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Histamine Sensitization of Mice by *Trichinella spiralis* Infection (38379)

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The increased sensitivity of mice to the lethal action of histamine induced by *Bordetella pertussis* cells or purified extracts obtained from them is well documented (1). Substances from a few other bacterial species have also been reported to increase the susceptibility of mice to histamine. These substances, however, have not induced this response with any degree of regularity and the physiological mechanism by which they induce histamine sensitivity may differ from that of the histamine sensitizing factor (HSF) of *B. pertussis*. HSF produces many interesting effects in mice (1). One of these is to stimulate reaginic antibody production to antigens given simultaneously with HSF (2). Stimulation of γE immunoglobulin is induced by certain nematode infections (3–9), such as trichinosis (4). It occurred to us that *B. pertussis* and *Trichinella spiralis* may have similar activities in mice. For this reason, the capability of this nematode to induce hypersensitivity to histamine was studied.

Materials and Methods. Mice. CFW male mice raised in our laboratory were used.

T. spiralis. Larvae of *T. spiralis* were obtained from infected muscle of mice and rats as follows: about 50 g of carcass (without skin, head or viceral organs) was blended in 500 ml of pepsin solution (18.2 g pepsin, 24.3 ml HCl, 1800 ml H_2O) for 1 min. The suspension was then gently agitated in an additional liter of pepsin for 2 hr in a shaking water bath at 37°. The suspension was next filtered through two layers of gauze and the larvae allowed to settle for 15 min. Most of the supernatant fluid was removed, and the sediment poured into a funnel fitted with rubber tubing and a clamp. After about 15–20 min, the sedimented larvae were removed in the bottom 10–20 ml of fluid and the supernatant fluid was discarded. The funnel was refilled with physiological saline, the larvae mixed in the saline, allowed to settle, and collected as before. This process was repeated (three or four times) until a clean suspension of larvae was obtained.

The concentration of larvae was determined by direct count under a dissecting microscope at 20× magnification, and the suspension was standardized to contain 100–150 larvae per 0.1 ml.

Infection of mice. Male mice, 6–9 weeks old, were infected with larvae by gastric intubation by means of a 1 ml tuberculin syringe fitted with a 20-gauge needle with a small piece of tightly-fitted plastic tubing at its end. Each mouse was securely held with one hand and the needle introduced via the eosophagus to the stomach and 0.1 ml of the larval suspension was deposited. The larval suspension was constantly stirred in a small beaker to maintain a uniform number of organisms per mouse dose. Only one dose at a time was taken in the syringe to prevent the rapid settling of larvae. Periodic counts were made during this process to check the number of larvae per mouse dose.

Histamine challenge. In the test for histamine hypersensitivity, each mouse was given ip 0.5 mg of histamine base (given as histamine diphosphate). The LD_{50} of histamine for normal CFW mice is about 16 mg and a dose of 0.5 mg normally kills only an occasional mouse. LD_{50}'s were calculated by the method of Reed and Muench (10).

Experimental results. CFW male mice, 7–9 weeks old, were infected with 100–150 larvae (four separate experiments in which mice were infected with 100, 120, 124 or 150 larvae). Uninfected mice served as controls. Groups of mice were challenged with histamine on days 1, 7, 9,

10, 11, 13, 15, 17, and 20 after infection. The combined results of these experiments are tabulated in Table I. A significant increase in sensitivity to histamine occurred 14 days after infection. Once sensitivity developed, it remained at a high level. Infected mice tested 133 days after infection were still sensitive to 0.5 mg histamine (six of ten mice died at this time from histamine challenge).

The degree of sensitivity induced by *T. spiralis* in CFW mice is similar to that observed when HSF is given to these animals (11). The LD_{50} of histamine in *T. spiralis* infected mice was 0.1785 mg (Table II) when tested in 6 week old mice infected with 125 larvae 20 days before challenge.

T. spiralis larvae fed to mice mature and mate in the intestine, and by day 7–11 the females deposit larvae (12). We found that in CFW mice the females began to deposit larvae by the 14th day, and larvae were seen in the diaphragm muscle by the 16th day. From this, it seems that the development of histamine sensitivity is first detected when larvae penetrate the intestinal wall of the host.

Infection with 100 larvae has consistently induced hypersensitivity to histamine after 20 days. Infection with 24 larvae did not sensitize mice to histamine 20 days after infection. Further work is necessary to establish the exact number of larvae needed for sensitization.

TABLE I. Development of Hypersensitivity to Histamine in CFW Mice Infected with 100–150 Larvae of *T. spiralis*[a].

Days after infection[b]	Histamine challenge: Infected D/T[c]	Infected %D	Normal D/T	Normal %D	Significance of difference (*P* values) by X^2 test
1	0/10	0	1/8	13	N.S.
7	3/30	10	1/28	4	N.S.
9	7/20	35	2/15	13	N.S.
10	0/10	0	2/10	20	N.S.
11	5/10	50	1/10	10	N.S.
13	2/10	20	0/10	0	N.S.
14	7/8	88	0/8	0	<.01
15	16/20	80	0/20	0	<.01
17	10/11	91	2/10	20	<.01
20	25/25	100	0/20	0	<.01

[a] Combined results of four separate experiments.

[b] Mice received 100–150 larvae in the stomach in a total volume of 0.1 ml. The ip challenge with 0.5 mg of histamine was done on the day indicated.

[c] *D/T* = deaths/total no. of animals.

TABLE II. Determination of LD_{50} of Histamine in CFW Mice Infected with *T. spiralis*[a].

Mg of histamine base given ip	Deaths/ total no. mice[b]	LD_{50} (mg)
0.5000	10/10	
0.250	7/10	0.1785
050.1250	2/10	
0.0625	1/10	

[a] Mice received 125 larvae orally 20 days before they were challenged with the indicated doses of histamine. Ten normal mice of the same age challenged with 0.5 histamine survived.

[b] The 12 surviving mice were rechallenged with 0.5 mg of histamine on the 37th day after infection and 11 died (92%).

Discussion. These experiments showed clearly that infection with *T. spiralis* markedly increased the susceptibility of mice to lethal effects of histamine. This increase in susceptibility to histamine was as great as that observed in mice receiving HSF from *B. pertussis*, and is first observed when the larvae penetrate the intestinal wall. In the dosages used, maximal sensitivity was reached about 16 days after infection when larvae were first seen in striated muscle (diaphragm). Preliminary experiments employing parenteral injection of larval suspensions or saline extracts of larvae in an effort to find a substance capable of inducing hypersensitivity to histamine have not yet been successful.

It is interesting that both *B. pertussis* and *T. spiralis* increase γE production and induce hypersensitivity to histamine. We have wondered whether these two phenomena are induced by similar mechanisms and by closely related substances. In the case of *B. pertussis* it seems that one substance is responsible for both effects (13, 14). Preliminary data from our laboratory indicate that some of the changes induced by HSF are not induced by *T. spiralis* infection; lymphocytosis, hypoproteinemia or hypoglycemia are not induced by *T. spiralis* infection, whereas these always are induced by HSF (1).

Interestingly, Briggs (15) observed that mice harboring *T. spiralis* infections were more sensitive than uninfected mice to serotonin. HSF also induces hypersensitivity to this amine (1). The

development of histamine hypersensitivity in CFW mice by *T. spiralis* infection may prove valuable as an indicator of experimental infection with this nematode, and thus it may be useful in evaluating effectiveness of larvacidal drugs or of drugs inducing sterility of the adult worms.

Summary. Infection of mice with *T. spiralis* larvae collected from infected muscle induce a state of histamine hypersensitivity in CFW mice. This hypersensitivity can be detected 14 days after oral infection with larvae, and coincides with the time when larvae from the adult female penetrate the intestinal wall and first appear in striated muscle. The LD_{50} of histamine for infected mice was 0.1785 mg, which is of the same order of magnitude as the LD_{50} in mice treated with histamine sensitizing factor of *B. pertussis*. The hypersensitivity induced by *T. spiralis* seems to be long lasting.

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