EXPERIMENTAL CONGENITAL TOXOPLASMOSIS

I. The Vagina as a Portal of Entry of Toxoplasma in the Mouse*

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Experiences with human toxoplasmosis during the past decade have tended to confirm the early impression that the disease is largely, although not exclusively, one of early life (1–5). Clinical and postmortem observations suggest that many, if not most, of the affected infants and children acquire the infection *in utero* during the latter part of gestation. At the time of birth advanced inflammatory and necrotizing lesions may already have developed in the central nervous system, eyes, and other tissues (6, 7). The children who survive often exhibit a characteristic group of clinical manifestations of which the most conspicuous are internal hydrocephalus, focal chorioretinitis, convulsive seizures and x-ray evidence of calcification in the brain (8). A variety of other neurological, mental, ocular, and somatic abnormalities may appear.

Although the clinical and pathological features of toxoplasmosis in the infant are gaining wider recognition, virtually nothing has been learned, as yet, of the natural mode of transmission of the infection to the fetus. While antibodies to *Toxoplasma* can be demonstrated in the mother's serum in many cases (6, 8–11), as well as in that of the infant, the mothers of affected infants have quite uniformly been in good health before, during and after pregnancy. It can be presumed that infection endemic in lower animals furnishes a source from which the parasite is somehow transmitted to the human being, but it has been impossible to determine with certainty in any case, the specific animal host involved, the portal of entry, and the nature of the pathological process, if any, in the mother. Moreover, an opportunity has not arisen, in studies of human material, to establish the presence of placental infection with *Toxoplasma* in histologic sections, or to demonstrate directly that transplacental passage to the fetus has occurred. Placentas of infected infants have not been available for examination; those obtained at subsequent deliveries of normal infants have been studied in a few instances with negative results (12).

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The work described in this series of reports was undertaken in an effort to determine whether experimental toxoplasmosis could be produced in the intact fetus by intermediate infection of the mother during pregnancy, and, if so, to study the conditions under which such transmission took place. In preliminary experiments various routes of infection were tried, including the intracerebral and intravenous, but these proved to be impractical because of the constant and early death of the mother animal. Further, it was thought desirable to select a route of infection which might perhaps serve as a portal of entry under natural conditions in the human being. For this reason the vagina was chosen. The use of the vaginal route also provided an opportunity to observe whether or not direct extension of toxoplasmic infection from the lower genital tract to the uterine contents occurred, a means of spread which has not been excluded in the human being.

The present report deals with the incidence and general characteristics of toxoplasmosis in the female mouse infected by the vaginal portal, while the companion papers are concerned with the pathology and pathogenesis of the disease in the adult animal (13), and with the transmission of toxoplasmosis to the placentas, fetuses, and offspring of mice infected by way of the vagina during pregnancy (14–16).

Experimental Procedure

Source of Toxoplasma.—Three strains of *Toxoplasma*, each originally derived postmortem from the brain of a human infant dying of toxoplasmic encephalitis, were available for use in the experiments. They included the original BD strain isolated in 1938 (1), the LM strain, isolated in 1939 (3), and the TR strain isolated in 1944 (12). Each had been propagated by consecutive serial passages in young white mice weighing 12 to 22 gm., and had undergone no apparent reduction in pathogenicity for this species during the ensuing years. Each passage was effected by the combined intracerebral (0.02 cc.) and intraperitoneal (0.2 cc.) inoculation of an approximately 10 per cent suspension of infected mouse brain in sterile physiological salt solution. Under these conditions the mice became ill or died of disseminated toxoplasmosis 5 to 6 days after injection. Thus, by maintaining several lines of these source animals, *Toxoplasma*-infected mouse-brain suspension was regularly available for the production of infection in pregnant and non-pregnant mice by the vaginal route. It soon became clear that there was no significant difference in the effectiveness of the three strains of *Toxoplasma* employed; therefore no further reference will be made to the particular strain used in the individual experiments.

Experimental Animals.—A stock of mature female white mice weighing 27 to 32 gm. or more constituted the source from which individual animals were selected for breeding and infection. In the early stages of the work, small groups of females were simply caged with a male until external signs of pregnancy, such as enlargement of the abdomen and nipples, became evident. In order to obtain animals in the early period of gestation, however, and to have more exact information as to the stage of gestation at which the mother animal was subjected to infection, it became necessary to make daily records of the estrous cycle of each stock animal by the vaginal smear method. When the smear showed an animal to be in estrus, it was caged overnight (usually 20 to 22 hours) with a normal male. The following morning, designated the 1st day of pregnancy, the female was removed and isolated in an individual cage for subsequent

use at some time during its 19 to 20 day gestatory period. Observations were made on a total of 365 mice subjected to vaginal infection with *Toxoplasma* at different periods with respect to the time of mating. The intervals selected varied from a few hours before, to 18 days after mating, but most of the animals were exposed between the 6th and the 10th day, inclusive. Observations were also made on a group of 62 virgin females infected by the same technique.

Method of Infecting Animals.—The animals were infected by the introduction of *Toxoplasma*-infected mouse brain suspension into the vagina. Two methods, the first less efficient, were employed. (*1*) This consisted of the insertion of a small pledget of gauze freshly saturated with the suspension, into the vaginal canal with the aid of two pairs of small thumb forceps, one to grasp the tampon, the other to gently dilate the vaginal orifice. The tampons measured approximately 5 to 7 mm. in length and 2 to 3 mm. in diameter, and were inserted about 1 cm. into the vaginal canal. This procedure was repeated on subsequent days, the preceding tampon being gently withdrawn prior to the insertion of a fresh one. Experience showed that long continued introduction of tampons tended to interfere with the completion of pregnancy and the number was therefore reduced to 2 to 4 usually given on successive days. While the mother was frequently infected by such use of tampons, it remained difficult to obtain living offspring, so that the method was abandoned after an extensive trial in favor of the syringe and cannula method. (*2*) The second method consisted of the simple instillation of *Toxoplasma*-infected mouse brain suspension into the vagina by means of a 1 cc. syringe equipped with a narrow blunt-tipped cannula. The cannula was introduced without force or trauma to the mucous membrane for a distance of about 1 cm. The vaginal cavity was then gently flushed with 0.2 to 0.3 cc. of the brain suspension and the excess exuding from the vaginal orifice removed with a clean cotton sponge. Although this procedure could be carried out daily for a long period without harm to the animal, it, too tended to diminish the chances of the completion of pregnancy, so that a routine use of 2 instillations on successive days was finally adopted. This proved to be adequate for the production of infection in many cases and pregnancy frequently continued undisturbed to term.

Manifest Signs and Incidence of Infection

Mated Females.—Included under this heading are all 365 mice which had been caged with a male during the period of estrus, whether or not they became pregnant. The effect of pregnancy itself on the development of the infection will be referred to in a later section. Following the introduction of the microorganism, the majority of the animals (241, or 66 per cent) remained active, alert, and showed no overt symptoms of toxoplasmosis or of vaginitis during the entire time of observation, an interval ranging from 1 to 11 weeks. The remainder of the mice (124, or 34 per cent) developed signs of infection and were eventually killed (41, or 11 per cent) or died (83, or 23 per cent). Such animals became less alert and were sluggish in response to painful stimulation. They tended to remain quiet and hunched up in a corner of the cage. At times the coat appeared rufflled, or respirations became slow and labored, suggesting the presence of pulmonary infection. Some animals developed symptoms pointing to involvement of the central nervous system, such as heightened irritability, generalized tremors, and weakness of the extremities. The gait became faltering or unsteady, and in the later stages the animal had difficulty in righting itself after having been placed on its back or side. Ataxic gait was at times revealed or intensified by twirling the animal by the tail. Convulsive seizures were not directly observed

but would appear to have preceded death in some instances to judge by the rigid extension of the extremities occasionally seen postmortem. No discharge of exudate from the vagina or alteration in the appearance of the vaginal mucosa was noted during the course of the infection. Mice exhibiting signs, or dying of the disease, almost invariably did so in the 2nd to 3rd week after the initial exposure to *Toxoplasma*. Most of the fatalities occurred in pregnant animals at the time of, or shortly after, parturition. In a few instances mild signs of infection, such as reduced motor activity or unresponsiveness to stimulation, appeared but were transient and did not recur. No signs of infection, or fatalities, occurred in mice surviving the first 4 weeks, although many of these animals showed microscopic evidence of having become infected.

Asymptomatic Toxoplasmosis.—The occurrence of external signs did not in itself afford a reliable indication of the true incidence of infection in these experiments inasmuch as postmortem examination often revealed the presence of toxoplasmosis in apparently normal animals. Histologic study of the nervous system and other tissues was made in a group of 130 of the 241 mice which had remained entirely free of obvious signs following exposure of the vagina to the microorganism. 80, or 61.5 per cent of these asymptomatic animals were found to have characteristic lesions of toxoplasmosis in the brain and other organs. Parasites were readily demonstrated in the lesions in many instances. The distribution and character of the pathological changes are described elsewhere (13).

Incidence of Infection in Mated Mice.—All the animals that died or manifested signs of disease, and were examined histologically, were found to be infected. Assuming that asymptomatic mice whose tissues were not examined microscopically were infected in the same proportion as symptom-free animals with verified lesions, the over-all incidence of the disease in the total group of 365 mice was approximately $\frac{124 + 61.5 \text{ per cent of } 241}{365}$, or 74.5 per cent.

Effect of the Number of Exposures on the Development of Infection.—The preceding estimate of the incidence of infection in the group of mated animals represents an over-all figure and disregards the possible influence of the number of exposures. It was found that while some mice could be infected by a single exposure to the microorganism, the results were uncertain and the incidence of toxoplasmosis relatively low. Thus, in a group of 22 animals exposed once, 3 to 10 days after mating, only 10 developed characteristic signs or were found to have specific lesions on microscopic examination. Less than half of the entire group, however, were mice in which mating resulted in conception and sustained pregnancy. As will be seen, single exposures might be expected to be relatively more effective in successfully mated animals. When the number of exposures was increased to two, the incidence of the disease was greatly increased, to about 84 per cent $\left(\frac{108}{129}\right)$. Augmenting the number of exposures

beyond two did not appear to increase materially the proportion of animals infected, although the number of mice so tested was too low to be of statistical value. Regardless of the number of exposures, some mice were resistant to infection by the vaginal route. This was true not only when the number of exposures was low, but also of certain animals receiving as many as 4 to 7 instillations or tampons. This resistance was verified histologically in 50 instances by the failure to find lesions or parasites in the vaginal wall and elsewhere at varying intervals after exposure.

Influence of Pregnancy on the Susceptibility to Toxoplasmosis.—During the course of the experiments the impression was gained that females which became pregnant and retained their fetuses through most or all of the normal period of gestation, were more susceptible to infection by the vaginal portal than animals in which mating was unsuccessful. In the group of 365 mated animals, 178 mice were known to have been fertilized as evidenced by the eventual delivery of offspring, the presence of fetuses in the uterus at autopsy, or the postmortem observation of implantation sites in cases in which the fetuses had been resorbed or aborted. The incidence of the disease was fairly accurately known in this series since a large proportion of the mice $\left(\frac{143}{178}\right)$ were studied histologically. 82 per cent of these animals were infected. In the remaining 187 mice there were no certain indications that conception had occurred or had continued for more than the early days of gestation. These negative results may have been due to a failure to copulate, to sterile mating, to spontaneous unobserved resorption or expulsion of the embryos, or to the experimental procedures themselves, the relative importance of these various factors being difficult to assess. In this "non-pregnant group" the exact frequency of infection was known with less certainty since only a minority of the mice $\left(\frac{66}{187}\right)$ was subjected to microscopic study. Of those so examined only 46 per cent proved to be infected and the rate for the total number was probably not greatly different from this figure. Considering that some of these mice were no doubt pregnant at the time they were exposed to *Toxoplasma*, but were not recognized to be so because of the early termination of pregnancy, the relatively low rate of infection in this group has added significance.

The great majority of the experiments were concerned with mice infected 6 to 10 days after mating, a period of high susceptibility. Most of the animals in which the disease was severe enough to manifest itself in abnormal signs or in a fatal termination were among those infected at this time. Of 123 pregnant mice first exposed to *Toxoplasma* on the 6th to 10th day, 70 (57 per cent) eventually developed signs of illness or died. On the other hand, only 29 (20 per cent) of a similarly treated group of 147 females which failed to become or to remain pregnant after mating, showed such external evidence of infection. It was a common

event for the pregnant animals to carry their fetuses for most or all of the normal period of gestation and to succumb at, or within a few days prior to, the expected time of delivery. In many cases the fatal outcome was the direct result of disseminated toxoplasmosis, while in others inability to expel the fetuses also appeared to play a part and the animals died during labor. Still other females gave birth to dead or living young only to become ill or to die after parturition. Most of the postpartum deaths occurred within 4 days following the birth of offspring. As has already been noted, many of the remaining females giving no indication of infection during or after pregnancy nevertheless proved to have toxoplasmosis when examined microscopically. Since comparatively few studies were made of animals exposed before the 6th or after the 10th day of pregnancy, additional observations would be necessary to determine more exactly relative differences in the incidence of infection following exposure in the early, middle, and late stages of gestation, respectively. It may be noted, however, that in the early period, occasional instances of maternal toxoplasmosis occurred in mice exposed just prior to mating, and on the 3rd to 5th, and 5th to 6th days after mating. In the late period, successes were obtained on the 12th to 14th, 15th to 17th, and 16th to 18th days after mating.

Toxoplasmosis in Unmated Females.—In order to obtain additional information bearing on the comparative susceptibility of pregnant and non-pregnant animals, observations were made of a series of 62 virgin females infected by the vaginal portal. 41 of these were mature mice weighing 27 to 32 gm., while 21 were immature females 12 to 16 gm. in weight. With the exception of 7, which were exposed once, the mice were all given 2 vaginal instillations or tampons on successive days. Only 1 mouse in the entire group died of the disease, and only 7 developed signs like those in the series of mated animals. The remainder exhibited no evidence of illness. All were examined microscopically after an observation period varying from 4 days to 5 weeks. Lesions of toxoplasmosis were found in only 17 instances, an incidence of about 27 per cent. 9 of the infected mice were immature and 8, mature animals. In consonance with the comparatively low rate of infection and with the infrequency of external signs, the pathologic changes in the tissues were less severe, in general, than in the pregnant animals. The phase of the estrous cycle obtaining at the time of exposure appeared to have no relation to the susceptibility to infection, since positive results occurred at various times in the cycle.

In summary, then, the incidence of toxoplasmosis in pregnant animals (82 per cent) was approximately 3 times, and the incidence in "mated" animals without sustained pregnancy (46 per cent), was almost twice the rate of infection in virgin mice (27 per cent).

DISCUSSION

The experiments described in this report show that the mouse is readily susceptible to toxoplasmic infection by the vaginal portal. So far as can be

determined, trauma to the epithelial surface of the mucous membrane did not appear to be a prerequisite for the occurrence of such infection. The introduction of gauze tampons into the genital passage might perhaps, have caused slight mechanical injury to the epithelium in some cases, but it is unlikely that trauma was a significant factor in the animals infected by the cannula method. Histologic examination of the vaginal wall showed no evidence of trauma. There was no discontinuity or ulceration of the epithelium, except in rare animals in which there was unusually severe, subjacent inflammation in the lamina propria (13).

The overt signs of the disease produced in mice under the conditions of these experiments have been outlined in a previous section. Of particular interest is the fact that many of the animals showed no external signs of illness and yet were found to have disseminated toxoplasmosis on pathological examination. Only about a third of the mice in the series of mated animals, and fewer in the unmated group became manifestly ill or died. Except for animals dying of dystocia at the end of pregnancy, outward evidences of infection, when present, were chiefly referable to the central nervous system and lungs. The absence of signs in the majority of the mice is similar to that recorded in many instances of spontaneous animal toxoplasmosis, and in some laboratory animals infected by various routes other than the vaginal. It seems probable that, whatever the portal of entry be, asymptomatic, perhaps transient, infections also exist in the human being, more particularly during pregnancy, and in non-pregnant individuals as well.

Another observation worthy of emphasis is the high susceptibility to vaginal infection shown by the pregnant mouse. Toxoplasmosis followed vaginal exposure approximately 2 to 3 times more often in the pregnant than in the non-pregnant animals. Indeed, it was only exceptionally that vaginal instillation in the middle third of pregnancy did not lead to toxoplasmic vaginitis and generalized toxoplasmosis. Whether this facilitating effect of pregnancy was mediated by an increased local susceptibility of the vaginal mucous membrane itself, or was the result of a general lowering of resistance, remains to be determined. In any case, the fact that the pregnant animal is a more receptive host, suggests that the same is, perhaps, true of the pregnant woman, whatever the mode of infection in her may prove to be. If so, conditions favorable for exposure of the fetus to the parasite may be presumed to arise, and an explanation is afforded for the relatively higher incidence in man of congenital toxoplasmosis, as compared with the lesser frequency of infection acquired later on in life. It would appear, however, that infection in the pregnant woman must be much less severe than in the mouse, since it has not been attended by overt symptoms, and that the fetus furnishes a more fertile soil for the growth of the parasite than does the mature organism. It may be recalled, in this connection, that some of the mice in these experiments gave birth to congenitally infected offspring, although they themselves remained to all appearances

normal, and were known to be infected only from microscopic examination of their tissues. Infection in the human mother may be more like asymptomatic toxoplasmosis in the pregnant mouse.

The ease with which toxoplasmosis may be experimentally transmitted to the mouse by introduction of the parasite into the vagina cannot in itself be accepted as convincing evidence that vaginal infection also plays a significant role in the human being under natural conditions. That the vagina cannot be the sole portal of entry in the human being is rendered certain by the occurrence of histologically authenticated, although as yet rare, cases of toxoplasmosis in older male children (17) and adults (18–20) in whom the question of congenital infection did not appear to arise. On the other hand, the results of the experiments in animals do at least add the possibility of vaginal infection in the human being to the more commonly entertained hypotheses of oral contamination or transmission by an invertebrate vector. The absence of clinical signs of vaginitis does not exclude this portal since manifest signs of inflammation, as in the mouse, may well be lacking. Two possibilities suggest themselves as to how such infection might occur. The first, acquisition of the disease from an infected male during intercourse, is perhaps the less likely. It presupposes the presence of active toxoplasmosis of the external or internal genitalia in men, for which there is as yet no evidence, and introduces in turn the additional problem of how such infection could be acquired by them. Although the animal experiments are in no sense conclusive, they seem in general to be opposed to the notion of mutual venereal transmission in the human being. A study of the pathology of the vaginal infection in the mouse has shown that parasites were present in the epithelial lining of the vaginal mucous membrane (13) during the first stages of the experimental disease and that microorganisms may have been cast off into the vaginal canal during delamination of the surface cells. As will be pointed out, however, the vagina did not appear to remain a focus of persistent disease which might continue to be a source of infection on contact. In limited experiments the introduction of *Toxoplasma* into the vagina of mice just prior to mating was not followed by toxoplasmosis in the males (12). A few attempts by Mesnil and Sarrailhé (21), and by us (12), to infect the preputial mucous membrane of male mice by direct exposure to *Toxoplasma*, have also been unsuccessful. Unless, then, further experimental evidence of venereal transmission can be provided, or active toxoplasmosis of the male genital organs can be shown to occur in the human being, it remains difficult to accept sexual transmission as a mechanism of infection in man. Further studies in this direction, however, are indicated.

A second means of human vaginal infection remains to be considered, nonsexual contamination as a result of unhygienic habits. If the feces provide a source of such contamination it seems necessary to presuppose (1) the occurrence of antecedent infection of the gastrointestinal tract (presumably as the

result of the ingestion of *Toxoplasma*-infected material), and (2) the presence of the microorganism in a potentially virulent form in the excreta. Direct evidence of the existence of human intestinal toxoplasmosis due to the intake of contaminated food or fluid is not at present available, although in the experimental animal infection may, at times, follow the feeding or cannibalism of *Toxoplasma*-containing tissue. In many instances attempts to produce toxoplasmosis in mice by the oral route are unsuccessful, and so far as we are aware, it has not been established that living parasites are present in the droppings of experimentally or spontaneously infected animals, although they have occasionally been demonstrated in lesions in the intestinal wall. In our experience, suspensions of the fresh feces of mice which had been repeatedly fed the BD, LM, and TR strains of *Toxoplasma* during the preceding 3 to 21 days, were ineffective in producing toxoplasmosis by vaginal instillation of virginal and pregnant animals (12). These limited experiments, however, do not exclude the possibility that the parasites might have been present in the feces in inadequate numbers, that they might appear in the excreta under other undetermined conditions, or that other strains of *Toxoplasma* more adapted to the gastrointestinal tract than those used in these trials, might be effective. Until the presence of living *Toxoplasma* in excreta is established, it seems premature to attempt to ascribe either oral or vaginal infection to this source. Contamination of the vagina by infected urine is also a possibility but implies the presence of urinary tract infection and leaves unanswered the question of the primary portal of entry. Other sources of vaginal infection remain conjectural at the present time.

SUMMARY

Toxoplasmosis can be transmitted to mice by the introduction of *Toxoplasma* into the vagina. Pregnant mice were more susceptible to infection than nonpregnant animals in the ratio of 3 to 1. Obvious signs of vaginitis were not observed. Many of the infected mice remained entirely free of external signs, while a minority showed neurological or respiratory disturbances. Pregnant animals, especially those infected 6 to 10 days following conception, often died in the terminal stages of pregnancy or shortly after parturition. The possibility that the vagina may serve as one of the portals of entry of *Toxoplasma* in the human being and that infection may occur by sexual contact or by contamination by feces or other *Toxoplasma*-containing materials is discussed. The high susceptibility of the pregnant mouse to toxoplasmosis under the conditions of these experiments suggests a possible explanation for the higher incidence of congenital as compared to postnatal human toxoplasmosis and for the associated asymptomatic maternal infection. The infected but clinically normal human mothers may be compared to some vaginally infected pregnant mice which remained symptom-free.

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