An Experiment on the Survival of *Toxoplasma gondii* in the Low Temperature Rooms of a Slaughterhouse

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Abstract

Examination was made of the survival period of *Toxoplasma gondii* in the low temperature rooms of a slaughterhouse. Materials for the examination were the mouse bodies themselves and the organs, such as the liver and brain, of mice infected with the RH or Beverley strain. These materials were stored in both of the refrigerating and the cold-storage rooms, then taken out of the rooms one after another at a short interval and examined on the existence of live Toxoplasma in them with the intraperitoneal inoculation into healthy mice.

In the first experiment, it was revealed that the survival of the proliferative form of Toxoplasma in an infected mouse body was 8 days in the refrigerating room and 4 days in the cold-storage room, but a putrefactive sign was manifesting slowly in the mice stored more than 13 days in the refrigerating room and 6 days in the other. In the following experiment, the livers excised from RH-infected mice and the brains from Beverley-infected ones were stored only in the refrigerating room. It was recognized as the result that cysts were capable of survival for as long as 67 days and proliferative forms could survive for 11 days in the room. A control experiment was attempted on the resistance of *T. gondii* to −14°C in a freezer and it was shown that both forms of this protozoa in the infected mouse organs could remain alive more than an hour but did not for 3 hours in a freezer of −14°C. Temperatures in both rooms were continuously measured by auto-recording thermometers. In the refrigerating room, it was 0.47°C in average and the cold-storage room always had 3 to 4°C higher temperature than the refrigerating room.

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Introduction

The edible flesh and organs of meat animals, especially pork in Japan, have been regarded as the most important source of Toxoplasma infection in man. Since many reports* have been so far contributed on the isolation of Toxoplasma from slaughtered animals, there might be no doubt about this opinion any longer.

The animal meat and organs treated at a slaughterhouse, in general, are once stored in a low temperature room for a few days, usually 4–6 days, then carried to a market. Accordingly, it can be considered most reasonable that an adequate measure for eliminating Toxoplasma from animal meat should be taken during the period of meat storage at a slaughterhouse.

There have been reported by several workers regarding the resistance of Toxoplasma to a low temperature. Jacobs *et al.* (1960) described that cysts could survive longer than proliferative forms and cysts survived as long as 68 days at 4°C. Fukazawa *et al.* (1964) reported the data indicating that proliferative forms in the peritoneal fluid of mice survived for 14 days and cysts in a mouse brain emulsion did for 40 days at 4°C, although both forms were destroyed within a day at −15°C. Robl (1965) stated that the longest survival of cysts was 50 days at 4°C in 5% glycerin saline and also 50 days at −20°C in milk. These experiments, however, were all carried out with use of a refrigerator and a freezer in their laboratories.

At the opportunity of making an examination on the distribution of Toxoplasma in meat animals at the Nagasaki City Slaughterhouse, the authors planned to examine on the survival period of Toxoplasma in the low temperature rooms of the slaughterhouse.

Since it was, however, difficult to use the flesh and organs of the large meat animals practically killed there, mice and their organs harboring Toxoplasma were used for this purpose.

Materials and method

Slaughterhouse employed

With permission of the managing authorities the Nagasaki City Slaughterhouse was used for this research during a given short period. Both of the refrigerating and the cold-storage rooms of the slaughterhouse were adjoined by a door, and 75.7m^2 and 41.6m^2 respectively. Temperature was adjusted to keep constantly approximately 0°C in the former room and 3°C in the latter. The temperatures, however, were expected to be waved up and down at the range of 2 to 3°C several times daily because of the periodical opening of the doors leading to the rooms and of carrying warm animal meat into them. It was the routine course of meat management that fresh meat was first carried in the refrigerating room, then moved into the cold-storage room after

*Refer to the publications quoted in this paper.

the meat was refrigerated, and the total storage period was usually 4 to 6 days. The whole period of experiment was from August 17 through December 10 in 1966.

Experimental materials

Materials used were both of the RH and Beverley strains of *Toxoplasma gondii* and mice weighing approximately 20 gm. RH-infected mice, the livers excised from them and the brains from Beverley-infected mice were employed for the exposure test to a low temperature at the slaughterhouse.

When mouse bodies themselves were used, the mice, which had been infected intraperitoneally with 10^6 proliferative forms of the RH strain in 0.1 ml physiological saline solution, were killed manually by the spinal cord splitting method* on the 4th day of infection, as the parasite number in the mice had reached the maximum. When the livers of RH-infected mice were used, they were excised with a possible aseptic procedure from the mice killed on the 4th day of infection and each was enclosed tightly in a small sterile tube with a rubber stopper. The brains of Beverley-infected mice, in which a great number of cysts had been probably formed by the time of their excision, were also excised aseptically from the mice later than the 30th day of infection and likewise put in small sterile tubes. Prior to the exposure of the materials to a low temperature, the random sampling of few materials was made from each group of materials and applied to an examination on the presence of live Toxoplasma in them. The results of this control experiment were described in the tables of this paper as the data at the storage time 0.

Since the materials to be stored in the low temperature rooms contained live Toxoplasma, precaution had to be paid for not causing the contamination of the rooms with Toxoplasma. To meet this inevitable request, the materials, either mouse bodies or the organs in small tubes, each was enclosed first in a three fold vinyl sack which was made to be each separately closed tightly with a rubber band, next in a clean small can with a tight lid and finally again in a vinyl sack. The outsides of the can and the outermost vinyl sack were satisfactorily wiped with 70% alcoholic cotton and dried. A set of enclosed materials was put together in a wooden box and locked. Approximately 30 materials were usually employed as a set in a experiment.

Temperatures in the refrigerating and the cold-storage rooms were continuously measured by auto-recording thermometers throughout the period of experiment.

Experimental method

Infected materials stored in both low temperature rooms were carried to the laboratory one after another everyday or at the interval of 2 to 4 days and subjected to an examination on the presence of live Toxoplasma in them by the mouse inoculation test. In the lst experiment, RH-infected mouse bodies were stored in both rooms, and the liver and spleen were excised aseptically from the mice carried from the slaughterhouse. In the 2nd, the livers of RH-infected mice and the brains

*The spinal cord splitting was made manually by pulling the tail of mouse, while fixing the neck with the fingers of another hand.

of Beverley-infected ones, both being stored in the refrigerating room, were carried to the laboratory.

Each of these organs was emulsified in a mortar with 4 ml saline for a liver and a brain, and 2 ml for a spleen. Penicillin G Kalium and Dihydrostreptomycin Sulfate were added to an emulsion to the concentration of 1,000 units per ml and 1 mg per ml. Subsequently, a sheet of small sterile gauze was spread to cover the surface of emulsion in order to sink any large bit of tissue under the gauze, otherwise they would plug a needle of syringe. The upper portion of emulsion over the gauze was used for the intraperitoneal inoculation into healthy mice with 2 ml syringe. The amount of inoculum to a mouse was 0.3ml, and the liver and brain emulsions were each injected to 3 mice and the spleen emulsion to one. Mice dead or dying within 30 days after the inoculation were subjected to the detection of Toxoplasma in them.

When mice survived more than 30 days, they were killed and examined for the same purpose. Microscopical examination was made of the fresh preparations with cover slips and the Giemsa stained smears of peritoneal fluid for the detection of proliferative forms, and the fresh emulsified preparations with covers and the Giemsa stained smears of the brain for cysts.

As a control experiment, a freezer in the laboratory, of which temperature was adjusted to −14°C, was employed for knowing the survival of Toxoplasma in the organs of mice, when they were exposed to a temperature as low as −14C°. The livers and brains of mice infected respectively with RH and Beverley were likewise applied to the examination in this case.

Experimental results

1. The first experiment

In this experiment, mouse bodies themselves, which had been infected with RH and killed by the spinal cord splitting method, were used in order to recognize the survival time of proliferative forms in them. Results obtained were given in Tables 1 and 2.

When the refrigerating room was used, RH proliferative forms were found to be capable of survival for 8 days in the liver of the mouse exposed to average 0.9°C in the room, from the fact that 3 mice, which were inoculated with the liver of a 8-days-stored mouse, died within 10 days and proliferative forms were microscopically detected in their peritoneal fluids. On the other hand, a mouse which was inoculated with the spleen of the same 8-days-stored mouse, survived over 30 days and no Toxoplasma was detected in the peritoneal fluid. It was, however, reasonably confirmed that live proliferative forms might still exist in the spleen of a 6-days-stored mouse, because a small number of them were detected in the peritoneal fluid of the mouse, which had been inoculated with the spleen of a 6-days-stored mouse and survived over 30 days thereafter. As to

Table 1. Survival days of *Toxoplasma gondii* in the RH-infected mice stored in the refrigerating room

Days stored	Mouse No.	Fate of mice inoculated with liver or spleen of a stored mouse	Average survival days of mice inoculated[1]	Detection of *T. gondii*[2]	
				Proliferative forms	Cysts
0	No. 1	3 D[4]	3.8	+	−
	2	4 D		+	−
	3	4 D		+	−
	4[3]	4 D		+	−
1	No. 1	4 D	4.8	+	−
	2	4 D		+	−
	3	4 D		+	−
	4	7 D		+	−
2	No. 1	5 D	5.5	+	−
	2	6 D		+	−
	3	6 D		+	−
	4	5 D		+	−
3	No. 1	5 D	5.5	+	−
	2	5 D		+	−
	3	5 D		+	−
	4	7 D		+	−
4	No. 1	6 D	6.0	+	−
	2	6 D		+	−
	3	6 D		+	−
	4	6 D		+	−
6	No. 1	8 D	8.0	+	−
	2	8 D		+	−
	3	8 D		+	−
	4	34 S[5]		+	−
8	No. 1	8 D	8.7	+	−
	2	8 D		+	−
	3	10 D		+	−
	4	34 S		−	−
9	No. 1	34 S		−	−
	2	34 S		−	−
	3	34 S		−	−
	4	34 S		−	−
10	No. 1	34 S		−	−
	2	34 S		−	−
	3	34 S		−	−
	4	34 S		−	−
11	No. 1	34 S		−	−
	2	34 S		−	−
	3	34 S		−	−
	4	34 S		−	−
13	No. 1	34 S		−	−
	2	34 S		−	−
	3	34 S		−	−
	4	34 S		−	−

Notes:
Temperature in the refrigerating room was 0.9°C in average and 2.5 to −1.1°C in range.
Experimental method: The liver and spleen were removed from the RH-infected mouse stored in the refrigerating room and emulsified in saline. Mice were inoculated with the emulsions and examined for the detection of Toxoplasma.

1) Calculation was made only from death cases.
2) The peritoneal fluids and brains were used for the detection of proliferative forms and cysts respectively.
3) No.4 mice were inoculated with spleen emulsions. Others with liver emulsions.
4) 3 D means the death of mouse on the 3rd day of infection.
5) 34 S means the survival of mouse for 34 days.

Table 2. Survival days of *Toxoplasma gondii* in RH-infected mice stored in the cold-storage room

Days stored	Mouse No.	Fate of mice inoculated with liver or spleen of a stored mouse	Average survival days of mice inoculated[1]	Detection of *T. gondii*[2]	
				Proliferative forms	Cysts
0	No. 1	4 D[4]	4.3	+	−
	2	4 D		+	−
	3	5 D		+	−
	4[3]	4 D		+	−
1	No. 1	4 D	6.3	+	−
	2	7 D		+	−
	3	8 D		+	−
	4	6 D		+	−
2	No. 1	5 D	6.5	+	−
	2	5 D		+	−
	3	7 D		+	−
	4	9 D		+	−
3	No. 1	6 D	7.3	+	−
	2	8 D		+	−
	3	8 D		+	−
	4	7 D		+	−
4	No. 1	6 D	8.3[6]	+	−
	2	9 D		+	−
	3	10 D		+	−
	4	21 D		−	−
6	No. 1	32 S[5]		−	−
	2	32 S		−	−
	3	32 S		−	−
	4	32 S		−	−
8	No. 1	32 S		−	−
	2	32 S		−	−
	3	32 S		−	−
	4	32 S		−	−
10	No. 1	32 S		−	−
	2	32 S		−	−
	3	32 S		−	−
	4	32 S		−	−
11	No. 1	32 S		−	−
	2	32 S		−	−
	3	32 S		−	−
	4	32 S		−	−

Notes:
Temperature in the cold-storage room was 4.2°C in average and 5.1 to 3.2°C in range.
1−5) Refer to the notes in Table 1.
6) 21 D in No. 4 mouse was not included in the calculation of average survival days.

a 13-days-stored mouse, discoloration to reddish black and slight putrefactive smell of the abdominal organs were noticed when the abdominal incision was given to the mouse. Since it was regarded as a sign of putrefaction in that mouse, the subsequent experiment was stopped.

In the experiment using the cold-storage room, it was found as shown in Table 2 that virulent proliferative forms of the RH strain could still survive in the liver of a 4-days-stored mouse and in the spleen of 3-days-stored one. In this series of experiment, a sign of putrefaction was observed to develop in the mouse stored for 6 days or more.

An interesting phenomenon was that the survival period of the mice inoculated with infected materials had a tendency to extend along with prolongation of the storage period of the materials as indicated in the column of the average sur-

ival days of mice in Tables 1 and 2. It was understood that this might be caused by reason of either the reduction in number of the surviving proliferative forms or the lowering of infecitivity of Toxoplasma in the mice exposed to a low temperature, or both.

Temperature in the refrigerating room was 0.9°C in average and 2.5 to −1.1°C in the range from the maximum to the minimum, and it was 4.2°C and 5.1 to 3.2°C in the cold-storage room during the period of experiment.

2. The second experiment

In this experiment, the livers taken from the mice infected with RH and the brains from ones with Beverley were tested only in the refrigerating room. This decision was lead from the facts that in the previous experiment, a putrefactive change was observed to develop in mice, if mouse bodies were used in the exposure test to a low temperture, and that, much difference in temperature was not recognized between both rooms employed, although the refrigerating room always indicated a little lower temperature than the other. Results obtained were shown in Tables 3 and 4.

Table 3. Effect of storage of the infected mouse livers in the refrigerating room upon survival of the RH proliferative form

Days stored	Mouse No.	Fate of mice inoculated with liver emulsions	Average survival days of mice inoculated	Detection of *T. gondii*	
				Proliferative forms	Cysts
0	No. 1	3 D	3.7	+	−
	2	4 D		+	−
	3	4 D		+	−
1	No. 1	4 D	4.0	+	−
	2	4 D		+	−
	3	4 D		+	−
3	No. 1	5 D	5.0	+	−
	2	5 D		+	−
	3	5 D		+	−
5	No. 1	6 D	6.0	+	−
	2	6 D		+	−
	3	6 D		+	−
7	No. 1	6 D	6.7	+	−
	2	7 D		+	−
	3	7 D		+	−
9	No. 1	6 D	7.0	+	−
	2	7 D		+	−
	3	8 D		+	−
11	No. 1	10 D	10.7	+	−
	2	11 D		+	−
	3	11 D		+	−
13	No. 1	43 S		−	−
	2	43 S		−	−
	3	43 S		−	−
15	No. 1	43 S		−	−
	2	43 S		−	−
	3	43 S		−	−
17	No. 1	43 S		−	−
	2	43 S		−	−
	3	43 S		−	−

Notes:
Temperature in the refrigerating room was 0.47°C in average and 3.2 to −3.5°C in range.
Other explanations were described in the previous tables.

Table 4. Effect of storage of the infected mouse brains in the refrigerating room upon survival of the Beverley cyst form

Days stored	Mouse No.	Fate of mice inoculatd with brain emulsions	Detection of *T. gondii*	
			Porliferative forms	Cysts
0	No. 1	12 D[1]	+	
	2	43 S[2]	−	+
	3	43 S	−	+
2	No. 1	10 D	+	
	2	30 S	−	+
	3	43 S	−	+
7	No. 1	6 D	+	
	2	12 D	+	
	3	14 D	+	
15	No. 1	12 D	+	
	2	18 D	−	+
	3	18 D	−	+
21	No. 1	12 D	+	
	2	30 S	−	+
	3	30 S	−	+
28	No. 1	34 S	−	+
	2	34 S	−	+
	3	34 S	−	+
35	No. 1	30 S	−	+
	2	30 S	−	+
	3	30 S	−	+
42	No. 1	39 S	−	+
	2	39 S	−	+
	3	39 S	−	+
49	No. 1	31 S	−	+
	2	31 S	−	+
	3	31 S	−	+
56	No. 1	12 D	+	
	2	12 D	+	
	3	30 S	−	+
59	No. 1	9 D	−	−
	2	40 S	−	+
	3	40 S	−	+
63	No. 1	39 S	−	+
	2	39 S	−	+
	3	39 S	−	+
67	No. 1	33 S	−	+
	2	33 S	−	+
	3	33 S	−	+
70	No. 1	36 S	−	−
	2	36 S	−	−
	3	36 S	−	−
73	No. 1	33 S	−	−
	2	33 S	−	−
	3	33 S	−	−
77	No. 1	30 S	−	−
	2	30 S	−	−
	3	30 S	−	−
80	No. 1	30 S	−	−
	2	30 S	−	−
	3	30 S	−	−
87	No. 1	30 S	−	−
	2	30 S	−	−
	3	30 S	−	−

Notes:
Temperature in the refrigerating room was 0.47°C in average and 3.2 to −3.5°C in range.
1) 12 D means the death of mouse on the 12th day of infection.
2) 43 S means the survival of mouse for 43 days.
Some of results of the experiment were excluded from this table.

The livers infected with RH were transported one by one every third or fourth day to the laboratory from the refrigerating room in order to examine on the existence of live Toxoplasma in them. The presence of virulent proliferative forms was confirmed in the livers which had been stored within 11 days. This confirmation was lead from the results that the mice inoculated with one of those livers were all died from infection within 10 days and that, proliferative forms were detected in their peritoneal fluids. While, all mice inoculated with the materials which had been stored more than 11 days survived over 30 days and no Toxoplasma was demonstrated either in the peritoneal fluids nor in the brains. It was observed in this experiment similarly as in the lst experiment that the survival days of the mice inoculated with RH-infected livers trended to prolong along with extension of the storage period of infected livers.

As for the experiment on storage of the brains of mice infected with the Beverley strain, it was distinctly demonstrated that cysts in the brains could survive for as long as 67 days in the refrigerating room. It was generally accepted that the mice infected with Beverley would mostly survive more than 30 days, harboring cysts in their brain, although a few mice would die from infection within the period. As shown in Table 4, 12 mice died within 30 days and proliferative forms were detected in the peritoneal fluids of all mice but one which seemingly had an accidental death on the 9th day. Of the mice inoculated with the brains which had been stored within 67 days, others than above-mentioned 12 were all killed after 30 days and examined for the detection of Toxoplasma. Since not a few cysts were detected with a microscope in the brains of all mice examined, it was concluded that live cysts still existed in the 67-days-stored brain. On the other hand, all mice inoculated with the brains which had been stored over 67 days in the refrigerating room, survived long and neither cyst nor proliferative form was detected in any of them.

Temperature in the refrigerating room for 87 days in the 2nd experiment was 0.47°C in average and ranged from 3.2 to −3.5°C, and approximately 3 regular wavings daily were recorded as Figure 1 gives an example of the curve.

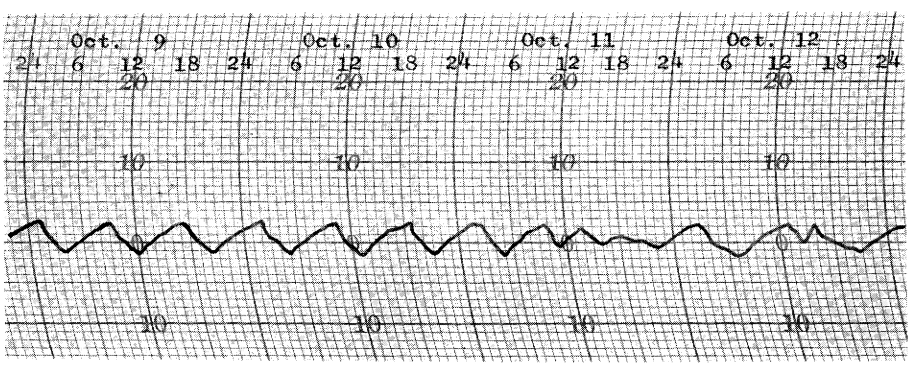

Fig. 1. Waves of temperature in the refrigerating room from 9 to 12, Octover

3. Control experiment with use of a freezer of −14°C

This control experiment was carried out with the object of knowing how long the survival time of Toxoplasma in the mouse organs could be in a temperature as low as −14°C. Materials used were the livers of RH-infected mice and the brains of Beverley-infected ones. Table 5 and 6 showed the results obtained in this experiment.

Table 5. Resistance of the RH proliferative form in the infected mouse livers to −14°C in a freezer

Time stored	Mouse No.	Fate of mice inoculated with liver emulsions	Average survival days of mice inoculated	Detection of *T. gondii*	
				Proliferative forms	Cysts
0	No. 1	5 D	5.0	+	−
	2	5 D		+	−
	3	5 D		+	−
5 min.	No. 1	6 D	6.0	+	−
	2	6 D		+	−
	3	6 D		+	−
15 〃	No. 1	8 D	8.0	+	−
	2	8 D		+	−
	3	8 D		+	−
30 〃	No. 1	9 D	9.0	+	−
	2	9 D		+	−
	3	9 D		+	−
1 hr.	No. 1	10 D	10.0	+	−
	2	10 D		+	−
	3	10 D		+	−
3 〃	No. 1	34 S		−	−
	2	34 S		−	−
	3	34 S		−	−
15 〃	No. 1	34 S		−	−
	2	34 S		−	−
	3	34 S		−	−
24 〃	No. 1	34 S		−	−
	2	34 S		−	−
	3	34 S		−	−

Notes:
Temperature in the freezer was −14°C in average and −13 to −15°C in range.
For explanations refer to the notes in the previous tables.

Three mice inoculated with the RH-infected liver which had been stored for 1 hour in −14°C, all died 10 days after the inoculation and proliferative forms were found in their peritoneal fluids. Whereas, 3 mice inoculated with the Beverley-infected brain which had been exposed to the same circumstance for 1 hour, survived more than 30 days after the inoculation and cysts were detected in the brain of only mouse of them. Other two mice, which demonstrated no cyst in their brains, were examined on the antibody titer of serum by the hemagglutination test in order to obtain a more detailed information of Toxoplasma infection. One of the two was positive in the serum test, demonstrating a positive response at 1,024 times dilution of serum, while the remaining one was negative. On the contrary, in both cases of RH and Beverley, the mice inoculated with the infected organs which had been stored for 3 hours or more in −14°C could all survive over 30

Table 6. Resistance of the Beverley cyst form in the infected mouse brains to −14°C in a freezer

Time stored	Mouse No.	Fate of mice inoculated with brain emulsions	Detection of *T. gondii*		Hemagglutination test
			Proliferative forms	Cysts	
0	No. 1	10 D	+		
	2	22 D	−	+	
	3	24 D	−	+	
5 min.	No. 1	32 S	−	+	
	2	32 S	−	+	
	3	32 S	−	+	
30 〃	No. 1	32 S	−	+	
	2	32 S	−	+	
	3	32 S	−	+	
1 hr.	No. 1	30 S	−	+	
	2	30 S	−	−	+
	3	30 S	−	−	−
3 〃	No. 1	30 S	−	−	−
	2	30 S	−	−	−
	3	30 S	−	−	−
15 〃	No. 1	34 S	−	−	−
	2	34 S	−	−	−
	3	34 S	−	−	−
24 〃	No. 1	39 S	−	−	
	2	39 S	−	−	
	3	39 S	−	−	
48 〃	No. 1	39 S	−	−	
	2	39 S	−	−	
	3	39 S	−	−	

Notes:
Temperature in the freezer was −14°C in average and −13°C to −15°C in range.
For explanations refer to the notes in the previous tables.

days and neither proliferative form nor cyst was detected in them. It was really of much interest that proliferative forms and cysts in the mouse organs could similarly survive only for less than 3 hours in a freezer of −14°C.

Temperature in the freezer revealed by an auto-recording thermometer was −14°C in average, accompanied by wavings between −13 and −15°C approximately 18 times daily.

Discussion

Since it is generally recognized at the present time that the flesh and organs of meat animals are the most significant sources of infection in human toxoplasmosis, it is needless to emphasize that the management of slaughtered animals has become greatly important for the suppression of Toxoplasma infection. There have been numbers of publications concerning studies on the isolation of this protozoa from slaughtered animals and on the distribution of animals in latent toxoplasmosis by means of the serological tests. In Japan, it is already ascertained that the swine has been playing a very important role in human infection with Toxoplasma.

To establish a control measure for preventing us from Toxoplasma infection, an examination should be first made on

the resistance of this protozoa in the animal meat and organs to environmental influences of various sorts. Speaking of the business of slaughterhouse on the management of animal meat, an examination on the resistance of Toxoplasma to a low temperature has a practical meaning. Whereas, in cooking the animal meat and organs, a treatment of them with a high temperature might be most important to kill Toxoplasma. Not a few reports have been published on the resistance of this protozoa to a low temperature, as already mentioned in the chapter of introduction. However, the low temperature which was applied for those experiments, was settled in a refrigerator or freezer at a laboratory and controlled to keep constantly a fixed temperature.

Summarizing the data so far published, it can be understood that in general, cysts can be more resistant to the temperature of refrigerator than proliferative forms and the cyst form of the Beverley strain in meat or in the brain is capable of survival more than 40 days in a refrigerator. Fukazawa's report (1964) was of interest, in which cysts and proliferative forms were both destroyed within a day at −15°C in a freezer. A more interesting result was obtained by Robl (1965), in which the freezing velocity and the medium suspending Toxoplasma might have a close relation to the survival of this protozoa and proliferative forms remained alive at −76°C in 5% and 10% glycerin saline for 200 days after slow freezing. His result also stated that the longest survival of cysts was 50 days at −20°C in milk as well as at 4°C in 5% glycerin saline. It should be, however, noticed that his result was not reproducible.

In the present experiment, the project was attempted to approach the actual condition of a slaughterhouse as much as possible. For this purpose, the low temperature rooms of a slaughterhouse, which were routinely used for storage of the meat and organs of killed animals, were employed instead of a refrigerator, and mouse bodies themselves and their organs harboring Toxoplasma were examined as materials for the survival test of this protozoa. Temperatures in the refrigerating and the cold-storage rooms were presumed to wave up and down to some extent daily owing to the frequent openings of doors and the transportation of large masses of warm meat into the rooms. As a matter of fact, the temperatures which were continuously checked by auto-recording thermometers showed a nearly regular waving three times daily during the experimental period. The range of waves, however, was not so much as expected.

Use of the meat and organs of large animals, such as swine, cattle and sheep, harboring Toxoplasma, was so much difficult for us that the mouse bodies infected with Toxoplasma, and their livers and brains were used as test materials. It could be expected to take at least several hours to completely refrigerate huge masses of the meat and organs of large animals in the refrigerating room. Therefore, use of the organs of a small animal like a mouse would be undesirable in this experiment, because it could never reveal the true feature of refrigeration

of large animal meat.

It was really noticiable that the cyst of Beverley in the mouse brain could remain alive for as long as 67 days and the proliferative form of RH in the mouse liver was capable of survival for 11 days in the refrigerating room, and temperature in the room was 0.47°C in average and 3.2 to −3.5°C in range during the period of experiment. These results can be acknowledged as a truth, in comparison with those obtained by the wokers previousely described, and it can be reasonably assumed that cysts in meat can survive long enough until the meat is brought to a kitchen. This assumption should be of much importance in the epidemiology of Toxoplasma infection.

In the first experiment using infected mouse-bodies, Toxoplasma could survive only for as short as 8 days in the refrigerating room. This probably was caused by the putrefaction slowly developing in the mouse bodies. This result, therefore, could not be accepted as the true fact which demonstrated the survival of Toxoplasma at the low temperature.

In the control experiment using a freezer at the laboratory, both forms of Toxoplasma were capable of survival only for an hour but not for 3 hours at −14°C. Considering from this result, it might be requested that all meat and organs of slaughtered animals must be kept at approximately −15°C for at least several hours in order to destroy Toxoplasma in them. But any measure which will be adopted for this purpose in the future, must be enforced within the limit in which the qualification of meat as a food should not be deteriorated.

Conclusion

With use of the refrigerating and the cold-storage rooms of a slaughterhouse, an examination was made on the resistance of *Toxoplasma gondii* to a low temperature. Materials employed for this experiment were the mouse bodies infected with Toxoplasma, and the livers and brains excised from them. The survival of live Toxoplasma in the test materials which had been stored in the low temperature rooms were examined by the intraperitoneal inoculation method into healthy mice. A control experiment was also carried out at −14°C in a freezer with the similar way. Temperatures in both rooms were continuously measured by auto-recording thermometers throughout the period of experiment.

Results obtained were itemized as follows:

1) Cysts of the Beverley strain could survive for as long as 67 days and proliferative forms of RH were capable of survival for 11 days in the refrigerating room. Temperature in the refrigerating room was 0.47°C in average and 3.2 to −3.5°C in range during the period of experiment.

2) In the experiment using the infected mouse bodies, the survival of proliferative forms of RH were 8 days in the refrigerating room and 4 days in the cold-storage room. This result, however, might fail to demonstrate the true sur-

vival of this protozoa in mice, because a putrefactive change which had developed slowly in the abdominal organs of mice in storage in the room, would influence to the survival of Toxoplasma.

The average temperatures were 0.9°C in the refrigerating room and 4.2°C in the cold-storage room during the period of this experiment.

3) Both forms, cyst and proliferative form, of Toxoplasma remained alive more than an hour but did not for 3 hours in a freezer of −14°C.

From the above-mentioned results, some considerations were contributed on the control measure of Toxoplasma infection at a slaughterhouse.

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屠場の低温室内における *Toxoplasma gondii* の生存期間に関する研究

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総括

Toxoplasma gondii の低温に対する抵抗性についての報告は少なくない．しかし，これらの研究では，実験室内の冷蔵庫またはフリーザーなどの精確に調節された温度条件下に実施されたものである．屠畜肉やその内臓中に潜在する本原虫が，もっとも重要な感染源と見なされている現在では，屠場の低温室中で畜肉中の本原虫が，どれ程の期間生存しつづけるかが疫学上重要な意味を持つことはいうまでもない．事実，屠場の冷却室あるいは冷蔵室の温度は，頻回の扉の開閉や大量の温かい大動物肉塊の搬入などのため，相当な変動を受けることが予測され，実験室の冷蔵庫内温度条件とはかなり異なるものであると考えられた．

本研究は，以上の趣旨に沿って長崎市営屠場内の低温室を利用し，*T. gondii* の増殖型および嚢子の生存期間を検討した．被検材料は，大動物肉塊や内臓を用いることができなかったので，RH および Beverley 株感染マウスおよびその臓器を使用した．

第1実験では，RH 株感染マウス自体を冷却室および冷蔵室に保存した．以後，毎日または隔日に保存マウス体を取り出し，その肝および脾乳剤を健常マウスに腹腔内接種し，そのマウスからの原虫検出を試みた．接種後30日以内に死亡したマウスは即時に，それ以上生存したものは屠殺して，その腹腔液および脳中の原虫の有無を顕微鏡下に検討した．その成績では，マウス体内の RH 株増殖型は冷却室中で8日間，冷蔵室中で4日間生存することを認めた．しかし，保存マウスの腹腔内臓器の腐敗が冷却室では13日め以後から，また冷蔵室では6日めから認められたことから，その成績にはマウスの腐敗が影響をおよぼしていることが想像された．実験中の室温は，冷却室では平均0.9°C，最高2.5°C，最低−1.1°C，冷蔵室では平均4.2°C．最高低巾は5.1～3.2°Cであった．

第2実験では，RH 株感染マウス肝と Beverley 株感染マウス脳を冷却室中に保存し，一定期間毎に取り出し乳剤として健常マウスに接種した．本実験により，マウス肝中の RH 株増殖型は11日間，マウス脳中の Beverley 株嚢子は実に67日間冷却室で生存することが判明した．実験期間中の冷却室温度は平均0.47°C，最高低巾は3.2～−3.5°Cにおよんだ．対照実験として，−14°Cのフリーザー中で第2実験と同一材料を用いて検査したが，その結果，嚢子，増殖型とも，1時間保存材料中に生存することを認めたが，3時間材料からは証明できなかった．

以上の実験で，屠場冷却室中で嚢子は67日間，増殖型は11日間生存し，かつ，感染力を保有していることが判明したが，大動物肉塊や臓器中では，より長期間生存することが想定された．