BIOCHEMICAL STUDIES OF INSECTIVOROUS PLANTS

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IV.

Occurrence of Antiproteases in the Larvae of the *Sarcophaga* Associates of *Sarracenia flava*

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The parasitic intestinal worms of man and the domestic animals contain antiproteases (antipepsin and antitrypsin), which effectively prevent the digestion of the parasite by the proteolytic enzymes in the digestive fluids of the host. This is especially true of *Ascaris* (1).

The pitcher liquor of *Sarracenia flava* contains a proteolytic enzyme (2). The larvae of certain species of *Sarcophaga* (*sarraceniae* Riley, *Rileyi* Aldrich, and *Jonesi* Aldrich) habitually occur in the pitchers of *Sarracenia flava*, where they are constantly bathed in the digestive liquor of the pitcher. This phenomenon suggested the examination of *Sarcophaga* larvae from *Sarracenia* pitchers for the presence of antiproteases. Live larvae obtained from open pitchers of *Sarracenia flava* were used in the study. Two series of experiments were made.

In the first series, 16 larvae (total weight 1.86 grams) were crushed, and ground with sand and 4.5 cc. of distilled water. The turbid solution and suspended tissue were removed by decantation, and were mixed with sufficient 95 percent alcohol to render the final concentration of the alcohol 60 percent. The precipitate which formed was collected on a filter and dried over calcium chloride in a dessicator. The filtrate was mixed with alcohol until the concentration of the latter was 85 percent; the precipitate which formed was negligible, although the antiproteases should have separated at this point.

The thought, that possibly the rather tough larval tissue had not been ground sufficiently to liberate the antiproteases, led to an examination of the first precipitate for these antienzymes. When thoroughly dry, the precipitate was separated from the filter paper, ground intimately with glass powder, and then triturated with 10 cc. of distilled water. A supernatant liquid was obtained by centrifugation; 2.5 cc. of this liquid and 2.5 cc. of a 1 percent solution of pepsin in 50 percent glycerol were mixed; and sufficient hydrochloric acid and trikresol were added to produce a concentration of 0.2 percent of each of these reagents. The resulting solution was allowed to stand for 2 hours at room temperature to permit the pepsin and the antipepsin (if present) to combine. A

control experiment was made in which 2.5 cc. of physiological salt solution were substituted for the solution derived from the larvae. Carmine fibrin (0.2 gram, weighed, then swollen in 0.2 percent hydrochloric acid) was added to both the experiment proper and the control, and both were then incubated at room temperature. In the control, the carmine fibrin was completely dissolved in 1.75 hours. In the experiment proper, the carmine fibrin was not dissolved at the end of 12 days, but had been completely dissolved at the end of 17 days. Therefore antipepsin, an antiprotease, was present in the larvae, since the solution derived from the larvae markedly retarded the peptic digestion.

In the second series of experiments, 82 larvae (total weight 8.30 grams) were used. From the same gathering of larvae a number were bred to the adult fly, and proved, by examination of the male genitalia, to be *Sarcophaga sarraceniae* Riley, the first recognized *Sarcophaga* associate of *Sarracenia*. The larvae were ground with glass powder to an intimate mixture, which was thoroughly triturated with distilled water. The pasty mass was subjected to a pressure of 50 kilograms per square centimeter in a Buchner press; 48 cc. of press juice were obtained. The press juice was so cloudy that the edestan and casein tests could not be applied in the examination for antiproteases, and only carmine fibrin was used as a substrate.

Antipepsin. In the experiment proper, 12 cc. of press-juice and 12 cc. of a freshly prepared 0.2 percent aqueous solution of pepsin were mixed and allowed to stand at room temperature for 30 minutes to permit the pepsin and the antipepsin (if present) to combine. Sufficient hydrochloric acid (2 percent) and trikresol (2 percent aqueous solution) were then added to make the concentration of each 0.2 percent in the resulting solution; lastly, 0.2 gram of carmine fibrin was added. A control experiment was carried out in exactly the same manner as the experiment proper, save that 12 cc. of distilled water were substituted for the press-juice. The temperature of incubation was that of the room. In the control experiment, the carmine fibrin was completely dissolved in 45 minutes; in the experiment proper, it was partly dissolved in 14 hours and completely dissolved in 17 hours.

Antitrypsin. In the experiment proper, 12 cc. of press-juice and 12 cc. of a freshly prepared 0.2 percent aqueous solution of pancreatin (owing its proteolytic power to trypsin) were mixed, and held at room temperature for 30 minutes to permit the trypsin and the antitrypsin (if present) to combine. Sufficient 4 percent solution of sodium carbonate and 2 percent solution of trikresol were added to make 0.4 percent of

the former and 0.2 percent of the latter reagent in the final solution; then 0.2 gram of carmine fibrin was added. A control experiment was made exactly like the experiment proper, except that 12 cc. of distilled water were substituted for the press juice. The incubation was made at room temperature. In the control experiment, the carmine fibrin showed signs of incipient digestion in 45 minutes, and had completely dissolved in 14 hours. In the experiment proper, the carmine fibrin was only partly dissolved at the end of 17 hours, but was completely dissolved at the end of 22 hours.

Since the press juice markedly retarded the digestion of carmine fibrin by both pepsin and trypsin, both antipepsin and antitrypsin were present in the larvae of *Sarcophaga sarraceniae*.

Thermo-stability of the antiproteases. The experiments proper were also carried out as described above, except that the 12 cc. portions of press juice were boiled and cooled to room temperature, then used without filtration. The protein in the press juice was coagulated by the heat on boiling. The digestion of carmine fibrin by both pepsin and trypsin was retarded to about the same extent as when unboiled press-juice was used. The coagulated protein of the press juice was dissolved completely by pepsin and by trypsin (pancreatin) only after digestion at room temperature for 7 to 8 days, the coagulum remaining long after the carmine fibrin had disappeared. These results indicate that the antiproteases—antipepsin and antitrypsin—of the larvae were thermostabile. They also indicate that the coagulated protein of the press-juice either adsorbed antiprotease and thereby resisted digestion, or else was in itself not readily digestible.

The methods used in the preceding experiments were based on those described by Fischer (1) and by Wohlgemuth (3) The trikresol served as a bactericide.

In this study, antiproteases have been found in the larvae of the *Sarcophaga* associates of the pitcher plant, *Sarracenia flava*. The larvae of other species of *Sarcophaga*, and of several other dipterous genera, are likewise able to live and escape digestion in an environment rich in proteolytic enzymes; probably these larvae also contain antiproteases which protect them from digestion. Thus *Sarcophaga haemorrhoidalis* Fall. can live in the human intestinal tract; Haseman (4) has recently published a detailed account of a series of cases of intestinal myiasis in man, due to the presence of the larvae of this species in the intestines; Aldrich (5) gives an additional and similar case in which the parasite

was also positively identified as *S. haemorrhoidalis*, and cites other records where this species may have been the one concerned.

LITERATURE CITED

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