

que. Fluorescence was observed using the IgG fraction of anti- $\alpha$ -chymotrypsin serum in mononuclear cells in the connective tissue. The cytoplasmic granules of these cells could be stained with Alcian blue. This finding suggests that mast cells contain an  $\alpha$ -chymotrypsin-like immunoreactivity. However, the cells with fluorescence numbered about half those stained with Alcian blue, and the extent of the reaction varied in different sections. It is possible that some mast cells contain too little antigen to be detectable by the present indirect immunofluorescent technique or that some antigen is eluted from the sample during the processes of fixation and section.

Fluorescence due to an  $\alpha$ -chymotrypsin-like immunoreactivity was observed when the sections were fixed with 10% formaldehyde, but not in the case of fixation with 95% ethanol or Bouin's solution. Regardless of the fixation, fluorescence was not demonstrated with various dilutions of antibody to trypsin. The fluorescence observed in this study was attributed to an  $\alpha$ -chymotrypsin-like immunoreactivity and not to a trypsin-like immunoreactivity because 1. the 2 antibodies have similar titers; 2. fluorescence was observed with the antibody of  $\alpha$ -chymotrypsin, not with that of trypsin; 3. in the antigen-antibody reaction in the gel, trypsin did not react with anti- $\alpha$ -chymotrypsin serum, even at higher concentrations. The  $\alpha$ -chymotrypsinogen also gave an antigen-antibody reaction. Its optical concentration in the reaction and its antigenicity were the same as those of the original antigen. Thus, it is uncertain whether the fluorescence detected was due to an  $\alpha$ -chymotrypsin-like or its zymogen form of immunoreactivity. However, in mast cells, an  $\alpha$ -chymotrypsin-like enzyme is present in the active form, not as a zymogen<sup>6,7</sup>, and it is bound ionically to heparin<sup>7</sup>.

The physiological function of the mast cell protease is not clear. The enzyme might be involved in the secretory activity of the mast cells or be secreted by the mast cells and act on some component of the connective tissue<sup>4</sup>. Attempts to show that cells stained with fluorescent antibody also

have an  $\alpha$ -chymotrypsin-like activity were not successful. Difficulty in the demonstration of the activity in bovine mast cells may be due to the existence of an aprotinin which inhibits trypsin, chymotrypsin and kallikrein. Aprotinin is an intracellular inhibitor<sup>18</sup>, and it readily binds to heparin because of its high basicity in vitro<sup>19,20</sup>. Thus in bovine mast cells, the  $\alpha$ -chymotrypsin-like immunoreactive materials might be regulated by aprotinin and/or heparin.

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## Gonadectomy and survival of *Ancylostoma caninum* (Nematoda) filariform larvae in mice

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**Summary.** Gonadectomy reversed the susceptibility response of male and female Swiss mice to *Ancylostoma caninum* infection. Orchiectomy decreased the survival of filariform larvae ( $p > 0.001$  to  $< 0.25$ ), whereas ovariectomy increased it significantly ( $p > 0.001$  to  $< 0.01$ ).

Numerous investigators have reported sex differences in infection with various parasitic worms, both in natural and experimental conditions<sup>2</sup>. This communication describes the influence of gonadectomy, i.e. removal of the source of sex hormones of the host, on the susceptibility of mice to *Ancylostoma caninum* infection.

**Materials and methods.** In 2 experiments 100 pairs of Swiss albino mice, 4 months old, weighing  $28 \pm 2$  g, were used. Half of them were gonadectomized and remaining half were sham-operated a month prior to infection. All were infected with  $1000 \pm 25$  filariform larvae of *Ancylostoma caninum*, cultured in the laboratory using the Petri dish technique of Sen et al.<sup>3</sup>. Animals were necropsied from 3 to 40 days post-infection, under ether anaesthesia. Larvae

were recovered and counted after digestion of visceral organs and muscle in artificial gastric juice, followed by the Baermann technique, according to Soh<sup>4</sup>. Student's t-test was used for determining statistical significance.

**Results and discussion.** Mortality was not greater than 6% in gonadectomized and control animals of both sexes. Orchiectomy in male mice depressed their susceptibility to parasitic infection, as is evident from the 4 to 31% decrease in the larval recoveries from castrated animals in comparison to sham-operated controls (table 1). On the 3rd and 7th day of infection this difference was insignificant, but on the 11th day it was significant ( $p < 0.05$ ) and from the 15th day onwards until the end of experiment it was highly significant ( $p > 0.001$  to  $< 0.001$ ). The average number of larvae

Table 1. The effect of gonadectomy on the larval recoveries from male and female mice infected with 1000 filariform larvae of *Ancylostoma caninum*, during the 40-day experimental period

Day post-inoculation	Number of larvae recovered (Mean $\pm$ SE)							
	Male Control	Orchiectomized	% decrease	p-value	Female Control	Ovariectomized	% increase	p-value
3	585 $\pm$ 19 (5)	562 $\pm$ 19 (5)	4.00	< 0.25	518 $\pm$ 15 (5)	592 $\pm$ 19 (5)	14.29	< 0.01
7	563 $\pm$ 23 (5)	528 $\pm$ 18 (5)	6.20	< 0.25	492 $\pm$ 16 (5)	609 $\pm$ 14 (5)	23.78	> 0.001
11	533 $\pm$ 28 (5)	461 $\pm$ 25 (5)	13.50	< 0.05	425 $\pm$ 20 (5)	533 $\pm$ 22 (5)	25.41	< 0.001
15	508 $\pm$ 13 (5)	435 $\pm$ 13 (5)	14.37	< 0.001	435 $\pm$ 24 (5)	550 $\pm$ 21 (5)	26.44	< 0.001
20	486 $\pm$ 11 (5)	373 $\pm$ 26 (5)	23.25	< 0.001	359 $\pm$ 16 (5)	497 $\pm$ 17 (5)	38.44	> 0.001
25	521 $\pm$ 13 (5)	401 $\pm$ 23 (5)	23.03	< 0.001	288 $\pm$ 14 (5)	471 $\pm$ 9 (5)	63.54	> 0.001
30	464 $\pm$ 16 (5)	337 $\pm$ 10 (5)	27.37	> 0.001	311 $\pm$ 14 (5)	426 $\pm$ 17 (5)	36.98	> 0.001
40	427 $\pm$ 19 (5)	294 $\pm$ 5 (6)	31.17	> 0.001	254 $\pm$ 17 (6)	444 $\pm$ 16 (4)	74.80	> 0.001

Table 2. Cross-comparison (p-values) between the larval recoveries from gonadectomized and control male vs female mice, infected with *Ancylostoma caninum*, during the 40-day experimental period

Duration of infection (days)	Male vs female Orchiectomized vs control	Control vs ovariectomized	Control vs control
3	=0.1	< 0.5	< 0.01
7	< 0.1	< 0.1	< 0.025
11	< 0.25	0	=0.01
15	0	< 0.1	< 0.01
20	< 0.5	< 0.5	> 0.001
25	< 0.001	< 0.01	> 0.001
30	< 0.1	< 0.1	> 0.001
40	=0.05	< 0.25	> 0.001

recovered from orchiectomized and control mice were (562–294) and (585–427) during the 40-day experimental period.

Ovariectomy in female mice significantly increased their susceptibility to infection, as revealed by a 14–75% increase in the survival and retention of infective larvae. But no sign of growth and development of filariform larvae was observed during these experiments. The average numbers of larvae recovered from ovariectomized and sham-operated-controls were (592–444) and (518–254) respectively, with ( $p > 0.001$  to  $< 0.001$ ) excepting ( $p < 0.01$ ) on post-infection 3 days (table 1).

The cross-comparison (in p-values) between the larval recoveries (table 2) from sham-operated control male vs

female mice revealed that males were significantly more susceptible ( $p > 0.001$  to  $< 0.025$ ) than females, throughout the infection period. The differences in the larval burdens between castrated male and control female groups were insignificant except on the 25th ( $p < 0.001$ ) and 40th ( $p = 0.05$ ) day of infection. The number of larvae recovered from control males was slightly greater than from ovariectomized females, but the differences were not significant, except on the 25th day ( $p < 0.01$ ). Thus removal of gonads eliminated the significant differences ( $p > 0.001$  to  $< 0.025$ ) in the size of larval populations shown between the sexes in normal mice, by remarkably altering the host susceptibility. An important factor in the pathogenicity of parasitic diseases is the sex of the host; pathogenicity depends at least to some extent on the sex hormones. The present findings clearly indicate the involvement of male and female gonadal hormones, testosterone and estrogen, in the susceptibility of mice towards *Ancylostoma caninum* infection, as extirpation of testes successfully decrease the susceptibility of the host and removal of the ovaries increased it.

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## Effect of rabbit antiserum to ovine LH on reproductive organs in male hamsters and guinea-pigs

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**Summary.** Administration of rabbit antiserum to ovine luteinizing hormone to immature hamsters and guinea-pigs resulted in a significant decrease in the weights of testes, seminal vesicle and ventral prostate.

Studies using antiserum to LH have shown that LH has an important role in regulating reproduction in both male and female mammals<sup>2-4</sup>. The neutralization of endogenous LH by way of active or passive immunization leads to atrophy of testes and accessory reproductive organs in rats and rabbits<sup>5-7</sup>. However, studies by Greenwald<sup>8</sup> using hypophy-

sectomized pregnant hamsters reported that in this species, unlike the rat, FSH and prolactin from the luteotropic complex and LH supplementation could not maintain pregnancy in hypophysectomized pregnant animals; this suggested that LH has no significant role in this species. In contrast, studies from this laboratory using specific antisera