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Induction of Adult Formation from Cysticercoid of *Hymenolepis nana* Established in Fischer (F344) Rat with Cortisone Acetate

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Introduction

This paper is the first of a series analysing the rat and *Hymenolepis nana* system from immunobiological point of view, and reports preliminary observation that adult formation never occurred in any Fischer (F344) rats given eggs of *H. nana*, except when they were treated with a total of 75 mg cortisone acetate exclusively from day 4 of egg inoculation, the day of cysticercoid maturation in the intestinal tissue.

Materials and Methods

Host Animals

Fischer (F344/DuCrj) inbred strain of rats were purchased from Charles River Japan Inc., Tokyo, as specific pathogen-free rats and bred under conventional but worm-free condition in the present laboratory. Five- to seven-week-old rats of both sexes were used throughout the experiments. Worm-free mice of both BALB/c inbred strain and dd random-bred strain (closed colony, Ito, 1982) were also used for a part of the experiments.

As donor mice of *Hymenolepis nana*, both dd mice described above and ddY mice (a colony different from dd mice, purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Japan) were used.

Parasites

Eggs of *Hymenolepis nana* were collected from the donor mice described above (Ito, 1982). *H. nana*, initially provided by Prof. K. Okamoto, Showa University, Tokyo, has been cycled exclusively through mice more than 10 years by the author himself. Shell-free eggs (Berntzen and Voge, 1965) of *H. nana* in about 0.5 ml 0.85%NaCl solution (5×10^4 eggs) or 0.2 ml (2×10^3 or 5×10^3 eggs) were administered into the rat stomach via stomach tube attached to tuberculin syringe under light ether anesthesia. Preparation or inoculation of rat-derived cysticercoids recovered from rats 4 days (96 hr) after inoculation with 1×10^5 shell-free eggs was carried out by the simple method (Ito, 1977).

Experimental Schedule

Experiment 1: Both Fischer rats and BALB/c mice were used. Previously uninfected five- to seven-week-old rats were

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divided into two groups at random: One was experimental group (R-Exp in Table 1) injected subcutaneously with cortisone acetate (Cortone, Merk-Banyu Co., Japan, 25 mg/ml) before or after each egg inoculation. The other was control group not treated but given an identical egg inoculation (R-Cont in Table 1). All animals were inoculated with 2×10^3 (Experiment I), 5×10^3 (Experiments II and III) and 5×10^4 shell-free eggs (Experiments IV and V), respectively, and killed either 4 days or 14 days after egg inoculation (AEI). Day 0 means the day of egg inoculation. In Experiments I, II and IV, both the experimental and control groups given an identical egg inoculation were each divided into two subgroups at random; one subgroup was killed 4 days AEI and the other subgroup was killed 14 days AEI. As experimental controls (M-Exp and M-Cont in Table 1), BALB/c mice were simultaneously given the same infection.

Experiment 2: Three litters of Fischer rats (24 rats) and one litter of dd mice (eight mice) were used. Twenty rats were divided into five groups of four rats each. All the rats were given an identical infection with 5×10^4 shell-free eggs and killed on days 4, 5, 6, 9 and 14 of infection, respectively. Rat-derived cysticercoids were collected on day 4 (96 hr) but not on day 5 from the other four rats given 1×10^5 shell-free eggs each. Recipient mice were each given about 100 rat-derived cysticercoids/0.2 ml of 0.85%NaCl solution and killed 10 days later.

Experiment 3: Fischer rats only were used, and given a total of 75 mg cortisone (25 mg/ml, 3 injections on alternate days). Every litter was divided into two groups (A and B) at random. Treatment with cortisone was started at different timing to the same infection between the two groups. The details of the cortisone treatment are described in Table 2. All rats were inoculated with 5×10^4 shell-free eggs and killed on day 14 of egg inoculation, except those of Exps. 7 and 8, which were killed on day 18.

Assay of infection

When the animals were killed 4 days AEI, following the method of Hunninen (1935a), the number of cysticercoids in the intestinal wall was determined under $\times40$ or $\times100$ magnifications. In addition, cysticercoid lesion at the liver surface, or adult tapeworms in the intestinal lumen were also looked for with the naked eye and microscope at autopsy. All worms, 2 or more mm long, were removed after examination with the naked eye and maturity of them was also determined under $\times100$ magnification (Ito and Yamamoto, 1977).

Results

Experiment 1. Effect of Cortisone Acetate on Cysticercoid or Adult Recovery from Fischer (F344) Rat

In preliminary work, it had been found that Fischer rats of 2 to 7 weeks old given a number of shell-free eggs ($\geqq 2\times10^4$ eggs) of *Hymenolepis nana* harbored a very small number of cysticercoids in the intestinal villi 4 days AEI, but did not harbor any adult worms 14 days later at all.

This experiment was designed to determine whether or not cortisone acetate increases the cysticercoid recovery rates or induces adult recovery from Fischer rat host. The details of the experimental design and the results are summarized in Table 1. In Experiments I, II and III, BALB/c mice either treated with cortisone or not were also given the same egg inoculation and used as experimental controls. Cortisone did not influence the cysticercoid recovery rates either from the rats or from the mice. All rats whether treated with cortisone or not harbored a very small numbers of cysticercoids (about 1% of eggs administered) exclusively in the small intes-

Table 1 Effect of cortisone acetate on the cysticercoid or adult worm recovery from Fischer rat given eggs of *Hymenolepis nana*

Experiment No. groups R: rat M: mouse		Cortisone treatment	Egg doses (day 0)	Cysticercoid recovery (day 4)		Adult worm recovery (day 14)	
				No. of animals infected	No. of cysticercoids Mean ± S.D.	No. of animals infected	No. of adult worms Mean ± S.D.
I	R-Exp	5 mg×3 (days−2, −1 and 0)	2000	5/5 (F)*	19.4± 7.0	2/5 (F)	14 ± 12.7
	R-Cont	no treatment (NT)	2000	5/5 (F)	12.8± 12.7	0/6 (F)	0
	M-Cont	NT	2000	5/5	345.6± 19.1	5/5	312.4± 42.5
II	R-Exp	5 mg×3 (days−2, −1 and 0)	5000	4/4 (M)†	52.8± 19.1	0/4 (M)	0
	R-Cont	NT	5000	4/4 (M)	59.5± 26.7	0/4 (M)	0
	M-Exp	5 mg×3 (days−2, −1 and 0)	5000	6/6	1600.3± 93.4	—	—
	M-Cont	NT	5000	6/6	1606.2±128.4	—	—
III	R-Exp	25 mg×3 (days−2, −1 and 0)	5000	4/4	56 ± 21.8	—	—
	R-Cont	NT	5000	4/4	46.8± 7.9	—	—
	M-Cont	NT	5000	4/4	1083.5± 88.2	—	—
IV	R-Exp	25 mg×3 (days−2, −1 and 0)	50000	4/4	601 ± 93.7	3‡/5	74.7± 23.6
	R-Cont	NT	50000	4/4	770.8±304.8	0/6	0
V	R-Exp	25 mg×3 (days 4, 6 and 8)	50000	—	—	5/5	597 ±296.8
	R-Cont	NT	50000	—	—	0/5	0

Animals of both sexes were used except (F)* and (M)†.
(F)*: only female rats, (M)†: only male rats, ‡: all of three females found infected.

tinal wall. There was no cysticercoid lesion at the liver surface even in any rats given cortisone. The percentage of the cysticercoids recovered from the rats was only 3 to 6% of cysticercoids recovered from the mice, even when egg doses were increased up to 5×10^4 and cortisone doses up to a total of 75 mg (Experiments III and IV). As previously found from preliminary work, no adult worms were recovered by day 14 from any control rats. In contrast, adult recovery occurred in some of female rats treated with cortisone (Experiments I and IV), but the number of adults recovered was much fewer than that of the cysticercoids established 10 days before (Experiment IV): In Experiment I, two of five females harbored a few adults; in Experiment IV, all three females harbored a few adults (only about 10% of cysticercoids established), whereas none of male rats harbored any adults. Adult recovery from both sexes of rats was induced successfully when the rats were treated with cortisone from day 4 of egg inoculation, just after maturation of cysticercoids in the intestinal tissue (Experiment V).

Experiment 2. Viability of Cysticercoids Recovered from the Rats

This experiment was done to determine whether the cysticercoids recovered from the rats were normal or abnormal in their viability. At first, the mode of a primary egg infection of the same batch was followed up from day 4 to day 14. The rats

Table 2 The fate of cysticercoids established in Fischer rat given eggs of *Hymenolepis nana*

Experiment No. (group)	Days of cortisone treatment (25 mg/day)	No. of rats infected	No. of worms Mean ± S. D.	Maturity No.	Maturity %
Exp. 1 (1A)	− 2, − 1, 0	1/4 (1F*/1F+3M†)	114	2/20	10
Exp. 2 (1B)	4, 6, 8	4/4 (2F+2M/2F+2M)	663.3±111.9	28/80	35
Exp. 3 (2A)	5, 7, 9	3/3 (1F+2M/1F+2M)	588 ±179.0	24/60	40
Exp. 4 (3A)	6, 8, 10	3/3 (1F+2M/1F+2M)	520.3±163.4	1/60	2
Exp. 5 (2B)	7, 9, 11	4(1‡)/4 (3F+1M(1M)/3F+1M)	55.5± 38.1	0/80	0
Exp. 6 (3B)	8, 10, 12	4(3‡)/4(3F+1M(2F+1M)/3F+1M)	31.8± 23.7	0/67	0
Exp. 7 (4A)	9, 11, 13	3/4 (2F+1M/2F+2M)	5 ± 3.5	3/15	20
Exp. 8 (4B)	10, 12, 14	1/4 (1F/3F+1M)	3	0/3	0

All rats were inoculated with 5×10^4 shell-free eggs/0.5 ml 0.85% NaCl solution on day 0, injected with a total of 75 mg cortisone acetate (25 mg/ml, thrice) and killed on day 14, except those of Exps. 7 and 8 killed on day 18. The same group number means the same littermates. When worm density was more than 20/rat, 20 worms were examined to determine their maturity and when it was less than 20, all worms recovered were examined.

F*: female, M†: male.

‡: No. of rats found infected exclusively with worms with no strobila, about 2 mm long.

of day 4 group harbored 498±231.2 (Mean ±S.D.) cysticercoids and those of day 5 group did 56±69.6 cysticercoids only, whereas neither cysticercoids nor worms (2 or more mm long, visible with the naked eye *in situ*) were found from any rats of day 6, day 9 or day 14 groups.

Rat-derived cysticercoids collected 96 hr AEI were administered into previously uninfected dd mice. Almost all rat-derived cysticercoids became excysted through the procedure of cysticercoid preparation. Rat-derived cysticercoids matured in all eight mice: The number of adults recovered, the fresh biomass and the egg output were 32.8±27.4 worms, 133.1±36.8 mg and $5.3\times10^5\pm1.7\times10^5$ eggs (Mean ± S.D.), respectively.

Experiment 3. The Fate of the Cysticercoids Established in Fischer Rat

This experiment was intended to visualize the fate of the cysticercoids established in the rat villi with varying the timing of cortisone treatment, since there was no evidence as to whether worms were lost from the intestine between days 4 and 6 (Experiment 2) or whether they were not detected by their very small size or lost through the washing process of the intestine. The details of the experimental design and the results are summarized in Table 2. When the rats of both sexes given eggs were treated with cortisone (25 mg/day ×3) from day 4 or from day 5, almost all the cysticercoids which had been established by day 4 developed into mature worms by day 14 (Exps. 2 and 3) at the posterior fifth of the small intestine. When the rats were treated with cortisone from day 6 (Exp. 4), all worms except one (1/60) found from the lumen remained immature.

On the contrary, when the rats given eggs were treated with cortisone from day 7 (Exp. 5) or day 8 (Exp. 6), only less than 10% of the established cysticercoids developed into adults. In addition, worms or three of four rats (1‡; Exp. 6) were recovered from one of four rats (3‡; Exp. 5) found remained those with no strobila, about 2 mm long, by day 14. These non-strobilated worms found by day 14, however, enabled to strobilate and mature when their development time after the onset of cortisone treatment in the rats were increased up to 9 days (Exp. 7). When the rats were treated from day 9 or day 10, no or few adults were recovered (Exps. 7 and

8). There was a tendency that female rats were more sensitive to cortisone than male rats.

Discussion

The present results clearly demonstrate that *Hymenolepis nana,* cycled exclusively through mice, did not infect Fischer (F344) rats to mature: In Fischer rat, eggs of *H. nana* developed into cysticercoids normally (Experiment 2), although the cysticercoids recovered were almost always only less than 1% of eggs administered and markedly fewer than those from mice (only 3 to 6% of cysticercoids established in mice), but thereafter they did not develop into adult worms at all. Cortisone treatment experiments revealed that excysted worms could survive in the rat lumen with no differentiation for only a few days, but the majority, invisible with the naked eye *in situ* (Experiment 2), was rapidly lost by day 7, and almost none remained by day 9 (Experiments 2 and 3).

Wardle and McLeod (1952) have described that there may be several strains of *H. nana* based upon Shorb's (1933) observation that mouse and rat strains of *H. nana* were physiologically as different from one another as they were from the human strain. Shorb (1933) found that eggs of mouse strain of *H. nana* inoculated into blackhooded rats, 6 to 7 weeks old, developed into cysticercoids in similar rate in mice, but *most of them* failed to mature. Similar results were also recorded by Hunninen (1935b) and Schiller (1959). Heyneman (1962) recorded somewhat lower rate in cysticercoid recovery from Sprague-Dawley rats, but found no adults from them.

The present results differ from these earlier reports, since cysticercoid recovery from Fischer rat was extremely less than that from mice and no adult formation occurred. However, it appears impossible, at present, to discuss such variation of worm maturity in various strains of rats used, since we have had little information except Shorb (1933) on variation of host susceptibility or variation of this parasite.

The mechanism by which cortisone, which does not influence the tissue phase, affects the early lumenal phase of *H. nana* in Fischer rat remains to be solved. Further works on this problem from the immunological point of view are now in progress.

Summary

Whether eggs of *Hymenolepis nana* collected from mice do or do not infect the Fischer (F344) rat was examined. In Fischer rats, 5 to 7 weeks old, only 0.6 to 1.5% of shell-free eggs administered developed normally into cysticercoids in the small intestine wall by day 4 of egg inoculation, but thereafter never developed into adult worms in the lumen, whereas in BALB/c mice 17 to 32% of the eggs developed into cysticercoids and almost all the established cysticercoids grew to mature worms by day 14. The low susceptibility to *H. nana* egg infection in Fischer rats, assessed by cysticercoid recovery, differed from the earlier observations by Shorb (1933) and Hunninen (1935b), and was not influenced by cortisone acetate injection (25 mg/day $\times 3$; days -2, -1 and 0) at all. However, when rats harboring fully developed cysticercoids or excysted worms with no strobila were treated with the same doses of cortisone (during day 4 to day 6), these worms began to differentiate into adults, but otherwise the worms with no strobila were rapidly lost. Therefore it may be probable that Fischer rat is not the natural host of *H. nana*, cycled through mice and used for the present study, and that the failure of adult development is due to a failure of cysticercoids to strobilate in the lumen, at least of this strain of rat. The mechanism by which cortisone, which does not influence the tis-

sue phase, affects the early lumenal phase of *H. nana* to differentiate into mature worms in Fischer rats remains to be solved.

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Fischer ラットにおける小形条虫の初感染像の解析

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マウスで継代している小形条虫はラットでは成熟しにくいことが Shorb (1933) によって観察されている．筆者は Shorb (1933) が用いた blackhooded ラット，6-7 週齢個体の代りに同週齢 Fischer ラットを用いて，この現象の再確認を試みたところ，上記の報告とかなり異なった成績を得た．即ち Fischer ラットでは本虫の成虫寄生は全く認められなかった．この実態に関してコーチゾン処置ラットを用いて若干の解析を加えた結果，本虫の虫卵は Fischer ラット小腸壁内で正常に擬嚢尾虫に発育（ただし擬嚢尾虫発育率はマウスにおけるそれの高々 3～6% にすぎなかった），脱嚢した後，ストロビラ形成をみることなく腸腔での成虫への発育分化が阻止され数日以内に消失することが観察された．ストロビラ未形成の虫体が生存可能な数日以内の腸腔期に総量 75 mg コーチゾンをラット皮下に注射することによって成虫化が誘導できたが，この機構の免疫学的解析は今後の課題である．