

## Proteolytic Activity in Trichinella spiralis Larval Secretions

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**OBJECTIVE:** One of the prevailing hypotheses concerning immunity in trichinosis is that secretions and/or excretions (here referred to as "secretions") of adult Trichinella spiralis contain antigen(s) which provoke(s) protective immunity in infected experimental animals<sup>1,2</sup>. It has been postulated that these antigen(s) consist of (or include) enzymes which are involved in the digestion of helminth nutrients, although there is no evidence for this at present. However, protease activity has been found in esophageal extracts of Ancylostoma caninum and this activity was inhibited by immune serum<sup>3</sup>. Furthermore, antibody inhibitory to larval lactic dehydrogenase has been obtained from rabbits infected with T. spiralis<sup>4</sup> and anti-enzymes have been described in other nematode infections. The objectives of this project are to (a) evaluate Trichinella spiralis secretions for protease activity and, if found, to (b) assess the ability of antisera raised in response to infection to inhibit this activity.

**DESCRIPTION:** T. spiralis larvae are isolated from the muscle of rats infected four weeks previously by a pepsin digestion technique and are incubated at 37°C in saline. Viability of larvae is assessed by microscopic sampling; larvae that are neither tightly coiled or motile are taken to be non-viable. Production of secretion is monitored by spectrophotometry at 280 and 260 m $\mu$ . The saline incubates are routinely cultured on blood agar and fluids showing bacterial growth are discarded and any data already obtained using such material rejected. Protease assays are performed using casein as substrate; the difference in absorbance at 280 m $\mu$ . between a trichloroacetic acid (TCA) extract of an unincubated control and that of the experimental reaction mixture is taken as an estimate of proteolytic activity. All protease determinations are performed in duplicate. Serum or serum fractions from rabbits infected with T. spiralis are tested for effect on protease activity by incubation with culture fluid for 30 minutes at 37°C prior to protease assay.

Gel diffusion tests are performed by standard techniques using both culture fluid and a somatic extract as sources of antigen.

**PROGRESS:** Essentially complete survival of the larvae is observed for the first 35 hours of incubation; after this, increasing numbers are found dead at each sampling. A significant amount of material absorbing at 260 and 280 m $\mu$ . can be detected as early as 15 hours and increases in an approximately linear fashion up until at least 35 hours. Routinely, culture fluid is harvested at about forty hours, when only a few larvae are nonviable. Protease activity has been found in both the culture fluid and in homogenates of the worms. TCA soluble material increases in the reaction mixtures for up to three hours, although linearity is not maintained. Since the activity is low, assays are routinely performed utilizing the three hour incubation period. The response obtained in this way is approximately linear with concentration of culture fluid (Fig. 1). The activity is abolished by boiling and appears to deteriorate on freezing and thawing. For this reason, most experiments are conducted with fresh material. Dialysis of the preparations in an

effort to reduce the amount of TCA soluble material absorbing at 280 m $\mu$ . also resulted in loss of activity. Determination of the pH optimum for the proteolytic reaction revealed a biphasic dependence of activity of pH (Fig. 2). One optimum, at pH 2, is very close to that of pepsin and may represent a contaminant retained from the larval isolation procedure, although washing of the larvae before culture is rigorous. The second optimum is at pH 6; at this pH, there appears to be no contribution from the pepsin or pepsin like material. Studies of pH—activity curves of the pepsin preparation employed in these studies gave no evidence of activity at pH 6 (Fig. 3). These findings provide assurance that the activity detectable at pH 6 in T. spiralis secretions is in fact a product of the organisms.

Gel precipitin tests on sera from rabbits infected with T. spiralis show a brisk serologic response. Preliminary to study of the effect of antibody on protease activity, the influence of normal serum was explored; whole normal serum inhibits protease, but this inhibitory effect can be circumvented by the use of the 50% ammonium sulfate insoluble fraction of the sera (Table 1). A preliminary experiment with immune ammonium sulfate precipitated globulins suggested partial inhibition of protease, but attempts to confirm this finding with other sera have not been successful. Serially collected rabbit sera are now being titrated for antibody by passive hemagglutination and will be systematically studied for inhibition of protease activity.

**SUMMARY:** Proteolytic activity, as measured by production of trichloroacetic acid soluble material absorbing at 280 m $\mu$  during incubation with casein, has been demonstrated in saline in which T. spiralis larvae have been incubated. The activity appears to be a linear function of concentration and is abolished by boiling. The pH optimum is approximately 6. The activity is completely inhibited by normal rabbit serum but not by its 50% saturated ammonium sulfate insoluble fraction. Preliminary experiments suggest partial inhibition of activity by immune globulin.

**REFERENCES:**

- (1) Campbell, C.H., J. Parasit. 41: 483, 1955.
- (2) Chipman, P.B., J. Parasit. 43: 593, 1957.
- (3) Thorson, R.E., J. Parasit. 42: 21, 1956.
- (4) Dusanic, D.G., Expt. Parasit. 19: 310, 1966.

Table I. Effect of normal rabbit serum and its 50% saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitate on T. spiralis protease activity

	Mean $\Delta$ A <sub>280</sub>
Saline	0.091
Whole serum	0
Heated serum	0.027
Dialyzed serum	0.027
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	0.124

Table II. Effect of the 50% saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitate of immune rabbit serum on T. spiralis protease activity

	Mean $\Delta$ A <sub>280</sub>	
	Exp. No. 1	Exp. No. 2
Normal Serum	—	0.100
Saline	0.107	—
Rabbit # 1	0.089	0.069
Rabbit # 2	0.071	0.070
Rabbit # 3	0.085	0.080

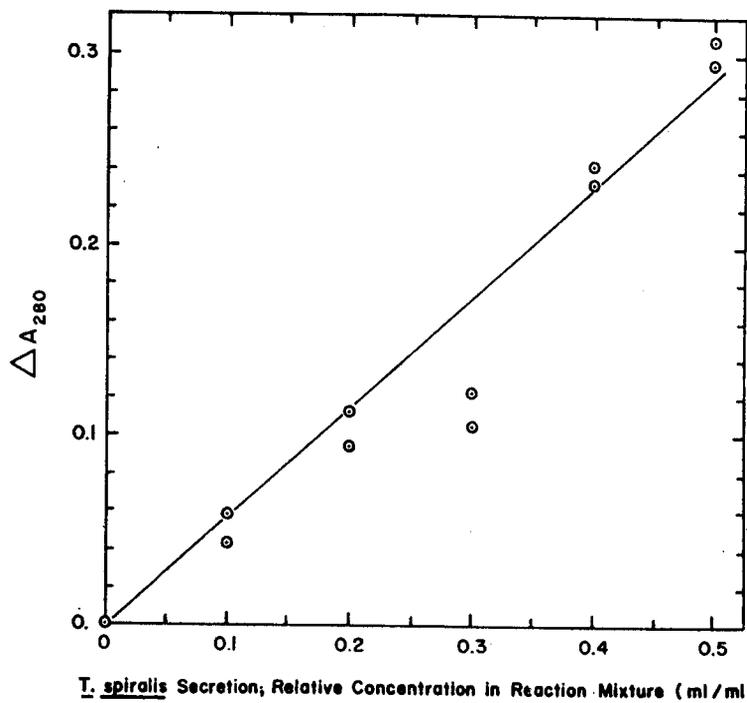


Fig. 1. Effect of concentration of *T. spiralis* secretion on production of TCA soluble material absorbing at 280 m $\mu$  on incubation with a casein substrate. Each point represents the difference in absorbance between the TCA extract of a single reaction mixture and its unincubated control.

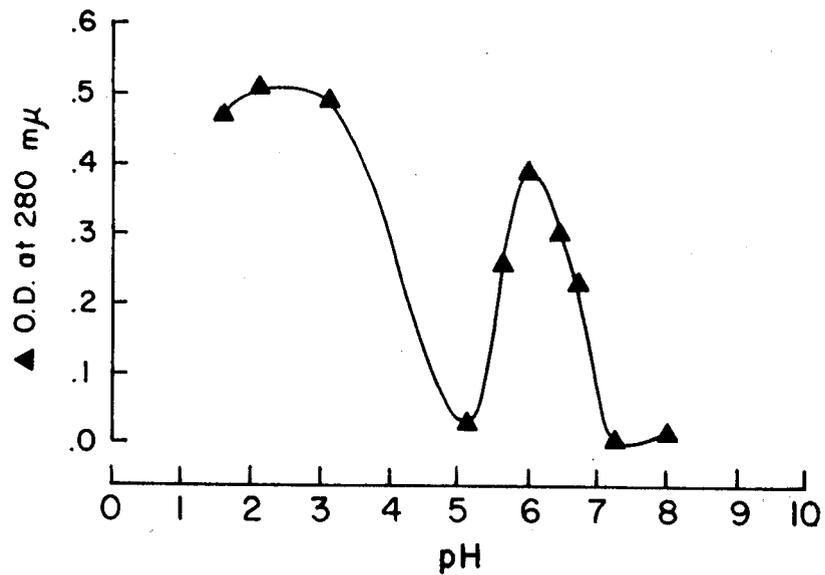


Fig. 2 Effect of pH on protease activity of *T. spiralis* secretion. Each point represents the mean of duplicate determinations.

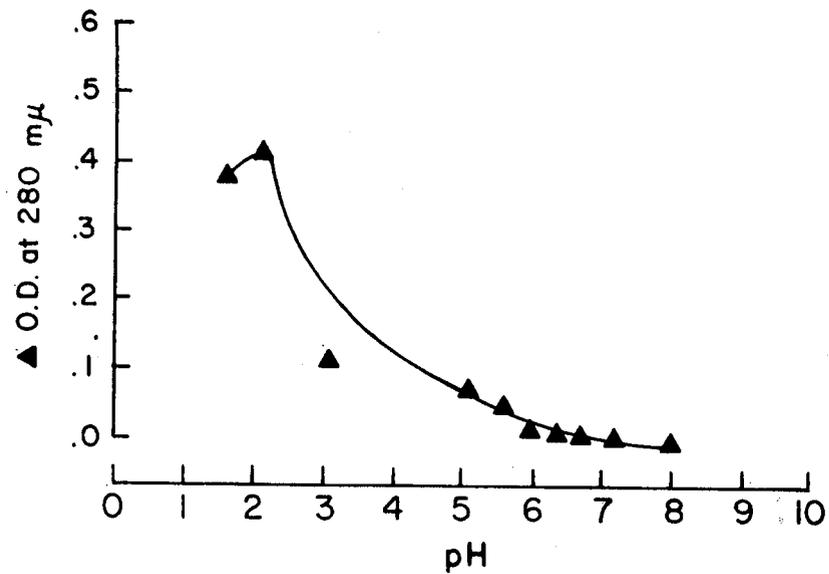


Fig. 3 Effect of pH on protease activity of the pepsin preparation used for isolation of *T. spiralis* larvae. Essentially no activity is detectable at pH6.