EXPERIMENTAL TOXOPLASMOSIS

I. Transmission of the Infection in Utero and Through the Milk of Lactating Female Mice

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The protozoan toxoplasma has long been known to be pathogenic to a large variety of mammals and birds, but interest in the organism was aroused only when Wolf, Cowen, and Paige firmly established that the parasite causes a disease in human infants. Pinkerton and Weinman subsequently showed that not only infants but also adults can have toxoplasmosis, a disease marked by variable clinical and subclinical manifestations. In very young children and babies, hydrocephalus, microcephaly, chorioretinitis, cerebral calcifications and psychomotor disturbances are observed most commonly, while in older children acute, nonsuppurative encephalitis is also seen. Relatively few cases of the disease in adults have been reported. A spotted-fever-like syndrome associated with atypical pneumonia appears to be the cardinal sign of the acute disease, while chorioretinitis is probably a manifestation of a more

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chronic course. There is now a considerable body of evidence to indicate that, in addition to the more acute disease, a latent or subclinical infection can occur in man. Such chronic, asymptomatic toxoplasmosis was experimentally produced in mice by Weinman and has been demonstrated to occur naturally in wild rats.

It is now generally accepted that only one species of Toxoplasma exists. Sabin found that organisms isolated from different hosts (a) cross immunize, (b) are morphologically indistinguishable and (c) can be successfully inoculated into every host reported so far to be susceptible to the infection. These facts suggested to Sabin that the reservoir of infection existing in a large variety of lower animals is of considerable importance in the little known epidemiology of the disease. At present, few concrete facts have been established concerning the transmission of the organism from host to host; these can be summarized as follows:

With man, the only natural proved method of transmission is the congenital transfer of the protozoon from mother to fetus in utero. Mothers of such infected children show no signs of illness and appear to suffer no ill effects from the presence of the toxoplasma in their tissues; with the infant, on the other hand, the infection often terminates fatally. A similar congenital transmission of the disease has been demonstrated experimentally in mice, but the incidence of infection among the off-spring was very low.

Insect vectors have been postulated, but attempts to transmit the disease by this route have been uniformly unsuccessful.

Sabin and Olitsky have shown that mice are susceptible to infection via the alimentary tract if fed food containing viable toxoplasmas. If mice are allowed to starve until they become cannibalistic and are then permitted to devour an infected mouse, toxoplasmosis will result in most of them. It is thus possible that omnivores and carnivores can become infected in nature by the ingestion of tissue harboring the parasite.

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Since transmission of the disease via the alimentary tract is possible, it appeared profitable to examine the possibility that the milk of lactating, infected mothers could serve as a vehicle for the transfer of the organism from mother to offspring.

EXPERIMENTAL INVESTIGATION

The Toxoplasma strain used throughout this investigation is of human origin, having been isolated originally by Dr. A. B. Sabin and maintained since then in mice.14

I transferred the organisms routinely in mice for over a year by intraperitoneal inoculation of ascitic fluid or of liver emulsion. Transfers were made regularly every three or four days. When obtained originally from the Harvard Medical School, the strain showed considerable affinity for the central nervous system, but after long-continued serial passage through the animals became so adapted to liver, lung, spleen, intestinal mucosa, kidney and heart tissue that even after subcutaneous inoculation most organisms became concentrated in these visceral organs, with only small and infrequent foci of infection occurring in nerve tissue. The disease in these mice was accompanied with acute pulmonary congestion, with edema as its principal sign. Hepatic and myocardial lesions were also severe, and the meninges were occasionally invaded. The picture presented thus resembles to some degree acute adult toxoplasmosis.

As Weinman8 has shown, mice fed or given injections of suitably small quantities of infected material do not succumb to the disease but there develops, after generalized parasitemia, a chronic infection without evident illness. Many of the animals used in this study continued to harbor organisms in pseudocysts distributed only in the lung, heart and spleen for about three months after the initial generalized systemic infection. Weinman, on the other hand, observed surviving pseudocysts mostly in the brain, where they persisted sometimes for over nine months without provoking any reaction in the surrounding tissue. This difference in the site of location of the pseudocysts is most probably due to the different tissue affinities exhibited by Weinman's strain and the one used in this study. Since immune reactions are at their lowest level in the brain, it is logical that pseudocysts will persist longest there.8

TRANSMISSION OF TOXOPLASMA BY MOTHERS IN ACTIVE STAGE OF THE DISEASE

Thirty-five healthy 3 month old virgin female mice were caged separately and fed a diet consisting of standard mouse food "purina." Their intake was restricted to an amount that would keep the animals hungry without any visible

14. The Toxoplasma strain was obtained through Prof. D. L. Augustine, of the Harvard Medical School.
sign of undernutrition. After five days, healthy adult male mice were introduced into each of the cages and the animals allowed to mate. The appearance of a vaginal plug in the female was taken as an indication of successful copulation, a method which has proved reliable. As soon as a vaginal plug was noted in one of the animals, the male was removed from the cage. On the seventh day after copulation, each of 20 females was fed a small amount (about 0.2 Gm.) of toxoplasma-infected mouse liver; 15 control females were given a similar amount of liver from healthy mice. This method of infecting the animals was chosen because (a) it probably resembles natural conditions more closely than injection and (b) because this dose of infected material had previously been found to produce regularly a nonfatal generalized infection within five to eight days, lasting a minimum of three weeks and followed by a chronic carrier state of several months' duration.

After having ingested the liver, the mice were allowed to feed ad libitum for the remainder of the experiment.

Thirty of the animals proved to be pregnant and gave birth to their litters seventeen to twenty days after fertilization. The mothers and their litters were divided immediately into four groups:

Group A: Five infected lactating mice, each nursing 5 of their own young
Group B: Ten infected lactating mice, each nursing 5 young born of healthy mothers
Group C: Five healthy lactating mice, each nursing 5 young born of infected mothers
Group D: Five healthy lactating mice, each nursing 5 of their own young

(Whenever it was necessary to remove a litter from the mother and transfer it to a foster mother, this was done within an hour after birth.)

It can readily be seen that the litters included in group A were exposed both to intrauterine and to milk-borne infection, while those of group B were exposed only to the latter. Group C was included to give an indication of the incidence of congenital infection. Group D represents the controls.

The young were allowed to remain with the females until weaned (about eighteen days); then they were caged separately and fed ground “purina” pellets. Any animal that died during the experimental period was examined at once for the presence of toxoplasmas, both microscopically and by inoculation of organ emulsions into healthy mice. Twenty-five days after birth, all surviving members of the various litters and all mothers were killed and examined in the same manner for the presence of the protozoon. All mothers fed infected liver were found to harbor toxoplasmas, while those given normal liver showed no sign of infection. The results are shown in table 1.

The high incidence of infection in group B strongly suggests the existence of milk-borne transmission of the toxoplasma; however, the possibility that the organisms may have been transmitted to the offspring by other routes must also be considered. The following experiment was, therefore, performed:

15. This was done so that the animals would readily eat the infected material given later; the slight degree of malnutrition has no influence on the course of infection.

An apparatus for the collection of milk from lactating mice was devised which consisted essentially of a sterile, small-bored, rubber-tipped medicine dropper. By placing the open tip of the glass tube over a nipple and gently massaging while suction was applied with a rubber bulb, milk could fairly readily be collected. Previous to every milking, the ventral surface of each mouse was carefully scrubbed with 70 per cent alcohol in order to eliminate any possible contaminating material.

**Table 1.—Experimental Toxoplasmosis in Four Groups of Lactating Mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Description of Group</th>
<th>Total No. of Young</th>
<th>No. of Young Infected with Toxoplasmosis</th>
<th>Avg. Period of Infection, Days</th>
<th>No. of Young Surviving Postnatal Age of 20 Days</th>
<th>No. of Young Infected at Age of 20 Days</th>
<th>Total Percentage Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Five infected lactating mice, each nursing 5 of their own young</td>
<td>25</td>
<td>10</td>
<td>7±3</td>
<td>3</td>
<td>12</td>
<td>52</td>
</tr>
<tr>
<td>B</td>
<td>Ten infected lactating mice, each nursing 5 young born of healthy mothers</td>
<td>50</td>
<td>4</td>
<td>16±2</td>
<td>8</td>
<td>38*</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>Five healthy lactating mice, each nursing 5 young born of infected mothers</td>
<td>25</td>
<td>7</td>
<td>4±2</td>
<td>2</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td>D (controls)</td>
<td>Five healthy lactating mice, each nursing 5 of their own young</td>
<td>25</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>25</td>
<td>—</td>
</tr>
</tbody>
</table>

*The total includes 2 mice which died during the experimental period of causes other than toxoplasmosis.

**Table 2.—Milk-Borne Transmission of Toxoplasma in Young Mice**

<table>
<thead>
<tr>
<th>Group</th>
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<th>Total No. of Young</th>
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<th>Total Percentage Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Young fed indirectly with milk from 5 infected lactating females</td>
<td>20</td>
<td>2</td>
<td>16,18</td>
<td>2</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Young fed indirectly with milk from 3 healthy lactating females</td>
<td>10</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Fifteen lactating mice infected during pregnancy in the same manner as those in the previous experiment were milked once or twice daily. The milk obtained was promptly fed to 20 suckling mice born of healthy mothers. The feeding was best accomplished by inserting the open tip of the milking device into their mouths and forcing the milk out of the tube by gradually compressing the rubber bulb. Between these one or two daily feedings, the young mice were permitted...
to obtain additional nourishment directly from 5 healthy lactating females. Ten normal suckling mice which received similar artificial feeding with milk from 3 healthy donors were included in these groups as controls.

Artificial feeding of the test mice was continued for ten days; then they were allowed to obtain all their nourishment from their healthy foster mothers until weaned. Mice dying during the course of the experiment were tested for the presence of toxoplasma as before, and all survivors, controls and the 5 healthy foster mothers were killed twenty-five days after birth of the young and similarly examined. None of the foster mothers were found to be infected. The other results are shown on table 2.

Substantially the same incidence of infection was obtained whether the litter was in direct contact with the infected foster mother or was fed indirectly with her milk. After birth, therefore, transmission of the disease by routes other than by milk would not appear to be an important factor.

Logically enough, the incidence of the disease is highest among those offspring in which both infection of the fetus in utero and infection of the young by milk is possible. A comparison of the results in groups B and C in table 1 shows that prenatal transmission of the toxoplasma occurs more readily than transmission through milk. However, a temporal relationship must be considered here. It is quite conceivable that if the mother became infected late in pregnancy the number of young acquiring the disease in utero would be small and, conversely, that infection of the mother earlier, or just before pregnancy, would give a higher percentage of intrauterine infection.

It should be noted that deaths among congenitally infected mice not only were more frequent but occurred sooner than among mice infected through milk. At autopsy, a much more diffuse and heavier parasitization was observed in the animals infected in utero; no organ, including the central nervous system, escaped invasion by the toxoplasma. On the other hand, with milk-infected animals, the digestive tract, liver, spleen and lungs were parasitized almost exclusively, and a much smaller number of organisms was present.

TRANSMISSION OF TOXOPLASMA BY MOTHERS IN THE CHRONIC CARRIER STAGE OF THE INFECTION

Fifteen female mice which were shown to be capable of transmitting toxoplasma to their offspring congenitally as well as through their milk under the conditions of the previous experiment (i.e., in the active stage of the disease) were allowed to mate again with healthy males forty to sixty days after they had been infected initially. The litters, totaling 75 animals, were permitted to obtain milk from their own mothers. The survivors and the mothers were killed ten days after weaning and examined for the presence of toxoplasmas by the same procedures as be-
before, an offspring whereby eliminated calculation, spleen, sparsely were but given can the toxoplasma mary thus necessary. All the parasites, swiftly produced infected uterus. Within the production of a transient parasitemia, since more organisms were introduced than the immune reactions of the host could effectively handle. Within a few days, however, balance was again restored. The parasitemia, nevertheless, lasted sufficiently long to allow the toxoplasmas to reach the uterus and mammary glands and thus lead to infection of the offspring. Preliminary evidence indicates that only a small degree of immunity to the disease is transmitted from the mother to the litter in utero through the placenta, but further work on this problem is necessary.

NATURE OF THE TOXOPLASMA FOUND IN MILK

There is no evidence to lead one to suppose that the organisms transmitted by milk differ from the forms usually encountered in tissue fluids. All facts indicate that the number of toxoplasmas in milk is small; extensive microscopic examination of preparations stained by the Giemsa method revealed only a few organisms. They were morphologically indistinguishable from those found in ascitic fluid, and their resistance to

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physical agents was likewise about the same: At 3 C. milk remained infective up to eight days; infectivity was lost in fifteen minutes at 50 C. and in ten minutes at 54 C.

Infected milk is capable of producing toxoplasmosis when administered to mice either parenterally or orally.

COMMENT

It would be unwise to apply unselectively data obtained by animal experimentation to human disease. However, as Weinman9 stated, "it is important to note that thus far the correspondence of the human and the experimental disease (toxoplasmosis) in mice is extraordinarily complete." In addition, it should be pointed out that a "fresh" strain of toxoplasma may not necessarily behave like the adapted, viscerotropic strain used in these experiments. There is undoubtedly considerable variation in behavior among different strains. With these facts in mind, the following points may be of interest to the clinician:

1. A new route of transmission of the toxoplasma from mother to child must be considered; the infant may escape intrauterine infection, but if breast fed may subsequently be infected. It follows that if the mother acquires the disease after pregnancy but during lactation, infection of the breast-fed infant is still possible. The statement that the child is in danger only if the mother harbors the organisms during pregnancy may, in the light of evidence presented here, require modification.

2. The possibility that the symptoms of the disease produced by ingestion of toxoplasmas may be different from those observed in cases of the congenital disease must be considered. These symptoms would, of course, not be evident at birth but would be noted later. Unless a very large number of organisms are ingested, a chronic, rather than an acute, form of the disease would most likely tend to occur, since human resistance is probably fairly high. Both intrauterine and milk-borne infection may be involved in some cases. Infants born with signs of toxoplasmosis should, therefore, not be breast fed by the mother.

3. These results suggest the possibility of human infection through ingestion of milk from infected cows or goats. There are no data on the incidence of toxoplasmosis in these animals, although they are known to be susceptible;17 definite information would be valuable. The labile nature of the organism would seem to indicate that such a route of transmission is not a common occurrence. Pasteurization would, of course, serve to render milk noninfective.

4. The fact that female mice can transmit the disease to their offspring in utero or through milk only while an active, generalized infection is in progress, rather than in the chronic carrier state, may have some bearing on the observation that in a large majority of known cases in human infants one sibling alone was infected, while the others showed no sign of present or past toxoplasmosis and gave negative serologic reactions.

SUMMARY

Experimental evidence is presented to show that in mice transmission of toxoplasmosis to the offspring via the milk of infected mothers is possible. This route of transmission exists in addition to a previously recognized one, namely, infection of the fetus in utero.

As compared in the congenitally acquired disease, fewer organisms are observed in the tissue of milk-infected mice, and the distribution of the parasites is more limited. These factors result in a relatively milder disease.

Congenital or milk-borne transmission occurs probably only while the mother is undergoing an active, generalized infection. Chronic carriers rarely, if ever, are capable of transmitting the organisms to their offspring, except when they are reinfected with heavy doses of toxoplasmas during pregnancy. The resulting parasitemia is of relatively short duration in these immune animals.

Methods of transmission from mother to offspring other than through milk do not play a significant role after birth, as judged from these experiments.

The toxoplasmas found in milk are morphologically indistinguishable from those occurring in other tissue fluids and possesses the same resistance to physical agents.

The application of these observations to the human disease is discussed.

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