits, guinea pigs and many white mice did not yield positive results. In not one case could we succeed in infecting any of these animals with the same strains transmitted fecally.

Experiments with kittens. A pair of 2-month-old kittens was fed on the brain of a white mouse carrying the avirulent form of Toxoplasma strain PDP. Within 3-5 days the feces of these animals showed many of the fecal forms of Toxoplasma. A large number of them were kept for 3 or 4 days.

On the 5th day there were fewer of them, and by the 6th to 7th day they had disappeared entirely. In several days the Toxoplasma again appeared in the feces of the kittens and quickly disappeared. The same picture was observed in several other infected kittens.

Two kittens from this series of experiments died within 2 days after inoculation. No toxoplasmas were found in their parenchymatous organs, nor were cysts found in their brains. In one case a general infection was found in a kitten which died on the 12th day. A large number of proliferative forms was found in the various organs.

Exogenous Development of the "Fecal Forms"

Cat feces taken on a day when large numbers of fecal forms were present were separated from the organic masses by flotation and centrifuging, then washed in tap water or physiologic NaCl solution and allowed to stand at room temperature (20-25 C).

On the first day--the day of exit from the intestine with the feces of the host--it was possible to see an exceptionally uniform type of oocyst-shaped organism (Fig. 1) measuring $10 \times 13-14 \mu$. The wall was soft and its stratification was hard to establish. A micropyle was never observed. The protoplasmic mass was concentrated in the center of the cell and was soft and granular. Protoplasmic structures such as the nuclei were not distinguishable (Fig. 2).

On the second to third day it was possible to observe dividing forms--sporogony--in the fecal culture. The protoplasmic mass of the cells divided into 2 sporocysts. They were most often spherical, but some were stretched or ovoid (Fig. 2). The spores were separate and had sharply outlined contours. There was no trace of a residual body.

On the third to fifth day some isolated sporocysts contained markedly opaque bodies. It was hard to establish their number, but they could be counted in some individuals. In others, however, the protoplasmic mass grew coarse, and many large, angular granular particles formed. They also were opaque.

Some sporocysts left the oocysts when a break occurred in the oocyst wall. In this instance their form strongly resembled that of the common Toxoplasma cysts (Fig. 3) (these observations require supplementary checking). Siim and Hutchison (1970) observed division of sporocysts in the manner of Isospora into 4 sporozoites in histosections of fecal forms.

Our observations, conducted after a month or more, enabled us to see new forms of development of the fecal forms. Instead of two sporocysts per oocyst, all within the same wall, we clearly saw a large number of rough, rather large bodies, randomly distributed and almost completely filling the cavity of the oocyst (Fig. 3). Under the light microscope it was hard to distinguish their structure, nor did we see a residual body or traces of a sporocyst wall. Digestive fluid did not rupture the wall of

such adult oocysts, but we did see movement and mixing of these bodies within the wall of the fecal form (oocyst). Apparently this phase of breakdown is the final stage in the exogenous development of the fecal forms.

Discovery of fecal forms of Toxoplasma--a phase of development in the intestine of the cat and outside the host organism-- enables us to close up the chain of the life-cycle of this species, consisting of complex phase-links.

The proliferative phase is the division of the trophozoite. It occurs in various tissues of the host. The encysted phase occurs in various tissues, most often the brain, diaphragm muscle and other organs, in the host. The phases of schizogony and gametogony with zygote formation occur in the epithelium of the small intestine of the cat (fastest of all predatory mammals and birds). The sporogony phase with formation of sporozoites occurs outside the body of the host. The sporozoites get into the body of a new host in the form of trophoblasts, and the cycle repeats itself anew. Is it possible to call cats the final hosts, since a new phase of development occurs in them, while all other mammals and birds are only intermediate? Apparently not, because in the cat, just as in all carnivores, asexual reproduction can take place. Moreover, Frenkel, Dubey and Miller (1970) succeeded in infecting a cat with feces from another cat containing Toxoplasma oocysts. It is not possible for one species of animal to be both final and intermediate host for the same species of parasite.

In order to establish whether these forms belonged to the toxoplasmas or the coccidia, we used them to infect susceptible animals.

In this series of experiments we used feces of kittens containing a large number of fecal forms. Feces from a kitten with a large number of fecal forms were placed in a culture. The same day after several hours material from the culture was used to infect two batches of white mice: one by feeding, the other by introducing it into the peritoneal cavity. Neither group of mice developed infections. No Toxoplasma were found in any of these mice when they were killed 10 days later.

Three weeks after the feeding on this same culture of fecal forms a new group of white mice was exposed. All of them became infected but none died. Toxoplasma cysts were found in the brains of all the mice when they were killed on the 15th day.

The experiment with susliks was especially indicative. Three susliks (Citellus fulvus) were exposed by feeding them material from a 2-week-old culture of cat feces. On the 6th day after exposure one of the susliks died; the 2nd died on the 7th day and the 3rd on the 3th day. An unusually large number of Toxoplasma trophozoites was observed in the peritoneal cavity and all parenchymatous organs of the dead animals. Toxoplasma was also found in scrapings from the small intestine.

In the same series of experiments we used rabbits, dogs, cats and hoofed animals. Two rabbits which had shown a negative CFR prior to exposure were fed material from a month-old culture of cat feces containing fecal forms. After 7 days they reacted clinically.

They were killed 15 days after inoculation. All of them reacted positively to the CFR, and their brains contained Toxoplasma cysts. Infection was confirmed also by bioprobe on white mice.

The same experiments were performed on hoofed animals (6 sheep, 3 goats, 2 calves, 2 donkeys, 3 swine, 6 saiga, 2 mountain goats, 3 arkhar, 2 roe deer) by our colleague A. Polomoshny; they also gave positive results with a marked clinical picture. Many of the animals died. Infection was confirmed by positive CRF and bioprobe on white mice. We did not succeed in infecting dogs fecally.

The results of our experiments give us the right to state that Toxoplasma has a unique form of sexual development, similar to that of coccidia, and that this form, being excreted with the feces from the host, passes thru an exogenous phase of development, the products of which are pathogenic for grass-eating animals (in our experiments for rodents, white mice, rabbits, hoofed animals, hamsters and especially for susliks). This is the path of Toxoplasma transmission from carnivores (this time cats) to grass-eaters which supplies the parasite with great possibilities for circulation in the wild and at the same time reveals the sources of infection of domestic grass-eaters. Apparently this happens thru contamination by cat feces, but possibly other animals, feed or water may play a part.

These studies support our expressed position that Toxoplasma has a sexual phase in its life cycle and that it belongs to the spore-bearing class. However, toxoplasmas are not coccidia.

True, coccidia and Toxoplasma share certain structural resemblances in their exogenous forms (oocysts), but their life cycles, as shown above, are completely different. Not one representative of the order Coccidiida has a phase of asexual development, proliferation and encystment as is seen in Toxoplasma. The phase of sporogony outside the body of the host in Toxoplasma proceeds in a slightly different manner from what we see in coccidium Isospora. The phases of schizogony and gametogony, which have been observed by Hutchison and Siim (1970) and Frenkel (1970) have hardly been studied at all, so that for the time being to talk of their being the same as the endogenous phases of coccidia is impossible. If we take into consideration here the narrow host specificity and territorial preference which are characteristic of representatives of the order Coccidiida, and their strict organotropism, which Toxoplasma does not display, then it becomes obvious that at the ordinal level coccidia and Toxoplasma are completely different. All this makes it possible to establish them as a separate order of the Sporozoa, Toxoplasmoda. The possibility is not excluded that many of the so-called toxoplasmod organisms will take their places in this order at the level of families or perhaps sub-orders. Toxoplasma, however, can properly be assigned to an autonomous family, Toxoplasmodae.

Thus, T. gondii gropes about for its place in the system of Protozoa. It retains its generic name Toxoplasma, which represents the family Toxoplasmodae of the order Toxoplasmoda of the class Sporozoa.

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Fig. 1. Oocyst forms of Toxoplasma in cat feces.

Fig. 2. Oocyst-like forms of Toxoplasma in cat feces on 3rd day after introduction. Dividing forms.

Fig. 3. Oocyst-like forms of Toxoplasma in cat feces. Multiple fractionization of oocyst protoplasm.