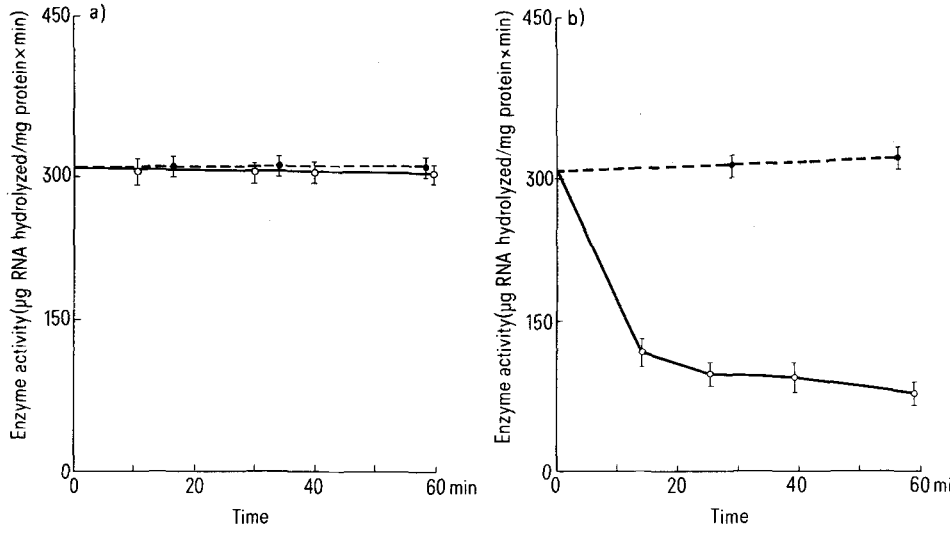

Effect of irradiation in the presence of rose bengal on the activity of acid ribonuclease. *a* At pH 5.6; *b* at pH 7.1. Data are the mean of 6 determinations. Vertical bars are standard errors of the mean. O—O, Experimental; ●---●, controls.

causes a marked decrease in activity over the first 30 min. The control samples (pH 7.1) show a very slight increase in activity during a 60-min incubation and it is thus likely that the total extent of inactivation is even greater than that recorded in the figure (b).

The resistance of the enzyme to photodynamic treatment at pH 5.6 is interesting. Methionine, cysteine and to a lesser extent, tryptophan, are photo-oxidized at this pH[2], and the results therefore suggest that these amino acids do not contribute directely to the enzyme activity. In pancreatic ribonuclease photo-oxidation of methionine lying on the surface of the enzyme molecule causes a significant reduction of activity[5]. The fact that this does not happen with pea cotyledon ribonuclease supports earlier findings that a small fragment of the native enzyme retains catalytic activity[3]. When the methionine residue in position 13 of pancreatic ribonuclease, is photo-oxidized the activity of the enzyme decreases by 80%[5]. This is because this particular methionine residue lies in the active site, adjacent to a histidine residue which participates in the enzyme reaction[6,7]. Our data thus suggest that methionine does not occupy such a position in pea ribonuclease.

The acid ribonuclease from pea cotyledons is very markedly inactivated by photo-oxidation at pH 7.1. At this pH, histidine is by far the most vulnerable amino acid, and cysteine, methionine and tryptophan are also photo-oxidized to a significant extent. The marked inactivation at pH 7.1 (taken with the lack of inactivation at pH 5.6) may thus be attributed to photo-oxidation of histidine. Cysteine is efficiently photo-oxidized by crystal violet in acidic media[8]. Tryptophan photo-oxidation is more rapid as the pH of the solution is raised from neutral[2]; the lack of inactivation at pH 8.1 suggests that this amino acid is not involved in the active site. Histidine, therefore, appears to be directly involved in the active site. The mode of action of pea cotyledon ribonuclease is thus likely to be very similar to that of pancreatic ribonuclease. In pancreatic ribonuclease, the histidine residues at positions 12 and 119 lie on opposite sides of the groove which forms the active site[6,9] and actually participate in the reaction. Modification of either of these residues by photo-oxidation[10], or by iodoacetate[9], causes a marked loss of enzyme activity.

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Effects of larval firefly extracts on molluscan cardiac activity[1]

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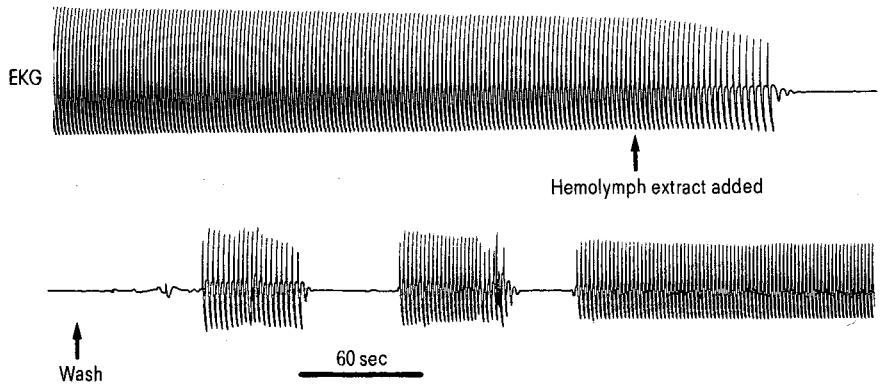
Summary. Larval firefly midgut extracts and larval firefly hemolymph extracts were found to produce a potent inhibitory effect when applied directly to the heart of a terrestrial mollusc. It is suggested that such substances could comprise an important part of the paralyzing toxin which is reportedly injected by larval fireflies into their prey.

The larvae of fireflies, bioluminescent members of the beetle family Lampyridae, have been reported to subdue their prey, much larger invertebrates such as terrestrial annelids, arthropods, and molluscs, by injection of a paralyzing toxin via their hollow fang-like mandibles[2]. A tubular canal is found within each mandible and this canal extends from near the mandible's tip, where it exits, to the mandible's base, where it opens into the mouth. Several



The electrocardiogram (EKG) of *Limax maximus* stops following addition of larval hemolymph extract to the bath.

investigators[3-5] have inferred from such anatomical evidence and from anecdotal, sometimes contradictory, behavioral evidence[2,6,7] that the midgut contents are injected into the prey via the larval mandibles. The physiological effect of the midgut contents has not yet been tested. In the present experiments untreated extracts were prepared from different compartments of the larval firefly body. Since a number of invertebrate poisons are known to effect excitable tissue[8], extracts were tested using an in situ myogenic heart preparation of the terrestrial mollusc *Limax maximus*.

Methods. Using 5–15 g *Limax maximus*, preparations were made in a Sylgard lined petri dish filled with a 19–21 °C saline containing 76.4 mM Na^+, 2.5 mM K^+, 3.42 mM Ca^{++}, 0.8 mM Mg^{++}, 81.94 mM Cl^-, 0.4 mM H_2PO_4, 5.0 mM HCO_3^-, 20 mM glucose. The pericardial sac was slit, allowing a small piece of ventricle to be grasped by a suction electrode[9]. The electrocardiogram (EKG) was AC coupled, amplified (WP-Instruments DAM 6A) and displayed DC on a polygraph (Grass 5D). Inhibition was scored as a reduction in EKG amplitude or frequency by more than 40% of control values or, alternatively, by complete cessation of heartbeat.

Larval extracts were prepared by crushing the relevant tissue (midgut, 4.8 mg; head, 1.6 mg; thorax-abdomen, 96.4 mg; mandibles, 47.8 µg; entire animal, 102.7 mg) in 1 ml of saline or by adding 1 µl of hemolymph to 1 ml of saline. Extracts prepared similarly from *Tenebrio molitor* served as control. Extracts, still containing tissue particles, were added to the 50-ml bath near the heart, where they remained for 30–240 sec until their removal and replacement with 150 ml of new saline. *Photuris lucicrescens*, *Photuris versicolor*, and *Photuris* sp. were used.

Cardiac inhibition produced by larval firefly extracts

Extract source	Trials with inhibitions (%)	Total trials	p*
Midgut			
Photuris	64	14	0.01
Tenebrio	0	9	
Hemolymph			
Photuris	63	30	0.01
Tenebrio	7	15	
Entire animal			
Photuris	42	26	0.05
Tenebrio	0	13	
Head			
Photuris	37	19	0.05
Tenebrio	0	12	
Thorax and abdomen			
Photuris	27	22	NS
Tenebrio	8	13	
Mandibles			
Photuris	29	18	NS
Tenebrio	0	10	

*Fisher-test, two-tailed.

Results and discussion. Limax heartbeat was inhibited during diastole by a number of different larval firefly extract types (table, fig.). The greatest inhibitory effect was produced by midgut or hemolymph extracts. The duration of inhibition was variable, from several sec to several min for all extract types. All effects were immediately reversable save some hemolymph extracts (n = 8) and most (n = 10) midgut extracts. These usually required several h to reverse completely. No species differences in the potency of extracts were observed.

Since all the *Photuris* species used here had hollow mandibles which were connected via the mouth to the digestive system, these results leave open the possibility that prey capture involves direct injection of midgut secretions and subsequent inhibition of cardiac (or other excitable) tissue in a molluscan prey. Such an effect would eliminate locomotion in molluscs, because hydrostatic pressure, necessary for pedal mucus secretion, is produced principally by the heart[10].

Some adult fireflies reflexly bleed their distasteful hemolymph in response to attack by vertebrate predators[11]. Recently steroidal pyrones called lucafubagins, with cardiotonic and emetic effects in avian firefly predators, have been found in 2 adult *Photinus* species[12]. The low molecular weight and lipid solubility of lucafubagins make these compounds reasonable candidates for producing such an effect. Whether the cardioinhibitory fraction of the larval hemolymph is also responsible for these cardiotonic and emetic effects is presently unknown. However, the possibility that one hemolymph constituent is responsible for both predatory and anti-predatory mechanisms is an attractive hypothesis that warrants further investigation.

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