Studies on the Passive Transfer via Serum of Immunity to *Hymenolepis nana* in the Mouse *Mus musculus*[1]

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ABSTRACT

Five experiments were conducted to investigate the passive transfer via serum of immunity to *Hymenolepis nana* in the white mouse. Serum collected from mice that received 5,000 *H. nana* eggs by mouth 14 days before bleeding (Type I serum) was used in 3 experiments. Serum collected on Day 28 from mice that received 1,000 eggs on Day 0 and 10,000 eggs on Day 14 (Type I serum) was used in the fourth experiment. Serum collected on Day 42 from mice that received 1,000 eggs on Day 0, 5,000 eggs on Day 14, and 10,000 eggs on Day 28 (Type III serum) was used in the fifth experiment.

The sera were injected intraperitoneally into 6- to 8-week-old mice, and the recipients were challenged orally with 10,000 eggs. Control mice were challenged after the injection of normal serum. Fewer cysticercoids developed in the mice treated with 1 ml of Type I serum immediately prior to the administration of eggs than in controls injected with normal serum before egg administration. The duration of the protection afforded by Type I serum lasted less than 6 hours and was not prolonged by increasing the amount of serum injected to 2 ml. The injection of Type II and Type III serum extended the protective period to 35 days. The extended period of resistance from Type II and Type III sera suggests an anamnestic response following a second exposure to eggs. The transient nature of the protection indicated that the passive resistance probably was antibody mediated.

INTRODUCTION

Hymenolepis nana, the dwarf tapeworm of man and rodents, is an exception among cestodes in that it does not require an intermediate host although it may utilize one in an alternate indirect life cycle (see Heyneman 1962a for a review). Eggs ingested directly by the vertebrate host hatch in the duodenum and release onchospheres that invade the mucosal lining and develop into cysticercoids in the intestinal villi. The cysticercoids become fully developed in 96 hours, begin leaving the villi at approximately 102 hours, migrate into the ileum, evaginate, attach, and develop into mature worms.

When an infection is induced by eggs, a tissue invasive stage is involved and host resistance to reinfection is acquired. An infection of just 200 to 500 eggs can elicit a lasting immunity, which is first discernible 9 hours after initial infection, marked at 12 hours, and practically absolute after 24 hours (Hearin 1941).

White mice were resistant to a challenge of *H. nana* eggs after intraperitoneal injections of serum from experimentally infected donors (Hearin 1941). It is not clear if this actually demonstrated passive transfer of immunity because the persistence of the protection was not investigated. Weinmann (1966) reported that serum from infected mice had varying degrees of protection against oral challenges of *H. nana* eggs. A humoral basis for the immunity was demonstrated by DiConza (1969) when he found that serum from mice infected with *H. nana* contained IgG (7S) immunoglobulin fraction which had a strong antiparasitic activity against subcutaneously injected, growing *H. nana* larvae. The sera of mice that received a single oral injection of eggs acquired significant immune activity within 7 days; the maximum level was reached at 14 days and maintained until Day 28. The present

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study was conducted to evaluate further the role of serum in the immunity to *H. nana* in mice and to measure the duration of the passively transferred protection.

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MATERIALS AND METHODS

A breeding colony of Swiss albino mice was purchased from Maxfield Supply, Cincinnati, Ohio, established, and maintained under *Hymenolepis* free conditions. Only those animals negative for *H. nana*, as determined by fecal examinations over a 6-week period, were used to establish the initial colonies. The experimental mice were isolated from the colonies when approximately one month old.

Initial infections of mature tapeworms were established from an exogenous source of *H. nana* eggs secured from Carolina Biological Supply Co., Burlington, N. C.; thereafter, hosts with patent infections of worms were killed to obtain eggs. Desired numbers of eggs for administration to experimental animals were prepared by Heynemen's (1962a) egg dilution count. Eggs were administered to the animals via stomach tube while mice were lightly anesthetized with ether. Cysticercoids that developed in the small intestine of infected mice were counted by the method of Hunninen (1935). The following nonparametric statistical tests were used to determine the significance of the observed differences in the numbers of cysticercoids recovered from the serum treated and the nontreated groups: (1) Wilcoxon Rank Sum Test (Wilcoxon et al. 1963) when comparing 2 groups, (2) Kruskal-Walis One-Way Analysis of Variance for Ranks (Spence et al. 1968) when comparing more than 2 groups, and (3) Dunn Multiple Comparison Test for Rank Sums (Dunn 1964).

Serum was obtained from blood drawn from mice by cardiac extravasation. Blood was allowed to clot at room temperature for 1 to 3 hours and the serum separated by centrifugation at 2,500 rpm for 10 min. Serum from within a group of mice was pooled and frozen at –20 C without preservatives. Before inoculation the serum was thawed and mixed thoroughly.

Serum was collected from mice 14 days after an initial infection of eggs or 14 days after a challenge dose of eggs was administered. Three schedules for drawing blood were coordinated with administration of *H. nana* eggs: (1) Type I serum was derived from blood collected on Day 14 from mice that received 5,000 eggs on Day 0, (2) Type II serum was prepared from blood collected on Day 28 from mice that received 1,000 eggs on Day 0 and 10,000 eggs on Day 14, (3) Type III serum was prepared from blood collected on Day 42 from mice that received 1,000 eggs on Day 0, 5,000 eggs on Day 14, and 10,000 eggs on Day 28. Uninfected mice were bled to provide normal sera.

The sera were injected intraperitoneally into 6- to 8-week-old mice. Those mice received an oral challenge of 10,000 eggs at various specified times following serum injection. Control mice were challenged after injection of normal serum. Ninety-six hours after the challenge doses of eggs were administered, cysticercoids in each group were counted as an index of the manifestation of the immune response.

Experimental Design for Type I Serum

In Experiment A, 3 groups of 5 mice each were used. Each mouse received 1 ml of serum. Type I serum was inoculated into the mice of Groups 1 and 2, and normal serum was injected into Group 3 mice. Eggs were administered to the mice of Groups 1 and 3 immediately after injection of serum and to the mice of Group 2, 6 hours after inoculation of serum.

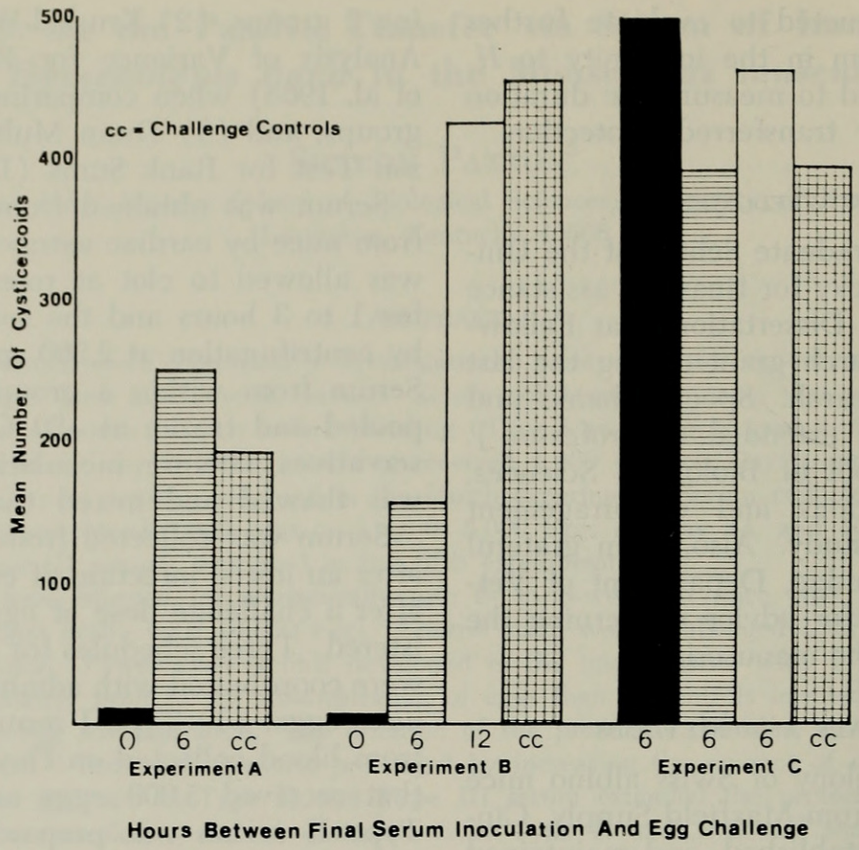

FIG. 1. Effect of intraperitoneal injection of Type I serum on the number of *H. nana* cysticercoids developing in the intestinal villi of white mice following a 10,000-egg challenge.

In Experiment B, 4 groups of 5 mice each were used. Each mouse was inoculated with 2 ml of serum. Type I serum was administered to the mice of Groups 1, 2, and 3, and normal serum was administered to the mice of Group 4. Eggs were administered to the mice of Groups 1 and 4 immediately after injection of serum; to the mice of Group 2, 6 hours after injection of serum; and to the mice of Group 3, 12 hours after injection of serum.

In Experiment C, 4 groups of 5 mice each were used. Each mouse was injected with 0.5 ml of serum on successive days. Group 1 mice received 2 injections of Type I serum; Group 2 mice received 3 injections of Type I serum; Group 3 mice received 4 injections of Type I serum; and Group 4 mice received 4 injections of normal serum. Eggs were administered 6 hours after the last dose of serum to the mice of all groups.

Experimental Design for Type II Serum

In Experiment D, 12 groups of 10 mice each were used. One-half of the mice were injected with 1-ml amounts of Type II serum and the other half were inoculated with 1-ml amounts of normal serum. Eggs were administered immediately, 12 hours, 1, 3, 7, 10, 12, 14, 21, 28, 35, or 42 days after sera were injected.

Experimental Design for Type III Serum

In Experiment E, 3 groups of 10 mice each were used. One-half of the mice were injected with 1-ml amounts of Type III serum and the other half were inoculated with 1-ml amounts of normal serum. Eggs were administered 28, 35, or 42 days after the sera were injected.

RESULTS

In Experiment A, there was a significant difference in the number of cysticercoids that developed among the 3 groups ($P = 0.01$). Fewer cysticercoids developed in those treated with the Type I serum immediately prior to egg inoculation (Fig. 1). The protective effect lasted less than 6 hours ($P = 0.1$).

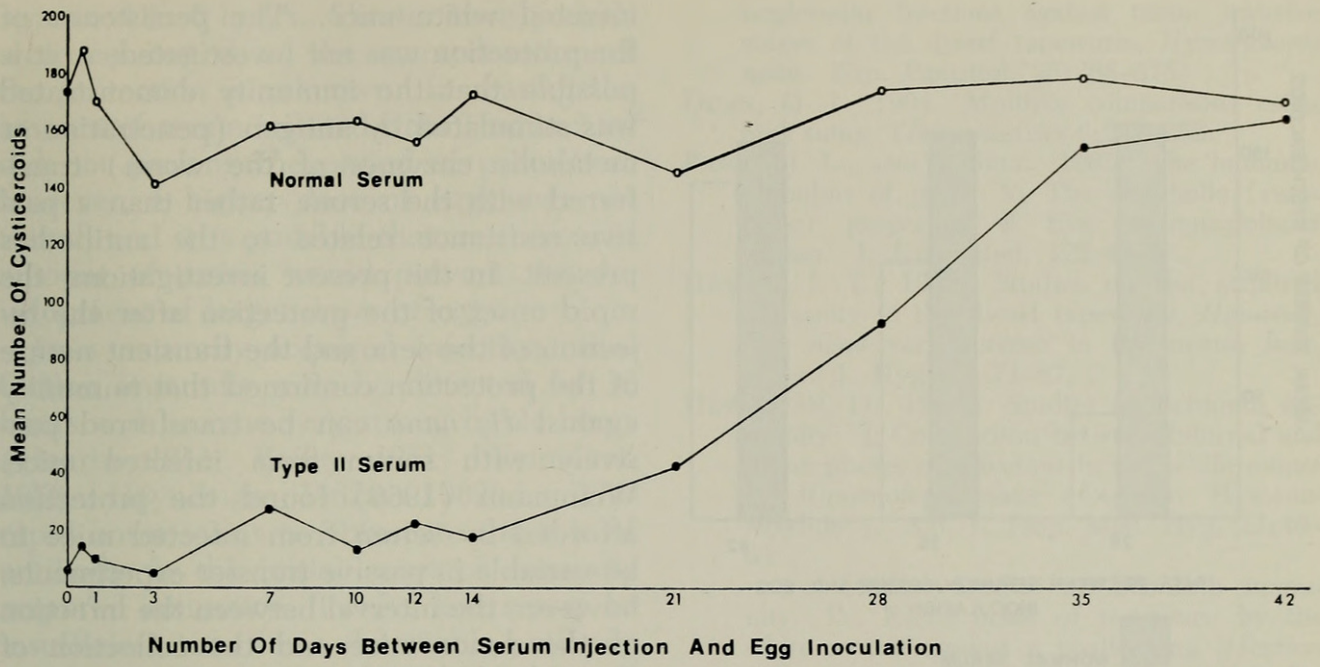

FIG. 2. Effect of intraperitoneal injection of 1-ml doses of Type II serum on the number of *H. nana* cysticercoids developing in white mice after a 10,000-egg challenge at intervals during a 42-day period after serum injection (Experiment D).

Similarly, in Experiment B there was a significant difference in the numbers of cysticercoids that developed among the 4 groups of mice ($P = 0.01$). Fewer cysticercoids developed in the mice of Group 1 which were treated with Type I serum and inoculated with eggs immediately thereafter ($P = 0.1$). As shown in Fig. 1, the number of cysticercoids was reduced in the group of mice given eggs 6 hours after the injection of serum; however, this was not statistically significant ($P = 0.1$).

The number of cysticercoids that developed in the mice of the 4 groups of Experiment C were not significantly different ($P = 0.1$); therefore, the multiple injections were not effective against the *H. nana* challenge (Fig. 1).

The mean numbers of cysticercoids that developed in each group of mice of Experiment D are shown in Fig. 2. The numbers of cysticercoids in the animals injected with Type II serum and inoculated with eggs immediately to 28 days later were significantly fewer than the numbers in the control animals that received eggs at the same time intervals after the injection of normal serum ($P = 0.01$). Although still detectable, that difference was not as marked at 35 days ($P = 0.1$), and had disappeared by 42 days ($P = 0.1$) (Fig. 2).

The mean numbers of cysticercoids that developed in each group of mice of Experiment E are shown in Fig. 3. A similar number of cysticercoids developed in each group that received the normal serum ($P = 0.1$), but there was a significant difference among groups that received the Type III serum ($P = 0.01$). Fewer cysticercoids developed in the animals treated with Type III serum 28 days ($P = 0.01$) and 35 days ($P = 0.1$) prior to the administration eggs than in the corresponding control groups. There was not, however, a significant difference between the numbers of cysticercoids that developed in the 2 42-day groups ($P = 0.1$).

DISCUSSION

Type I serum collected from donor white mice 14 days after an initial dose of eggs and injected intraperitoneally into homologous recipients was effective for less than 6 hours in decreasing the number of cysticercoids that developed following a chal-

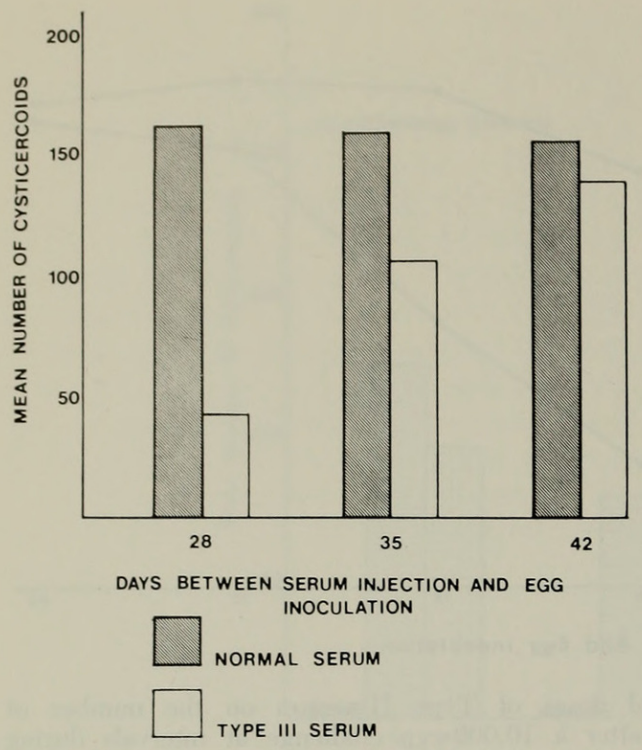

FIG. 3. Effect of intraperitoneal injection of 1-ml doses of Type III serum on the number of *H. nana* cysticercoids developing in white mice after a 10,000-egg challenge at intervals during a 42-day period after serum injection (Experiment E).

lenge dose of eggs. The protective value of Type I serum was transient and so low that daily injection of 0.5 ml was ineffective. One ml of the serum was a sufficient quantity to protect mice against a challenge exposure of 10,000 eggs.

Both Type II and Type III sera, collected after additional challenge doses of eggs, extended the period of protection to 35 days, and the protection was dissipated gradually. The increased efficacy of these sera suggest an anamnestic response following a second exposure to eggs. The prolonged period of protection and the decrease in total numbers of cysticercoids developing in the animals treated with Type II or Type III sera as compared to the recipients of Type I serum indicates that the former response could occur in naturally acquired immunity against *H. nana.*

Hearin (1941) transferred resistance against a challenge dose of *H. nana* eggs to susceptible mice by prior injection of them with multiple doses of serum from infected white mice. The persistence of the protection was not investigated; so it is possible that the immunity demonstrated was stimulated by antigen (penetration or metabolic enzymes of the worm) transferred with the serum, rather than a passive resistance related to the antibodies present. In the present investigations, the rapid onset of the protection after the injection of the sera and the transient nature of the protection confirmed that immunity against *H. nana* can be transferred passively with serum from infected mice. Weinmann (1966) found the protection afforded by serum from infected mice to be variable in passive transfer experiments; however, the interval between the infection of the donor mice and the collection of their blood varied from 2 to 6 weeks rather than the 14-day interval indicated by DiConza (1969).

Serum injected into the peritoneal cavities of mice should reach the circulation by way of lymphatics in a very short period of time (Weiss 1972). In the present study, it is presumed that the time necessary for the protective factors of the immune serum to reach the intestinal area was compatible with the time it took the eggs to hatch and the onchospheres to reach the intestinal villi. In active immunity acquired by infection, the majority of onchospheres in a second dose of eggs are unable to penetrate the intestinal villi (Bailey 1951). According to Weinmann (1966), immune serum also inhibits the onchosphere at the mucosal surface, presumably by extravasation of passively transferred antibody into the intestinal lumen or by adsorption of the antibody onto the tissues of the host. Therefore, the protective property of the serum would have to be present within at least the first 12 hours after the administration of eggs.

The half-life of IgG, IgA, and IgM is on the order of 4, 1.2, and 0.5 days, respectively (Fahey and Sell 1965). If, indeed, the protection observed in the present investigations was due to antibody, the titer of Type I serum was low, as indicated by the fact that the protection had dis-

appeared in 6 hours. Shorter time periods were not tested, so the exact duration of the protection is unknown. Increasing the amount of serum injected from 1 ml to 2 ml did not prolong the period of protection; however, a higher dose of eggs was not tested, and it is possible that an increased amount of serum would prevent the development of larger doses of eggs.

In a naturally acquired infection, reinfection immunity may be detected by 12 hours and become very strong by 24 hours (Hearin 1941; Bailey 1951; Weinmann 1958; Heyneman 1962a, 1962b). This rapid onset seems to obfuscate the role of antibody in the initial onset of naturally acquired immunity. Twelve hours may be insufficient time for a detectable antibody response. Heyneman (1962b) proposed that detection of rapid antibody production in serum is delayed by the large dilution factor, but in cases where the intestinal mucosa is directly challenged in the absence of a blood borne transport mechanism, a 12- to 24-hour period may be possible.

Okamoto (1970) and Okamoto and Koizumi (1972) demonstrated that the thymus was involved in the development of acquired immunity to *H. nana*. Levine and Claman (1970) indicated that more than one cell type was necessary for at least some of the antibody responses in the mouse. The present study shows that protective humoral factors (possibly protective antibody) are present in mice following *H. nana* infection. Although antibodies may not be the major agent working against *H. nana* challenge in naturally occurring infections, a T-cell-dependent antibody response is possible and should be investigated further.

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