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Investigation on the congenital transmission of toxoplasmosis in chronically infected mice which were reinoculated during pregnancy

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It is generally believed that the congenital transmission of human toxoplasmosis occurs mostly in the acute stage of infection in mothers, namely when they acquire the primary infection during the pregnancy. In the acute stage of infection, the proliferative forms of the parasite multiply in various organs and appear in the blood. It is quite natural to assume that the parasites have more chances to penetrate into foetus in the acute stage than in the chronic stage of infection in which most of the parasite have produced cysts. Different opinions have been expressed by many authors as to the possibilities of the congenital transmission during the chronic infection of mothers. Apart from this problem, there is another problem that pregnant women having the chronic infection may receive a new infection of *Toxoplasma* and may transmit the infection to their fetuses. Nakayama (1966) reported in the previous paper that a high virulent strain of *Toxoplasma* could multiply actively in tissues of mice chronically infected with a low virulent strain for 3 days after reinoculation. The main purpose of the present study is to elucidate whether the parasites inoculated into chronically infected pregnant mice can be transmitted into fetus.

Materials and Methods

Two strains of *Toxoplasma gondii*, RH and S-273, were used. The latter was originally isolated from a pig in Japan, and is avirulent to mice. All mice inoculated with S-273 strain are able to survive the infection and only a few cysts are generally found in their brains.

ICR mice weighing about 20 g were used throughout this study. The brain of mice infected with S-273 strain was removed aseptically and was added with 5 ml of saline and emulsified. After the number of cysts in the emulsion was injected into clean mice intraperitoneally in amount of 0.2-0.5 ml. Those mice which survived the infection for more than 4 weeks were considered to be chronically infected. They usually do not succumb to the challenge inoculation of the RH strain. For the challenge inoculation, RH-trophozoites were harvested from the peritoneal cavity of mice which had been infected for 3 days. The numbers of the trophozoites were estimated by a hemocytometer and they were injected into blood vessel of the experimental animals.

For the detection of the antibodies in experimental animals, hemagglutination test after Hanaki *et al.* (1963) was applied, in which sheep erythorcytes were available after sensitization with the aid of bis-diazobenzidine. A swine serum with a high

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HA-titer was served as positive control in serological test.

Results

1) Detection of *Toxoplasma* from fetuses in chronically infected mice which were reinoculated with RH-trophozoites during the pregnancy

Female mice first inoculated with 8–20 cysts of S-273 strain. When they became pregnant by mating with clean males, they were again inoculated with 2,000 RH-trophozoites, usually 1–6 months after the first inoculation. The date of conception could not be determined at the time of reinoculation. The mice were killed 3 to 10 days after the RH-injection and their fetuses, placentas, and livers were removed. These materials were dipped in 75% alcohol for a moment and washed throughly with normal saline. Then each material was emulsified and injected intraperitoneally into clean mice. The heart blood of the mother mice was also injected into clean mice in amount of about 1 ml. The presence of RH-*Toxoplasma* in a test material can be recognized by the appearance of a huge number of trophozoites in the peritoneal cavity of subinoculated mice which die of the infection within a week.

Altogether 44 pregnant mice were examined after the RH-inoculation. Results are shown in the Table 1. RH-*Toxoplasma* was found in 30 mice (68%) inoculated with heart blood and in 35 mice (79.5%) with liver, while 8 mice were negative in both blood and liver.

On dissection of 44 pregnant mice, 310 fetuses were obtained. Liver emulsion of each fetus was injected into one clean mouse. Thus, only one fetus among them was found to have been infected with RH-strain, because the subinoculated mice died of acute toxoplasmosis 10 days after, having numerous trophozoites in the abdominal cavity. The mother mouse of this fetus was killed 5 days after the RH inoculation and had 9 fetuses of which only one was infected. Blood, liver and all of 9 placentas of this mother mouse were infected with RH-strain. On the other hand, the organism was found in 172 (55.5%) of 310 placentas. These results seem to indicate that the transmission of challenged *Toxoplasma* to fetus through placenta is very difficult to occur in cases of immune mice.

The mice which survived for more than 4 weeks after the subinoculation of fetus material were examined for the presence of infection with S-273 strain which had been inoculated into pregnant mice. Out of 309 mice, 104 were sacrifised and their brains were examined microscopically to detect cyst. All were negative. The remain-

Table 1 Transmission of RH-*Toxoplama* to the fetuses from immune pregnant mice after challenge inoculation of RH-trophozoites intravenously

Days after chall. infect.	Days between 1st infection and challenge	No. of pregnant mice		Detection of living trophozoites in organs :					
				pregnant mice		placenta		liver of fetus	
		No. exam.	No. posi. for transm.	No. posi. in blood	No. posi. in liver	No. exam.	No. posi.	No. exam.	No. posi.
3	64 to 114	5	0	4(10.6)	5(12.0)	38	17(10.7)	38	0
4	40 to 125	10	0	6(8.2)	7(8.6)	65	29(8.7)	65	0
5	34 to 181	10	1*	9(9.8)	9(8.9)	73	58(9.1)	73	1(10.0)
6— 7	30 to 163	13	0	8(11.0)	11(10.5)	88	47(9.8)	88	0
8—10	35 to 104	6	0	3(12.7)	3(10.0)	46	21(9.0)	46	0
	Total	44	1	30	35	310	172	310	1

Figures in parenthesis indicate the average days of survival of mice subinoculated.
1* indicates to have a RH-infected fetus.

ing 205 mice were challenged with RH-trophozoites to detect the presence of antibo dies in them and 3 survived the challenge. These three must have been infected with S-273 strain, because all control mice inoculated with RH-trophozoites succumbed to the infection without exception. In the brains of these 3 mice, however, no cyst was found by close microscopical examinations. The results indicate that the transplacental transmission of S-273 strain rarely took place. According to several investigators (Beverley, 1959, Remington *et al.* 1961), the congenital transmission is recognized fairly frequently in mice chronicaly infected with Beverley strain. Wildführ (1954) also reported frequent congenital transmission in rats. The main reason why S-273 strain was rarely transmitted to fetus may be attributed to its poor growth in mice. According to Nakayama (1967), the number of cysts produced by S-273 strain in mouse brain is about 1/30 of that produced by Beverley strain. In the present study, the frequency of infection in uterus was compared between these two strains: the uterus emulsions of mice inoculated with S-273 or Beverley strain were subinoculated into clean mice to detect the parasite (Table 2). The period of examination was extended from 3 weeks to one year after the inoculation. Uteri from mice inoculated with Beverley strain had living parasites far more frequently than those from S-273 mice. That is to say, living parasites were demonstrated from uterus of 17 (54.8%) out of 31 S-273 mice while they were found from 19 (86.4 %) out of 22 Beverley mice. The difference is statistically significant. It is reasonable to conjecture that the number of the parasite in each mouse would be smaller in cases of S-273 infection than in Beverley strain infection. These considerations will explain why the congenital transmission occurred rarely in S-273 infections.

As stated above, RH strain inoculated into immune pregnant mice were rarely transmitted into fetus. The immunity in pregnant mice would probably play an important role in the protection against the congenital transmission. To confirm this assumption, clean pregnant mice were inoculated with RH-trophozoites and examined for the frequency of congenital transmission as a control test. One group of mice was inoculated with a large number (50,000–2,500,000) of the parasite and another group was inoculated with a small number (2,000) of the parasite. Mice from each group were examined daily for 6 days after the inoculation (Table 3). Transmis-

Table 2 Comparison of the survival of parasites in uterus and blood between S-273 and Beverley strains

Periods examined after inocul.	S-273			Beverley		
	No. of mice exam.	No. of mice having Tp in: Uterus	Blood	No. of mice exam.	No. of mice having Tp in: Uterus	Blood
3 weeks	3	2	1	3	3	0
4 〃	3	2	0	3	2	1
5 〃	3	3	0	—	—	—
6 〃	5	4	2	5	4	1
7 〃	5	1	0	—	—	—
8 〃	3	0	0	—	—	—
9 〃	5	2	1	3	3	0
13 〃	1	0	0	3	2	0
18 〃	2	2	0	3	3	0
25 〃	—	—	—	2	2	0
1 year	1	1	1	—	—	—
Total	31	17 (54.8%)	5 (16.1%)	22	19 (86.4%)	2 (9.1%)

Table 3 Transmission of *Toxoplasma* to the fetus in non-immune pregnant mice intravenously inoculated with RH-trophozoites

Days after inocul.	Inoculum	No. of pregnant mice		Detection of living trophozoites in organs:					
				pregnant mice		placenta		liver of fetus	
		No. exam.	No. posi. for transm.	No. posi. in blood	No. posi. in liver	No. exam.	No. posi.	No. exam.	No. posi.
1	Small	2	0	2(10.0)	2(8.5)	18	11(10.6)	18	0
	Large	3	0	3(8.4)	3(6.0)	21	21(11.7)	21	0
2	Small	2	0	2(8.0)	2(7.5)	13	13(10.2)	13	0
	Large	3	0	3(7.5)	3(6.0)	22	22(7.5)	22	0
3	Small	5	0	5(7.4)	5(6.4)	46	46(8.1)	46	0
	Large	10	4	10(6.3)	10(5.1)	66	66(6.7)	66	7(16.9)
4	Small	11	5	11(6.8)	11(5.5)	92	92(7.9)	92	10(12.0)
5	Small	13	6	13(6.4)	13(5.5)	85	85(7.4)	85	11(10.5)
6	Small	12	11	12(6.5)	12(5.5)	101	101(6.9)	101	24(11.3)
Total		61	26*	61	61	464	457	464	52

Figures in parenthesis represent the average days of survival of subinoculated mice.
Inoculum small : 2×10^3 trophozoites, large : 50×10^3—2.5×10^6 trophozoites.
* These pregnant mice were shown to have one or more infected fetuses.

sion to the fetus was first recognized 3 days after the inoculation of the large inoculum and 4 days after the inoculation of the small inoculum. All pregnant mice had parasites in their blood and liver and almost all of their placentas. Thus, among 464 placentas examined, only 7 were negative. All of these negative placenta were obtained from mice which were inoculated with 2,000 parasites and sacrificed 24 hours after the inoculation.

In contrast to the high percentage of infection in placenta, the infection in fetus was not so frequent, only 52 out of 464 fetuses being found infected. The infection in fetus was far more frequent as compared with that of immune mice. The average period of survival of mice inoculated with liver emulsion of infected fetus varied from 10.5 to 16.9 days. This is relatively a long period of survival as compared with routine inoculation in mice and may indicate that the number of parasite in the liver of fetus was small.

It is probable that the location of fetus in uterus may have som erelation to establishment of congenital transmission of parasite. Infection of fetus was examined by dividing them in three groups according to their locations in uterus. Incidence of infection of each group was as follows: 10.9% of fetuses located in the central part or near the *Portio vaginalis* of the bicornate uterus, 13.0% of fetuses situated in the right horn and 9.2% of fetuses situated in the left horn. Thus, no significant differences were recognized between these groups as to the incidence of infection. This suggests that the parasites may be distributed evenly throughout the uterus.

It was impossible to determine what time of pregnancy would the congenital transmission occur. Relatively small fetuses, however, were obtained from both non-immune and immune pregnant mice. They were estimated to be within a period less than 10 days of pregnancy. Eleven out of 25 small fetuses obtained from 3 non-immune mice were found infected. These pregnont mice had been infected with 2,000 RH-trophozoites and were sacrificed 4-6 days after the inoculation (Table 4). This indicates that the transmission can take place fairly early stage of pregnancy. None of 45 small fetuses obtained from 6 immune pregnant mice was found infected.

As the congenital transmission occurred

Table 4 Transplacental transmission of *Toxoplasma* to the fetus in the early stage of pregnancy

Days examined after inoculation	Non-immune pregnant mice inoculated with RH			Immune pregnant mice challenged with RH		
	No. of mice		No. of posi. fetus / No. of fetus exam.	No. of mice		No. of posi. fetus / No. of fetus exam.
	exam.	posi.		exam.	posi.	
4	1	1	2/9	2	0	0/13
5	1	1	4/9	1	0	0/7
6	1	1	5/7	—		—[1]
7	—		—	1	0	0/9
8	—		—	1	0	0/9
10	—		—	1	0	0/7

not infrequently in normal pregnant mice inoculated with RH-trophozoites, their tissues of several organs were examined for the presence of histopathological changes. They were inoculated with 2,000 RH-trophozoites and were sacrified 5 days after the inoculation. To confirm the presence of infection, emulsion of liver and placenta and blood of these pregnant mice were inoculated into clean mice. Liver emulsions of fetuses were likeweise examined. Histological sections of liver, spleen and placenta of these pregnant mice were fixed in 10 % formal and stained with hematoxylin-eosin.

One of the pregnant mice had 14 fetuses and 4 of them were found infected with RH-trophozoites. Days of survival of mice subinoculated with liver emulsion of these fetuses extended from 9 to 10 days, indicating that the number of RH-trophozoites in fetus was rather small. In the histological sections of liver of this pregnant mouse, clusters of trophozoites were found frequently. Prominent degenerations and necrosis were found near the clusters. Many small granulomas due to proliferation of fibroblasts were also recognized (Fig. 1). In spleen sections, many trophozoites and small foci of necrosis were found scattered in the tissues (Fig. 2).

In striking contrast to the prominent histopathological lesions in liver and spleen, placentas showed little changes. What was different from normal placenta was that *Toxoplasma* trophozoites were found near blood capillaries (Figs. 3 and 4). Other experimental animals of this series indicated also similar findings.

2) Infections in young born to non-immune and immune mice which were inoculated with RH-trophozoites during pregnancy

In the preceding experiments, examinations were made on fetuses taken out of uterus. In this second series of experiment, pregnant mice were inoculated with 2,000 RH-trophozoites 1–5 days before the delivery in cases of non-immune (control) mice and 3–9 days before the delivery in cases of immune mice. Young born to these mice were examined for the detection of infection 1–5 days after birth. Although they were allowed to suck mother's milk, those born to control mice often died during the examination period, because their mothers died of RH-infection. Young, if died, were examined as soon as possible after death. Mice which had been inoculated with 8–20 cysts of S-273 strain previously were used as immune mice. They were inoculated with RH-trophozoites when they became pregnant and they did not succumb to the RH-infection. Young born to these immune mice were allowed to suck milk and were sacrificed 21–28 days after birth. The results are shown in the table 5. Thirty one control pregnant mice, inoculated with RH-trophozoites gave birth to 157 young in total and 4 of them were found infected. These 4 young were born to 3 mice, two of which gave birth

Table 5 Prenatal transmission of RH-*Toxoplasma* to the young born of non-immune and immune mice

Pregnant mice	Days from inoculation to delivery	Days of lactation	No. of mothers Posi./exam.	Detection of RH in young	
				exam.	posi.
Non-immune	1 to 2	1 to 5	0/12	L*54, D**7	0
	3	1 to 4	2/9	L 16, D 18	L 2(8.0), D 1(16.0)
	4	1 to 3	0/8	L 35, D 15	0
	5	1	1/2	L 2, D 10	L 0 , D 1(9.0)
	Total		3***/31	L107, D 50	L 2 , D 2
Immune	3	21 to 28	0/5	L 21	0
	4 to 5	22 to 28	0/3	L 18	0
	6 to 9	21	0/4	L 18	0
	Total		0/12	L 57	0

L* and D** indicate the living and dead young respectively at the time of examination.
Figures in parenthesis indicate the average day of survival of subinoculated mice succumbed to acute toxoplasmosis.
3*** : These mothers were shown to have had the infected young.

to young 3 days after inoculation and the remaining one 5 days after inoculation. It is, however, not clear whether these young had been infected before birth or they got the infection by lactation after birth. Immune mice, 12 in number, gave birth to 57 young in total and none of them were found infected.

In another series of experiment, mice were inoculated with 12–24 cysts of S-273 strain and were again inoculated with 3,000 RH-trophozoites 59–76 days later. Then they were mated with clean males and were delivered of young which were reared at the breast. Infection in these young was examined by sacrificing 5–6 litters every week after the birth during a period of 8 weeks (Table 6). None of the young examined during a period of 2 weeks after birth were found infected. Some of the young examined at 3, 4, 5 and 7 weeks periods after birth were found infected. They were 12 in number and the parasites isolated from them were all S-273 strain, producing cysts in brains of subinoculated mice. RH-strain was never found from these

Table 6 Transmission of *Toxoplasma* and its antibody checked by HA test to the young which were born and given the breast of immune mice

Periods after delivery	No. of mother		Young				Mother	
			Infection		HA titer		Posi. HA titer at the time of :	
	exam.	Tp posi.	exam.	Tp posi.	exam.	posi.	Pregnancy	examin.
Within 1 day	5	0	41	0	41	13(31.7%)	2	5
3 〃	6	0	58	0	58	25(39.7%)	4	6
1 week	5	0	37	0	37	20(54.1%)	3	3
2 〃	5	0	42	0	42	22(52.4%)	3	4
3 〃	5	3	40	5	35	16(45.7%)	2	5
4 〃	5	2	43	4	39	25(64.1%)	1	3
5 〃	5	1	38	1	37	0	4	5
6 〃	5	0	38	0	38	0	4	4
7 〃	6	1	44	2	42	2(4.8%)	6	4
8 〃	5	0	35	0	35	0	4	5
Total	52	7	416	12	404	123	33(63.5%)	44(84.6%)

Table 7 Transmission of *Toxoplasma* to the young born and given the breast of infected mother mice

Period after delivery	Young			Coresponding mother			
	HA titer		Cysts in brain	HA titer			Cysts in brain
	exam. time	4 wks. after exam. time		during pregnancy	exam. time	4 wks. after exam. time	
3 weeks	4096	64	+	(−)	4096	256	+
	1024	1024	+				
	1024	4096	+ same litter	(−)	4096	4096	+
	4096	1024	+				
	256	(−)	+	4096	1024	1024	+
4 weeks	4096	64	+	(−)	1024	64	+
	(−)	(−)	+				
	(−)	(−)	+ same litter	(−)	(−)	(−)	+
	(−)	(−)	+				
5 weeks	(−)	(−)	+	256	4096	not exam.	+
7 weeks	(−)	(−)	+ same litter	1024	64	256	+
	(−)	1024	+				

young, because none of them died of acute toxoplasmosis in a short period. As the infection in young was first evidenced later than 3 weeks after birth.

Antibody in sera of mothers and young were tested with Hanaki-Nobuto's hemagglutination test. Titers 1 : 64 or higher were regarded as positive (Table 6). More than a half of mothers (63.5%) showed positive titers during the pregnancy and most of them (84.6%) were positive after delivery when their litters were sacrificed for examination. Table 7 indicates the HA titers of 12 young which were infected and those of their mothers. It is noteworthy that one mother and 3 young of its litter gave negative titer of HA test by repeated examinations in spite of the presence of cysts in their brains.

Among 404 young from which the parasite was not found, 123 showed positive HA titer. Almost all of them were those examined during the period from one day to 4 weeks after birth. Only 2 positive cases were found 7 weeks after birth. Among 41 young examined within one day after birth 9 had a titer as high as 1 : 4096 or higher. This antibody must have been transmitted from mother mice through placenta. The percentage of positive cases increased gradually from one day to one week after birth, attaining around 50% and this level was maintained until 4 weeks after birth. At the fifth week and thereafter, all young were HA test negative except at the seventh week when only two out of 42 young were positive. Young sucked the breast during 3–4 weeks after birth. Soon after they stopped the suckling, the antibody disappeard from their serum. These results suggested that the antibody may be transmitted from mother to young through milk as well as through placenta. Young born to clean mother mice were likeweise examined as controsls. They were all HA test negative.

Discussion

It is an established fact that in murine toxoplasmosis the congenital transmission can take place in the chronic stage of infection of mothers, while this is a very important problem to be solved in cases of human toxoplasmosis. Even in cases of murine toxoplasmosis, however, it is reasonable to conjecture that the congenital transmission may take place more readily in the acute stage than in the chronic stage of infection, because the parasites are

circulating in the blood stream and can reach the uterus very often in the acute stage, while they are mostly enclosed in the chronic stage of infection. The present author (1966) recognized in tissue sections an active multiplication of a high virulent strain which were inoculated into chronically infected mice. This active multiplication, however, was seen only for 3 days after the inoculation and dividing forms became to be difficult to find out thereafter. Thus, mice survived the infection of the high virulent strain. The main purpose of the present study is to investigate whether the high virulent strain inoculated into chronically infected mice may be transmitted readily into fetus. Experimental results indicated that the transmission occurred rarely, while the same strain inoculated into non-immune pregnant mice was transmitted into fetus very often. This difference in the frequency of congenital transmission in non-immune and immune pregnant mice can be attributed to the limited growth of the parasite in immune mice.

Although the congenital transmission in chronic stage of *Toxoplasma* infection in mice has already been definitely demonstrated by several investigators (Beverley 1959, Remington *et al* 1961), it was rarely recognized in cases of S-273 strain infection. This strain is avirulent to mice, producing a few cysts in brain. The paucity in number of organisms reproduced by this strain may be main reason why the congenital transmission is rare.

How can *Toxoplasma* get into fetus in the chronic stage of infection of mothers? There are two possibilities to be supposed. One is that the parasitemia is recognized from time to time in the chronic stage of murine toxoplasmosis (Jacobs *et al.* 1950) and the parasites may reach placenta and have chances to penetrate into fetus. Another possibility is that cysts located in uterus may be ruptured by the invasion of trophoblasts into endometrium and freed parasites may have chances to penetrate into fetus. Whatever the process may be, the more in number of the parasites in mothers are, the more chances they may have to penetrate into fetus. The presence of lesions in placenta may not always be necessary for transplacental infection, because it was demonstrated in this study that the parasites have penetrated into fetus without any recognizable lesions in placenta in cases of acute infection of mothers. Remington (1961) also suggested the possibility of pentration of *Toxoplasma* into fetus without first establishing a focus in placental tissues.

There is still divergence of opinions as to the possibilities of congenital transmission in the chronic stage of human toxoplasmosis. Provided that it does occur, the possibility may be much influenced by the virulence or the rate of reproduction of the strain concerned. This may be true with the congenital transmission in the acute stage of infection. Strains of high virulence produce far more parasites than those of low virulence and may have more chances of transplacental infection than low virulent strains.

Lactation seems to be an important factor of transmission of parasite to young. Eichenwald (1948) demonstrated the transmission of *Toxoplasma* to young through lactation. Remington (1961) also recognized the infection via milk by allowing foster young to suckle on a mother which had been infected 6-8 days before delivery. In the present studies young born to mice chronically infected with S-273 strain were often found infected 3-5 weeks after birth, while the prenatal infection was quite rare. These infections must have been contracted through lactation. It is, however, rather strange that a strain which is rarely transmitted to fetus through placenta can be liberated in milk and transmitted to young frequently. This problem seems to be well worthy of futher studies.

Antibody was often transmitted to

young from chronically infected mother mice. It disappeared, however, in a short period when young were weaned.

Conclusion

1. The high virulent RH strain of *Toxoplasma* was often transmitted to fetus when clean or non-immune pregnant mice were inoculated with this strain. The transmission was first recognized 3 days after the intravenous inoculation. RH strain inoculated into pregnant mice which had been infected with a low virulent strain was rarely transmitted to fetus.

2. In the chronic infection of avirulent S-273 strain in mice, the congenital transmission occurred rarely. This exhibits a striking contrast to Beverley strain which is readily transmitted to fetus during the chronic infection in mothers.

3. RH strain inoculated into clean pregnant mice can penetrate into fetus in the absence of appreciable lesions of placental tissuse.

4. Although the prenatal infection occurred rarely by S-273 strain infection, the postnatal infection through lactation seems to be not infrequent. Antibody may be often transmitted to young through placenta as well as lactation.

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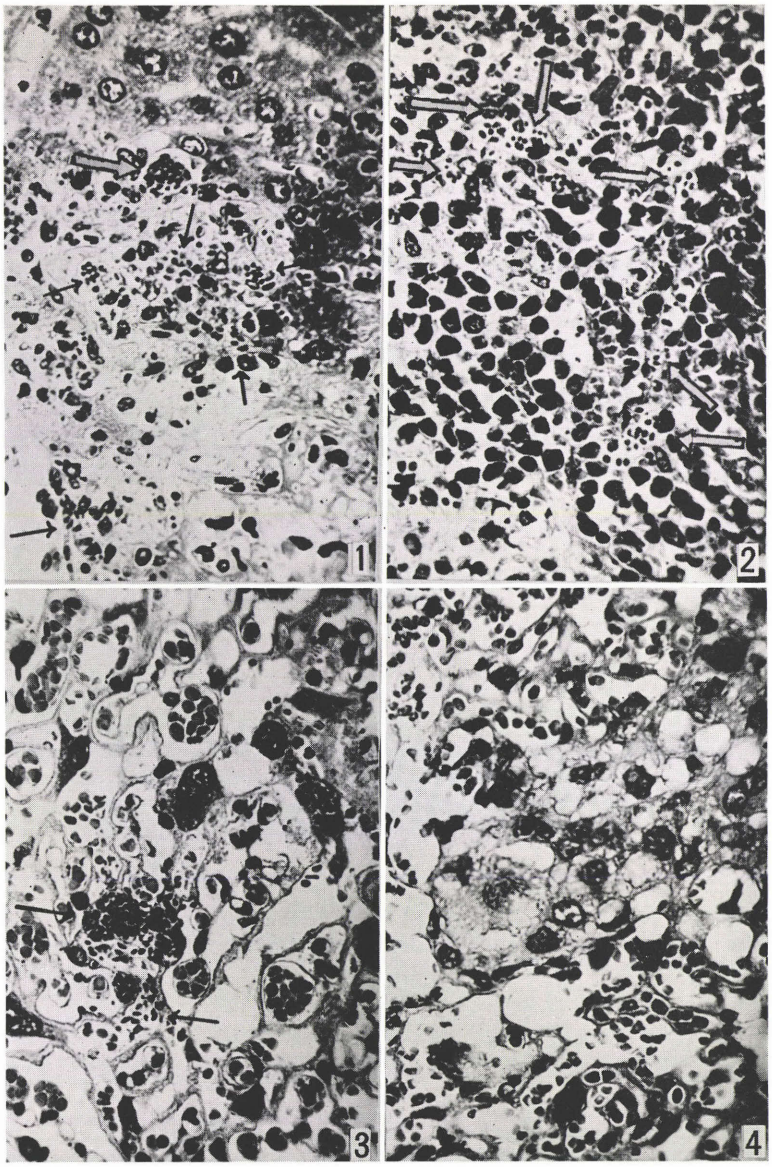

Explanation of Figures

Figs. 1, 2, 3 are tissue section of a pregnant mouse which was intravenously inoculated with 2,000 RH trophozoites and sacrifised 5 days after the inoculation. Fig. 4 is from normal pregnant mouse (control). Allows indicate *Toxoplasma* organism. (×400)

Fig. 1 : Liver. Advanced degeneration and necrosis of liver cells and many small granulomas formed by the proliferation of fibroblast are recognizable. Clusters of trophozoites are also recognizable.

Fig. 2 : Spleen. Many amall foci of necrosis and numerous trophozoites are seen everywhere in the section.

Fig. 3 : Placenta. Fetus attached to this placenta was infected with *Toxoplasma*. No lesion was seen. A few clusters of trophozoites can be seen, but the number of trophozoites in a cluster is small.

Fig. 4: Normal placenta (control).

妊娠慢性感染マウスにトキソプラスマが再接種された場合の先天性感染に関する研究

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ヒトのトキソプラスマ（以下 Tp と略記）症で慢性感染の時期に先天感染がおこるか否かは未解決の問題であるが，マウスの場合にはそれが比較的頻繁におこることがよく知られている．このことに関係して，慢性感染期にある妊娠マウスが強毒な Tp 株に再感染した場合，その強毒株が胎児に移行するか否かは興味のある問題であり，本研究の主要目的である．強毒株としては RH 株を，慢性感染をおこすための弱毒株としては S-273 株を用いた．この株は，一般に弱毒株として慢性感染をおこすのに用いられている Beverley 株よりも遙かに弱毒であり，脳内に検出されるシストの数も Beverley 株に比して遙かに少ない．

S-273 株に感染していて妊娠したマウスに RH 株を接種し，その胎児を取り出して検査した場合には，RH 虫体は極めて稀に胎児に移行したにすぎない．即ち 44 匹の妊娠マウスから取り出した 310 匹の胎児中，RH 虫体の検出されたのは僅かに 1 例にすぎなかった．これは妊娠マウスがすでに免疫をもっていて，そこに接種された虫体はその増殖が著しく阻止されるからであろう．対照として行った非感染妊娠マウスに RH 株を接種した場合では 66 匹から得た 464 匹の胎児中，感染していたものは 52 匹に達した．

慢性感染している S-273 株もまた胎児には甚だ移行し難いもので，本株の虫体の見出されたものは 1 例もなかった．併し，これらの胎児の肝の接種をうけた正常マウスを 1 カ月放置した後 RH 株の攻撃接種を行った場合，それに堪えて生きのびたものが少数あり，これらには S-273 株の先天感染がおこっていたことが想像される．いずれにしても Beverley 株に比して S-273 株による先天感染は甚だ少ないものである．これらのことはいづれも母体内における虫体の増殖の顕著な場合ほど先天感染もおこり易いことを示している．

胎盤の病理組織標本を検査した結果，胎児に先天感染がおこっていても，胎盤には何等の損傷も認められない場合が多いことから，Tp は胎盤に病変をつくることなく胎児に移行し得るものがあると思われる．また，子宮内で胎児が着床している位置が虫体の移行の難易に影響するということも認められなかった．

胎盤経由と授乳によって AH 抗体が仔に移行することが確認され，生後 4 週間までに約 50% の仔に抗体が証明された．離乳とほぼ一致してこれらの抗体は消失した．