Attempt to Change the Virulence of RH and Beverley Strains of *Toxoplasma gondii* by Drug Treatment

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ABSTRACT : In the present study, the author attempted 1) to change the virulence of RH strain of *Toxoplasma gondii* to that of Beverley type by the treatment of antitoxoplasma drugs and 2) to change the virulence of Beverley strain of *T. gondii* to that of RH type by the treatment of cortison or Kenacort (triamcinolone). The following results were obtained.
1) Mice inoculated RH strain died within 4～5 days in control without treatment, and mice treated with spiramycin or sulfathiazole-N^4 died of an acute infection, but survival periods were slightly longer than those of non-treated control mice. Mice treated orally with sulfadiazine or Policydal (sulfamethopyrazine) for 10～20 days survived for a long period, and from few of the mice, cysts were found in their brains. Proliferative forms were also found in several organs of the survival mice including brain. As a result, in the RH strain modification of the virulence by drugs did not succeed. 2) To enhance the virulence of Beverley strain, mice were treated with cortison or Kenacort just before the inoculation of parasite. Five days later a fluid which was taken by washing with a physiological saline of the peritoneal cavity of each mouse was inoculated to each fresh mouse which was treated with cortison or Kenacort. After the same treatment and inoculation were repeated up to five passages of mice, the washing fluid of last passage mouse of each series was inoculated to fresh mouse without the treatment. After stop of the treatment, some mice died of an acute infection, within 4～5 days and even in the non-treated control series, one case became a virulent type for several passages. But, if once the virulence of Beverley strain enhanced like RH type, often the parasites of "virulent" Beverley type turn again to avirulent Beverley type (cyst-cyst type) after serial passages.

There are well known laboratory strains of *Toxoplasma gondii* of which virulence is considerably different from each other, RH and Beverley strains. RH strain is highly virulent. Intraperitoneal inoculation of the strain results in death of the mice within 5～8 days in accordance with the number of parasites inoculated, and the infected mice develop 1～2 ml of serous ascites in which about 10^4～10^5 banana-shaped parasites per mm^3 are counted microscopically. While the case of an avirulent Beverley strain, inoculated mice can survive for a month or more, but in their brains, spherical cysts surrounded by the definite walls

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are observed showing characteristic of chronic infection. The parasite-stages in those infections in mice are called as "extra-intestinal stages", and in all host animals except Felidae, only such stages are reported. In the experimental infections of mice, the virulence of each strain, RH or Beverley, becomes stable respectively and their virulences do not change without the intervention of experimental procedures.

The treatments of RH infected mice or other animals with several anti-toxoplasma drugs have been reported by many workers, for example, spiramycin or acetyl-spiramycin by Nakayama and Matsubayashi (1963) and Aoki (1969), sulfa-drugs by Frenkel (1953), Eyles and Coleman (1953) and Nakayama and Matsubayashi (1961), SDDS by Oshima *et al.* (1967) and Oshima and Kumata (1974), and pyrimethamine by Summers (1953). Jacob (1973) also has reviewed recent works on treatments. After such treatments, sometimes mice, which survived for a long period, still had toxoplasmas in their tissues especially in their brains as reported by Frenkel (1953), and Nakayama and Matsubayashi (1961 and 1963). According to Nakayama (1964), mice which were previously infected with a non-virulent strain were challenged with the virulent RH strain 6 weeks later, and their brains proved positive of the RH for up to 7 weeks after the challenge. Those parasites were usually within cysts, but in some cases the parasites might be present in tissues or cells of mice without the cyst as pointed by Frenkel (1953).

In the present study, the author intended to change the virulence of each strain by treatment with several chemicals. Although the virulence of RH or Beverley strain could not be changed permanently, some interesting informations obtained have been discussed.

MATERIALS AND METHODS

1. The following strains of toxoplasma were used: Beverley, RH, N-130, and N-108. The last two strains were isolated from pigs in Nagasaki by Nakabayashi *et al.* (1969). Mice (DDK) weighing approximately 20～25 g were used for inoculation.

2. To obtain chronic infection, the mice inoculated with the RH strain were treated with orally or intraperitoneally with the following anti-toxoplasma drugs: Spiramycin-Kyowa, Theradiazine (sulfadiazine ; 2, (p-aminobenzon-sulfonamido)-pyrimidine), Sulzol S (sulfathiazole-N^4; sodium dextrosesulfonate), SDDS (2-sulfamoyl-4, 4′-diaminodiphenylsulfone), and Policydal (sulfamethopyrazine). The first time treatment was done at same time of the inoculation and the treatments were repeated daily or every the other day as shown in Table 1. The extension of survival period (days) of the treated mice, and the detection of cysts in their brains were recorded. The inoculation of the emulsion of the brains was further attempted to fresh mice to examine whether the chronic infection of mice can be continued again in the second passage or turn to acute infection.

3. To reduce resistant ability of inoculated mice against the infection with Beverley strain, the following substances were given intramusclarly to the mice just before the inoculation: Cortison (cortison acetate), and Kenacort-A (Squibb triamcinolone acetonide aqueous suspension, 9α-fluoro-16α-hydroxyprednisolone acetonide).

Terminology of each stages: Before going to report results, the author must describe his opinion on terminology for extra-intestinal stages. The life cycle of *Toxoplasma gondii* (Nicolle and Manceaux, 1908), had been known almost completely in the enteroepithelial cells of cats (Hutchinson *et al.*, 1968, 1970, and 1971). The enteroepithelial stages such as schizogony, gametogony, and oocyst formation are observed only in animals belonged to Felidae, from which the following species were reported as true host, *Felis felis*, *F. yagouarounde*, *F. pardalis*, *F. bengalensis*, *F. concolor*, and *Lynx rufus* (see Jacob, 1973, and Frenkel, 1974). While both extra-intestinal and enteroepithelial stages were reported in Felidae, only extra-intestinal stages were known in other animals. Hoare (1972) has suggested to use the following terms for the extra-intestinal stages of toxoplasma: *endozoite* for the rapidly multiplying forms within pseudocysts, and *cystozoite* for those which develop within cysts, because of existence of morphological differences between endozoites and cystozoites. They are "distingushable by the position of the nucleus, which is typically central in the endozoites but terminal in the cystozoites" (Zypen and Piekarski, 1967a and b). Frenkel (1973) has also proposed the two terms as follows: *tachyzoite* for the rapidly multiplying extra-intestinal forms of the acute infection, which reproduces by endodyogeny and eventually destroy their host cells; and *bradyzoite* for the more slowly multiplying (by endodyogeny) encysted forms, characteristic of the chronic infection. Both of the authors used the term "cyst" in the same meaning as commonly used. According to Jacob (1973), pseudocyst ("group" in the term by Frenkel) means the aggregation of rapidly dividing tissue forms (tachyzoites or endozoites) within a host cell. The terminology may not be conclusive up to now, and the present author can not identify whether each banana shaped parasite found from tissues or ascite is tachyzoite (endozoite) or excysted bradyzoite (cystozoite) if parasites are freed cyst. Then, in the present paper, he used proliferative form for banana shaped parasites which were found outside of cyst, and cyst in the same sense of Hoare and Frenkel.

RESULTS

1. *Attempt to change the virulence of RH strain to Beverley type by the treatment of anti-toxoplasma drugs*

Approximately 5×10^4 parasites which were obtained from ascites of RH inoculated mice were injected to each mouse intraperitoneally. Before the inoculation, each mouse was pre-treated with one of anti-toxoplasma drugs, then, the treatment was repeated every day or every other day after inoculation of RH strain. The drugs, its dosage per day, the method of treatment, and the daily observation of the mice were summarized in Table 1.
The mice treated with the daily dosage of 5 mg or 10 mg of spiramycin died of an acute infection, and the survival periods of the mice were slightly longer than those of non-treated control mice. In the case of sulfathiazole-N^4, the result obtained was similar to those of spiramycin, and the survival periods were also slightly longer, but all mice died of the acute infection within 10 days. With the oral treatments of 4 mg SDDS per day for 7 days, mice died of the acute infection; with intraperitoneal treatment, however, mice survived for

up to 30 days in chronic infection.

In mice treated orally with 2.5 mg sulfadiazine per day for 10 days, the survival days were markedly prolonged, however, the mice died of a sub-acute infection. Then, in the next attempt, mice were treated orally with 2 mg sulfadiazine per day for 20 days, and almost all the mice survived for up to at least 30 days in the chronic infection as shown in Table 2. Twenty mice, except one which died of an accidental injury to its stomach with syringe, survived for a long period, and cysts were not found in 17 mice, but the emulsion of the brains infected to fresh mice as shown in Table 2. Each emulsion of the brains obtained from mouse No. 6, 10, 18 and 19 in Table 2 was inoculated to fresh mice intraperitoneally. About 10 days later, the mice died of the acute infection. Mouse inoculated with the emulsion of brain taken from mouse No. 20 survived for a long period without any sign of infection. The brain of mouse No. 11 from which cysts (Fig. 1) were detected microscopically was inoculated to fresh mice orally or intraperitoneally. All the mice inoculated intraperitoneally died, but in the mice given orally, only two of them died of the acute infection, and the other 8 mice survived for a long period. About one month later, from one of the survival mice, cysts were found in the brain. Again the brain emulsion was inoculated to a mouse, but the mouse did not infect. All mice, which were inoculated intraperitoneally with the brain emulsion of mouse No. 17, died of the acute infection, but in the mice, which were given the emulsion orally, survived without any sign of the infection

Table 1. Experimental treatment of RH inoculated mice with several anti-toxoplasma drugs

Drugs	Daily Dosage (mg) and Days and Method of Treatment	Survival Days of Mice		Type of Infection and Other Observations
Spiramycin	10 mg, 10 days, orally***	(5)*	9.4	Acute infection
	5 mg, 10 days, orally***	(7)	7.6	Acute infection
Sulfadiazine	2.5 mg, 10 days, orally	(7)	24.1	Sub-acute infection, no cyst
	2 mg, 20 days, orally	(20)	30<	Chronic infection, cysts (2/20), see Table 2
Sulfathiazole-N[4]	4 mg, 7 days, orally	(8)	8.3	Acute infection
SDDS	4 mg, 7 days, orally	(5)	11.4	Acute infection
	4 mg, 7 days, intraperitoneally	(5)	30<	Cure or chronic infection
Policydal	2 mg, 14 days, intraperitoneally	(10)	30<	Cure or chronic infection
	4 mg, 7 days, intraperitoneally	(10)	22.7	Sub-acute infection, no cyst
	4 mg, every the other day for 14 days, intraperitoneally	(10)	30<	Cure or chronic infection
	6 mg, 7 days, intraperitoneally	(7)**	21	Sub-acute infection } see Table 3
		(2)**	30<	Chronic infection, cyst(+) } see Table 3
Control	no treatment	(5)	6.0	Acute infection

* (5) 9.4 means average 9.4 survival days in 5 inoculated mice.

** 7 out of 9 mice died within 30 days after inoculation and rest 2 mice survived for longer than 30 days.

*** Some of mice died before finish of the 10 days treatment.

Each mouse used in this experiment was inoculated intraperitoneally with 5×10^4 proliferative forms of RH strain.

Table 2. Experimental treatment of RH inoculated mice with sulfadiazine (2.5 mg/day for 20 days, orally)

Mouse No.	Survival Days	Cyst Detection	Result of Sub-inoculation with Brain
No. 1	1.5 died		
2	22.5 〃	No	
3	31.5 〃	No	
4	34.5 killed	No	
5	34.5 〃	No	
6	36.5 〃	No	Brain*→ip. 2 mice (P+, 9D)
7	39.5 〃	No	
8	39.5 〃	No	
9	41.5 〃	No	
10	42.5 〃	No	Brain→ip. 2 mice (P+, 6D & 8D)
11	53.5 〃	Yes	Brain→ip. 10 mice (P+, Ave.** 8.8D)
12	53.5 〃	No	orally, 2/10 mice (P+, 10D)
13	53.5 〃	No	
14	53.5 〃	No	See Note
15	53.5 〃	No	
16	53.5 〃	No	ip. 5 mice (P+, Ave. 9D)
17	57.5 〃	Yes	Brain→orally, 1/5 mice (P+, 9D), 4/5 Neg
18	57.5 〃	No	Brain→ip. Mice (P+, D)***
19	57.5 〃	No	Brain→ip. Mice (P+, D)
20	57.5 〃	No	Brain→ip. Mice (P Neg)****

Note

5 brains from Mouse No. 12 to No. 16 were pooled and emulsified, then inoculated to fresh mice.

Brains No. 12–16
- ip. 9/10 mice (P+, 11D) & 1/10 mouse (P Neg)
- orally, 9/10 mice (P & cyst Neg) & 1/10 mouse (cyst + in brain)

Retreatment with sulfadiazine

- ip. 2/10 mice (cyst+in brain)→Brain→ip. 1 mouse (P+, D) & orally, 1 mouse (Neg)
  Brain→orally, 1 mouse (Neg)
- orally, 10/10 mice (Neg)

* Brain of No. 6 was inoculated intraperitoneally to 2 fresh mice, and the mice died after 9 days and proliferative forms (P) were detected mainly from peritoneal cavity of the mice.

** Ave. means average survival days of 5 inoculated mice.

*** D means death of inoculated mice within 10 days.

**** Neg means negative of cyst and proliferative form.

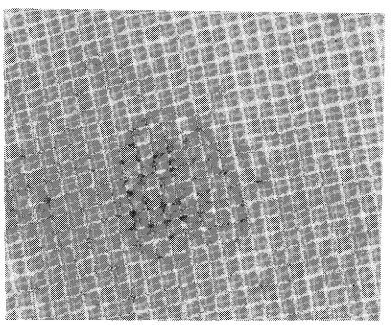

Fig. 1 Cyst of RH strain obtained by treatment with sulfadiazine (No. 11 in Table 2) (diameter about 30μ).

except one mouse which died of the acute infection. The pooled emulsion of the brains taken from 5 mice (mouse No. 12～16) was given intraperitoneally or orally to each 10 mice respectively. All the mice inoculated intraperitonealy died of the acute infection except one which survived for a long period, and the mice given orally survived without the infection except one from which cysts were detected in the brain. The same emulsion was given orally or intraperitoneally to another fresh mice, and the mice were treated with sulfadiazine just same as the initial treatment. The mice, which were given the emulsion orally, survived for a long period, and from 2 of 10 mice which inoculated intraperitoneally, cysts were observed in their brains. The brain emulsions from these 2 mice were inoculated intraperitoneally to fresh mice, which died of the acute infection, but the mice to which the emulsion was orally survived. As a result, even if mice survived with a chronic infection of RH strain after the treatment of sulfadiazine, the virulence character of RH strain did not change to Beverley type (cyst-cyst type) consistently, namely, after 1～2 passages of the cyst-type RH strain, the strain enhanced to highly virulent nature.

In Policydal, except a group of mice which died of sub-acute infection after the intraperitoneal treatment with 4 mg per day for 7 days, the other groups of mice survived with the chronic infection or without any sign of the infection. The results of a group which was treated intraperitoneally with 6 mg per day for 7 days, were shown in Table 3. Seven out of 9 inoculated mice died, in which the proliferative forms were detected within 20 days in ascites of the mice. From the brains of 3 mice out of 7, the proliferative forms were also observed with the examination of a fluorescent microscope after acridin orange staining (modified from Sakamoto, 1966). Two mice survived for longer than a month, but finally one of them died 43 days after the inoculation, and in its ascites, some proliferative forms were observed. The last mouse was killed to be examined 48 days after the inoculation. Some proliferative forms were still found in ascite of the mouse. From its brain, too many cysts and few proliferative forms were detected. In the case of Policydal, the treatment

Table 3. Experimental treatment of RH inoculated mice with Policydal (6 mg/day for 7 days, intraperitoneally)

Mouse No.	Survival Days	Proliferative Forms in Peritoneal Cavity	Proliferative Forms & Cysts in Brain*
No. 101	19 died	Yes*	No*
102	19 〃	Yes*	No*
103	20 〃	Yes*	No*
104	22 〃	Yes*	P: Yes**
105	22 〃	Yes*	No*
106	22 〃	Yes*	P: Yes**
107	23 〃	No*	P: Yes**
108	43 〃	Yes (few)**	P: Yes**, C: Yes**
109	48 killed	Yes (few)**	P: Yes**, C: Yes**

* Fresh preparation was examined without staining.

** Acridin orange staining perparation was examined by fluorescent microscope.

P : Proliferative form C : Cyst

with 4 mg or 6 mg per day for 7 days did not give rise to better curative effect than with 2 mg per day for 14 days, and in the latter treatment inoculated mice survived for a long period.

From the present experiments with RH strain, even if the inoculated mice survived with the chronic infection, it was rather difficult to find out cyst in the brain, while the brain emulsions were able to infect fresh mice. Then it seems likely that proliferative from can be present within some tissues or cells of the mice without cyst. Then in the next experiment, such the possibility was examined. Table 4 shows results of examination of the suspect tissues in which the proliferative forms or the cysts might be hiden in the mice of chronic infection. In this experiment, the mice No. 17 and No. 19 in Table 2, and the mice inoculated with N-130 and N-108 strain respectively, were used, and their tissues were emulsified separately in physiological saline and inoculated intraperitoneally with antibiotics to fresh mice. The parasites still existed even in the heart blood of mouse No. 19 which survived for 57 days in a chronic infection. From brains, livers, and spleens of all four mice examined, the parasites were detected, but from hearts, the parasite was not found except one case which inoculated N-108 strain. Unfortunately, its heart blood was not examined.

2. *Attempt to change the virulence of Beverley strain to that of RH type by the treatment of cortison or Kenacort*

To enhance the virulence of Beverley strain, mice were injected with cortison (1.25 mg or 2.5 mg/mouse) or Kenacort (2 mg or 4 mg/mouse) intramusclarly. Then, each mouse was inoculated intraperitoneally with 10～20 cysts. Five days later, a fluid which was taken by washing with a buffered saline solution of the peritoneal cavity of each mouse was inoculated intraperitoneally to each fresh mouse which was also treated with the same chemical used to the mouse of first passage. After the same treatment and inoculation were repeated up to five passages of mice, washing fluid of each mouse of the last passage was the inoculated to each fresh mouse without the treatment. In the case of Kenacort most of

Table 4. Parasite detection from various organs of chronic infection mice with RH type by sub-inoculated method to fresh mice.

Organ	RH(17), Cyst + 57.5 killed*	RH (19),** Cyst- 60 killed	N-130, Cyst + 60 killed	N-108, Cyst + 90 killed
Heart blood	No	Yes***	−	−
Heart	No	No	No	Yes
Lung	No	Yes	Yes	Yes
Liver	Yes	Yes	Yes	Yes
Spleen	Yes	Yes	Yes	Yes
Kidney	No	Yes	No	Yes
Brain	Yes	Yes	Yes	Yes

* RH (17), Cyst+, 57.5 killed means Mouse No. 17 (in Table 2) which was killed after 57.5 days from inoculation and cysts were detected in brain.

** RH (19) means Mouse No. 19 in Table 2.

*** Yes means inoculated mouse died of acute infection.

Table 5. Enhancement of virulence in Beverley strain by using of cortison or Kenacort

Drug & Dosage		Mouse No.	Passages in Treated Mouse	Passages in Untreated Mouse
Kenacort	4 mg	No. 201 ~ 206	6/7 1~3 passages, Bact. Cont.	
		207	1/7 P 2.0~3.7, 5~6D	
	2 mg	208	$\overset{8D}{2.8}\rightarrow\overset{7D}{0.8}\rightarrow\overset{7D}{0.2}\rightarrow\overset{8D}{0.4}\rightarrow\overset{7D}{2.0}$	$\rightarrow\overset{6D}{2.7}\rightarrow\overset{4\sim5D}{P+}\rightarrow$ 7 passages<
		209	$\overset{7D}{1.0}\rightarrow\overset{9D}{1.1}\rightarrow\overset{7D}{0.2}\rightarrow\overset{9D}{0.7}\rightarrow\overset{8D}{3.1}$	$\rightarrow\overset{6D}{1.5}\rightarrow\overset{5\sim7D}{P+}\rightarrow$ 7 passages<
		210	$\overset{6D}{4.6}\rightarrow\overset{6D}{3.4}\rightarrow\overset{7D}{0.4}\rightarrow\overset{7D}{+}\rightarrow$Bact. Cont.	
		211	$\overset{8D}{5.2}\rightarrow\overset{6D}{0.3}\rightarrow\overset{7D}{0.3}\rightarrow\overset{8D}{0.1}\rightarrow\overset{8D}{0.6}$	$\rightarrow\overset{5D}{P+}$
		212	6D Bact. Cont.	
		213	7D Bact. Cont.	
Cortison acetate	1.25 mg	214	$\overset{10D}{0.2}\rightarrow\overset{7D^{1)}}{1.5}\rightarrow\overset{7D}{0.7}\rightarrow\overset{C+}{P+}\rightarrow9D$	[1] 3/3 P + & D → 2/2 C + → C +
		215	$\overset{C+}{0.01}\rightarrow\overset{C+}{0.06}\rightarrow\overset{C+}{0.17}\rightarrow\overset{C+}{0.02}\rightarrow C?$	
		216	$\overset{12D}{0.02}\rightarrow\overset{C+}{0.02}\rightarrow\overset{C+}{0.02}\rightarrow\overset{C+}{P+}\rightarrow C+$	
		217	$\overset{13D}{0.02}\rightarrow\overset{C+}{0.04}\rightarrow\overset{C+}{0.05}\rightarrow\overset{C+}{P-}\rightarrow C?$	
		218	$\overset{C+}{0.03}\rightarrow\overset{C+}{0.02}\rightarrow\overset{C+}{0.05}\rightarrow\overset{C+}{P-}\rightarrow C+$	
	2.5 mg	219	$\overset{10D^{1)}}{0.01}\rightarrow\overset{7D}{2.07}\rightarrow\overset{14D^{2)}}{1.02}\rightarrow\overset{12D}{0.35}\rightarrow6D$	[1] Cyst-cyst type, [2] P+ & D 7 passages
		220	$\overset{C+}{0.02}\rightarrow\overset{C+}{P-}\rightarrow\overset{C+}{0.04}\rightarrow\overset{C+}{0.12}\rightarrow\overset{10D}{P+}$	
		221	$\overset{C+}{0.01}\rightarrow\overset{C+}{0.02}\rightarrow\overset{C+}{0.07}\rightarrow\overset{C+}{0.42}\rightarrow C+$	
		222	$\overset{C+}{0.01}\rightarrow\overset{C+}{0.29}\rightarrow\overset{C+}{0.01}\rightarrow\overset{C+}{0.01}\rightarrow C+$	
		223	$\overset{10D}{0.39}\rightarrow\overset{6D^{1)}}{1.38}\rightarrow\overset{22D}{3.07}\rightarrow9D$	[1] 2 passages P + & D → Cyst-cyst
Control		224	$\overset{7D}{0.2}\rightarrow\overset{C+}{0.1}\rightarrow\overset{C+}{P+}\rightarrow\overset{C+}{P-}\rightarrow\overset{C+}{P-}$	
		225	$\overset{C+}{P?}\rightarrow\overset{8D}{0.03}\rightarrow\overset{C+}{P-}\rightarrow\overset{C+}{P-}\rightarrow\overset{C+}{P-}$	
		226	$\overset{11D}{1.5}\rightarrow\overset{7D}{1.6}\rightarrow\overset{8D}{0.4}\rightarrow\overset{18D}{P-}\rightarrow\overset{7D}{0.7}$	→ 4 passages P + & D
		227	$\overset{10D}{3.0}\rightarrow\overset{5D}{P+}$ Bact. Cont.	
		228 ~ 233	C+→C+→C+→C+→C+	

$\overset{8D}{2.8}$: First passage mouse died of an acute infection after 8 days, and the volume of ascite was 2.8 ml.

C+ : Cyst positive in the brain of survival mouse

P+ & D : Proliferative form positive and death of an acute infection

Bact. Cont. : Bacterial contamination

mice injected 4 mg died of bacterial contamination, but with 2 mg dose, the mice of two out of 6 cases died of the acute infection within 4～5 days even after stop of the treatment (Table 5). Even in the non-treated control series, one case became a virulent type for several passages as shown in Table 5.

In the case of cortison, as shown in Table 5, any case did not change to the virulent type except one.

The number of proliferative forms in ascite was counted, but usually even in the acute infection, the parasites were not so many as compared with the case of RH strain. Table 5 summarizes the result of seven passages of each series after the stop of the treatment. Even if once the virulence of Beverley strain enhanced like RH type, often the parasites of virulent Beverley type turn again to avirulent Beverley type (cyst-cyst type) after serial passages.

DISCUSSION

In the present study, the author attempted to change the virulence of RH strain to a less virulent type (cyst-cyst type). However, as already mentioned above, the modification of virulence in the RH strain by drugs did not succeed. From this experiment, some interesting findings were obtained: in many cases of RH chronic infection, cysts were never detected in mouse brains, but the brain emulsions were infective to fresh mice after the intraperitoneal inoculation. By using a fluorescent microscope and acridin orange staining, banana-shaped parasites (proliferative forms in this paper) were detected in the brains and the peritoneal washing of the mice with RH chronic infection. In the case of brain of course it might be possible that the proliferative forms escaped from a destroyed cyst during a procedure for the staining. In the case of the peritoneal washing, however, the proliferative forms were found in certain free cells in the fluid. Furthermore the brain emulsion could easily infect fresh mice by intraperitoneal injection, but the same emulsion was rather difficult to infect fresh mice by oral treatment. This phenomenon also supports that the proliferative forms have been present in certain cells without the cyst wall which is protective against digestive fluid. According to Motomura (1967), when 1～5 cysts of Beverley strain were given to mice orally, about 70% of which became parasite positive, but in the case of oral inoculation with RH proliferative forms, at least 10^3 parasites were neccessary to infect mice. From one of the RH chronic infection mice, the parasites were found from heart blood in the present study. In the case of human chronic infection, the positive detections of toxoplasma from blood were rare as reported by Aoki (1973). To understand delayed parasitemia, there are two possible explanations as follows; 1) "flame-up" from raptured cysts and 2) the parasite, not cyst, survives in small number and multiples slowly. It might be possible that the proliferative forms could survive and multiple in certain cells for a long period during a chronic infection.

An attempt to enhance the virulence in Beverley strain by the use of cortison acetate was reported by Simizu *et al.* (1967). Results obtained by the present author are also similar to those by the previous authors. Concerning to suppress the immune response of

infected mice, the workers of Keio University have written many papers, for example Stahl *et al.* (1966). From the present author's results, it might be said that Beverley strain became to high virulence (RH-type) by using cortison or Kenacort for several passages, and infected mice died of acute infection within 6～8 days, but the virulence easily turn to the initial character of cyst-cyst type.

According to the present results and previous experiences (Nakabayashi *et al.*, 1969), the following four types of the toxoplasma infection in mice might be distinguished.

Type 1. This includes so called RH-type (virulent type) and parasites give rise to acute or subacute fatal infections. The infected mice have some symptoms such as standing-hair, diarrhea, and producing peritoneal exudate of about 1～2ml in which a number of parasites are present, and finally the mice die within 5～18 days after the inoculation. In this type, the mice die before raising of the titer of HA or Dye test. In the author's experience, the strains isolated from pigs were avirulent at first, but they gradually changed to virulent type during serial passages. The inoculated number of the parasites might also be important, and if a small number was inoculated to mice, some of the strains isolated from pigs produced a chronic infection. Even in Beverley strain, which were inoculated as cysts, sometimes mice died of acute or subacute infection.

Type 2. This infection type includes Beverley type (cyst-cyst type), and parasites give rise to a chronic infection, and the infected mice survive for longer than one month, and from their brains many typical cysts are detected. In this type, proliferative forms are observed in peritoneal washing fluid during early stages of the infection, but the forms disappeared possibly after raising of immune reactions. By drug treatment, the author could produce this type of the infection in RH infected mice.

Type 3. This is also chronic, but cyst is difficult to find out in infected mice. The proliferative forms might survive certain tissues or cells in small number and might be able to multiple slowly. The proliferative forms are easily detected in peritoneal washing fluid during early stages of the infection, but later the parasites disappear because of, possibly, raising of immune reaction. But by using of fluorescent microscope and acridin orange staining, even from mice which survived for longer than 40 days after the inoculation, the proliferative forms were detected in certain free cells of the peritoneal cavity and in the brains.

Type 4. In this type, once mice infect with toxoplasma, but later the parasite disappeare completely without any drug treatment, and hence the brains or other organs do not infect fresh mice. According to the author's experience, some mice inoculated with a pig material, in which many proliferative forms were found, showed an increase of HA titer, whereas the parasite could not be detected.

Usually types 2 and 3 were recognized as a chronic infection, and in these infection type, it might be believed by many workers that cysts were produced in the brains or other tissues. The author's opinion is, however, type 3 might be present because of above discussing results. In this type, only proliferative forms were detected.

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薬剤投与によるトキソプラズマ RH 株および Beverley 株の毒性を変える試み

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実験室で用いられているトキソプラズマの RH 株および Beverley 株は、それぞれ毒性が比較的安定しており，前者は強毒株として知られ，感染マウスは数日で死ぬが，後者は慢性感染をおこし，マウスは長く生存し，その脳内にシストができる．この実験の目的は，それぞれの株の毒性を全く反対の性質に変えようということである．得られた成績は次の通りではる．　1．RH 株接種マウス群では，スピラマイシンやサルゾール S (sulfathiazole-N^4) を与えた場合，無処置対象群よりもマウスの生存期間がやや長くなるが，結局急性感染死する．しかしサルファダイアジンやポリサイダール（サルファメソピラジン）で，長期間治療を続けるとRH感染マウスの多くは30日以上の長期間生存し，脳内にシストの検出された例もあった．しかしこのシストを別のマウスに接種すると，2代目マウスは急性感染死し，結局 RH 株を弱毒化することはできなかった．なお慢性期においてもRH株では，必ずしもシスト壁につつまれずに虫体が生残しわずかではあるが増殖している可能性がある．　2．Beverley 株接種マウスにケナコルト（トリアムシノロン）またはコーチゾンを筋注し，急性感染をおこさせ，接種5日後に腹腔洗液を次代の同じ処置をしたマウスに接種する．このような操作を5代継続し，6代目から無処置マウスに変えて，Beverley 株が RH 株のように強毒株に移行するかどうかを調べた．結果は1部処置群のみならず無処置対照群でも，強毒化するものがあった．しかしこの強毒化した性質はきわめて不安定で，継代を続けるうちにしばしばもとの Beverley 株本来の弱毒な毒性に戻った．　3．結局両株とも実験株として，長いマウスによる継代の間にその毒性がすっかり安定しており，両株ともその毒性を逆転させることはできなかったが，この実験中興味深い観察が得られたので，それについて論じた．

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