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THE ACTION AND COMPOSITION OF THE SALIVA OF AN ASSASSIN BUG *PLATYMERIS RHADAMANTHUS* GAERST. (HEMIPTERA, REDUVIIDAE)

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(With Plate 1)

INTRODUCTION

The predatory habit is widespread in the Heteroptera; over half of the families whose biology is known live by the capture of living invertebrate prey.

All Hemiptera must ingest liquid food and as a consequence their salivary secretions play an important part in feeding. This is perhaps most clearly seen in the predatory Heteroptera where the saliva has assumed the function of a venom, and among these it is the assassin bugs of the Reduviidae that have most extensively exploited the habit. Their ability to paralyse their captures rapidly has long been known to students of natural history; Degeer (1773), writing of *Reduvius personatus*, observed: 'La Mouche une fois piquée mourut promptement, ce qui denote assez, que la Punaise dois sans doute verser dans la playe quelque venin, dont l'effet très actif.' Later writers have suggested the salivary glands (Miller, 1953), and the maxillary glands (Poisson, 1925) as the source of the venom, but its composition and mode of action have not been examined.

Two examples will suffice to illustrate the efficacy of assassin bug venom. The first instar larva of the harpactocorine reduviid *Rhinocoris carmelita* is able to paralyse within 10 sec. a final instar larva of *Ephestia kuhniella*, over 400 times its own weight. Again, the large reduviine *Platymeris rhadamanthus*, provided with a cockroach, *Periplaneta americana*, puts an end to the convulsive struggling of its prey within 3–5 sec., and abolishes the last flickering movements from its appendages within 15 sec. To do this it injects 10–12 mg. of saliva, and thereafter removes the products of external digestion, amounting to between 40 and 60% of the prey's live weight, at a rate of 1·5–2 mg/min.

It is the purpose of this paper to describe the action of assassin bug saliva on whole animals, selected organs and tissues, and to examine components of the saliva that play a part in paralysis and external digestion.

MATERIALS AND METHODS

(1) *Source of saliva*

Platymeris rhadamanthus saliva was obtained in quantity by exploiting the spitting behaviour of the species (Vanderplank, 1958; Edwards, 1960). For routine

collections, adults that had fed 3–5 days previously were placed under a 10 cm. diametrically broken Petri dish. The animal was molested by tapping its thorax with a seeker, through the break in the Petri dish, thus inducing it to spit saliva from its rostrum on to the glass above. After a series of animals had been extracted to exhaustion the saliva was allowed to dry and was then scraped from the surface with a steel blade and stored over $CaCl_2$. The effects of injecting solutions of saliva so collected, at appropriate concentrations (10–15%), had effects indistinguishable from natural predation, and it is assumed that the saliva secreted during spitting behaviour is the same as that injected into its prey. The term 'saliva solutions' refers to saliva powder freshly dissolved in boiled distilled water at concentrations of 1% and above, and in saline (Hoyle, 1953) at concentrations below 1%.

(2) *Pharmacological preparations*

The *Periplaneta* heart-dorsum provided a quickly made assay preparation. An adult male was pinned dorsal side down to a cork slab and all but the terga with the attached heart was dissected away. The 'bath' formed by the abdominal terga was washed with saline, then almost emptied, and placed in a damp chamber. It was used after regular beating resumed, usually within 20 min. of preparation. With occasional changes of saline this heart preparation would continue regular beating for over 16 hr.

Periplaneta also provided a central nerve cord preparation. After their heads had been removed, adult males were attached dorsal side up to a cork slab with cellulose tape. The abdominal terga and viscera were removed, and the ventral diaphragm gently torn. The nerve cord was then freed between the last and penultimate ganglia to allow the insertion beneath the cord of a pair of recording electrodes. The majority of tests were of short duration and were completed within 30 min. of dissection. The recording apparatus consisted of Tektronix type 122 and 53/54 C preamplifiers, and Tektronix type 520 cathode-ray oscilloscope. Test materials were delivered from a graduated pipette to the region of the last abdominal ganglion. A neon stimulator of variable frequency and voltage was used.

(3) *Electrophoresis*

(*a*) *Tiselius.* A bulk sample of 65 mg. of saliva powder collected over a period of 6 weeks from twenty adult *Platymeris* was dissolved in 2·5 ml. 0·1 M acetate buffer, pH 5·6, and dialysed against buffer overnight at 3° C. A small protein precipitate amounting to 2 mg. was removed by centrifugation. The remaining solution was subjected to Tiselius electrophoresis in a Perkin Elmer Model 38 apparatus.

(*b*) *Zone electrophoresis.* The starch gel method of Smithies (1955) was used with a discontinuous buffer system (Poulik, 1957). The gel was prepared with veronal buffer pH 8·6, and 0·3 M borate buffer pH 8·6 was used in the electrode tanks. Electrophoresis was carried out at room temperature for 16 hr., with a potential gradient of 6 V./cm. After electrophoresis the gel was stained with amidoschwarz.

(4) *Enzyme assay*

(*a*) *Protease.* The activity of the salivary protease was estimated by the method of Charney & Tomarelli (1947). Sulphanilamide azocasein was prepared from commercial 'light white' casein after de-fatting with acetone. 2% stock solutions were prepared by dissolving the azocasein powder in a small volume of 1% Na_2CO_3, neutralizing with 0·1 M-HCl and making up to volume with distilled water. Stock solution was stored under toluene and used within 6 days of preparation. The final buffer concentration in most experiments was 0·1 M phosphate or borate. Optical densities were read at 450 mμ with a Unicam SP. 600 spectrophotometer.

(*b*) *Hyaluronidase.* Preliminary tests were made using the 'ACRA' test of Burnet (1948), as modified by Evans, Perkins & Gaisford (1951). Viscosity reduction of synovial fluid was further examined using an Ostwald viscometer of about 0·5 ml. capacity and water-time of 14 sec. Synovial fluid collected from astragalotibial joints of newly slaughtered cattle was pooled and centrifuged at 8000 g. for 30 min. It was stored over chloroform at $-15°$ C.

(*c*) *Lipase.* The methods of Cole (Baldwin & Bell, 1955) and Bier (1955) were used with olive oil emulsion and Tween 20 respectively as substrates.

(*d*) *Esterase.* The action of saliva on ethyl butyrate was examined by the method of Harrer & King (1941), and on acetylcholine by Glick's (1938) electrometric titration method.

(*e*) *Adenosine triphosphatase.* The method of Bailey (1942) was used.

(*f*) *Serotonin.* Serotonin was tested for by the method of Jepson & Stevens (1953).

RESULTS

Action of Platymeris *saliva on the whole animal*

The saliva proved to be toxic to a wide range of insects representing seven orders. Their own species are immune: application of 0·1 ml. of 1% *Platymeris* saliva to the heart and gut of a 5th instar *Platymeris* nymph after removal of the abdominal terga caused no marked alteration in their rhythmic contractions over a period of more than 3 hr. The toxicity of 1% saliva solutions toward the *Periplaneta* heart-dorsum preparation is not reduced by previous mixture with an equal volume of *Platymeris* haemolymph. The haemolymph alone is non-toxic, but the crop contents do preserve the toxicity of the saliva for at least 2 days.

Enteral and topical applications of saliva are not toxic to arthropods; the saliva must enter the haemocoel. *Periplaneta* drank 1% saliva solution without ill effect and *Calliphora* larvae were unharmed by 6 hr. immersion in 1% solution.

In the experience of the writer the bite of an assassin bug causes intense localized pain and swelling and leaves a long-standing necrotic pit. The dried saliva powder is an irritant of eye and nose membranes, causing oedema, vasodilatation, copious mucous secretion and respiratory disturbances similar to those caused by viper venom (Stanic, 1956).

The LD 50 of *Platymeris* saliva for *Periplaneta americana* was determined by

injecting a volume of saliva similar to that delivered in natural predation (*c.* 0·01 ml.) through the left prothoracic pleuron. Successive dilutions from a stock of 10% saliva were injected into groups of ten animals. The LD 50 for 18 hr. at 28° C. was 10·25 mg./kg.

Action of saliva on heart-dorsum preparation

Platymeris saliva at concentrations approximating to the natural concentration (5–15%) causes immediate and violent contraction and cessation in systole, together with general contracture of the tergal musculature. A slow relaxation of the inexcitable tissue follows after about 30 sec. and continues for 2–3 min. At a dilution of 10^{-1} (w/v) the action is as described above except that the relaxation phase is extended to between 5 and 10 min. At concentrations of 2×10^{-2} and below, an initial brief acceleration in rate of heartbeat precedes an irregular slowing, leading to cessation at between 30 and 70 sec. A lower limit to definite activity is reached at concentrations of about 10^{-6}. This concentration induces a slight increase in rate of beating in most preparations, followed by a decrease in rate and amplitude. The beat becomes irregular and contractility disappears after 10–20 min.

Extracts of both the anterior and posterior lobes of the principal salivary gland are toxic when applied alone, but the contents of the accessory salivary gland have no toxic action.

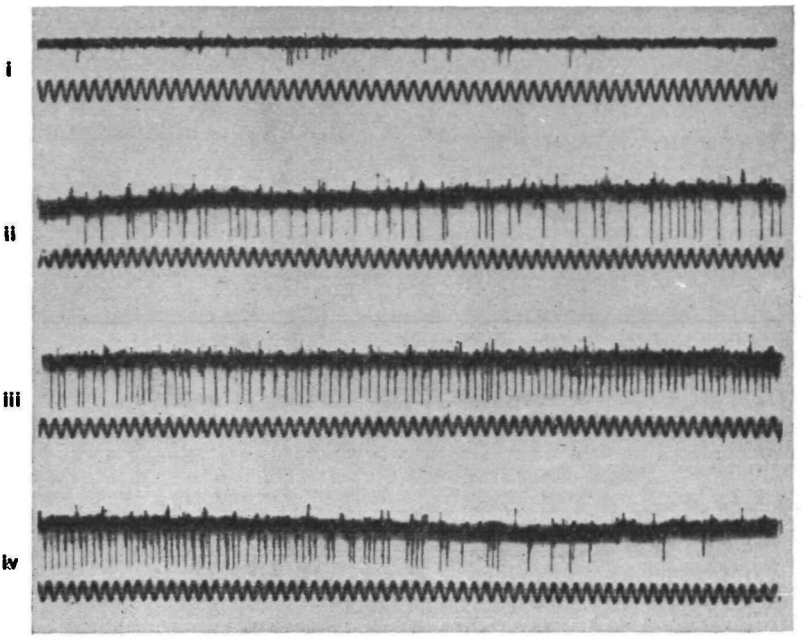
That potent toxicity is a special property of predatory bugs within the Heteroptera is demonstrated by the results shown in Table 1, where the action of homogenates of the salivary glands of a range of species is compared.

Table 1. *Toxicity of Hemipteran salivary gland homogenates*

Species	Quantity applied	Action
Platymeris rhadamanthus *Rhinocoris carmelita* *Reduvius personatus*	0·01 parts single gland in 0·1 ml. saline	Immediate cessation in systole. General contracture
Rhodnius prolixus *Triatoma protracta*	Entire pair glands in 0·5 ml. saline	No action
Naucoris cimicoides	0·01 parts single gland in 0·1 ml. saline	Immediate cessation in systole. General contracture
Oncopeltus fasciatus *Pentatoma rufipes*	Entire pair glands in 0·05 ml. saline	Slow decrease in amplitude Slight increase in rate, cessation after some minutes

Action of saliva on nerve

Application of 0·1 ml. of 5–10% saliva solution in the region of the last abdominal ganglion causes within a few seconds an increase in electrical activity leading to intense repetitive discharge of giant fibres after 10–15 sec. The bout of intense activity terminates abruptly and thereafter the cord ceases to conduct; the post-synaptic response to electrical and mechanical stimulation of the cercal nerve is abolished. Excerpts from a typical record of the activity are shown in Text-fig. 1*a*. At lower concentrations the action is similar but the latent period preceding the activity is prolonged (Table 2).



a

Text-fig. 1 (*a*). Extracts from oscillograph records of the action of *Platymeris* saliva on the abdominal nerve cord of *Periplaneta americana*. (i) Record from last abdominal connective before application of saliva. (ii) and (iii) Onset of repetitive discharge after application of 0·1 ml. 1 % saliva in the region of the last abdominal ganglion. (iv) Decline of activity. Time marker: 50 c.s.

Table 2. *Latent period and duration of the discharge of* Periplaneta *nerve cord exposed to* Platymeris *saliva at various concentrations*

Concentration of saliva (%)	Latent period	Duration of discharge (sec.)
10	3–6 sec.	50–60
1	110–240 sec.	60–90
0·1	180–500 sec.	120–600
0·01	>45 min.	

Pricking the neural lamella under 0·01 % saliva produces a general discharge readily distinguishable from localized damage discharge. It appears that the neural lamella and/or perineurium provide an adequate barrier at low concentrations of saliva.

When a 0·1 % solution is applied to the cord, seven to nine separate successive bursts of repetitive activity could frequently be distinguished, each occupying 3–5 sec. In these cases the giant fibres were discharging singly. During the brief burst the frequency of discharge rose from 10 per sec. to a peak of *c.* 500 per sec. before becoming silent. At lower concentrations persistent repetitive discharge at various frequencies was frequently recorded (Text-fig. 1 *b*).

The action of *Platymeris* saliva on a non-synaptic preparation was examined using a connective between the last thoracic and first abdominal ganglia of the locust *Schistocerca gregaria*. A length of connective was functionally isolated by crushing the cord adjacent to both ganglia until through conduction was abolished. The isolated connective was stimulated anteriorly and recordings were taken from the posterior end. 1 % saliva solution caused a decay in conduction beginning 80 sec. after application, and finally abolishing conduction after 120 sec.

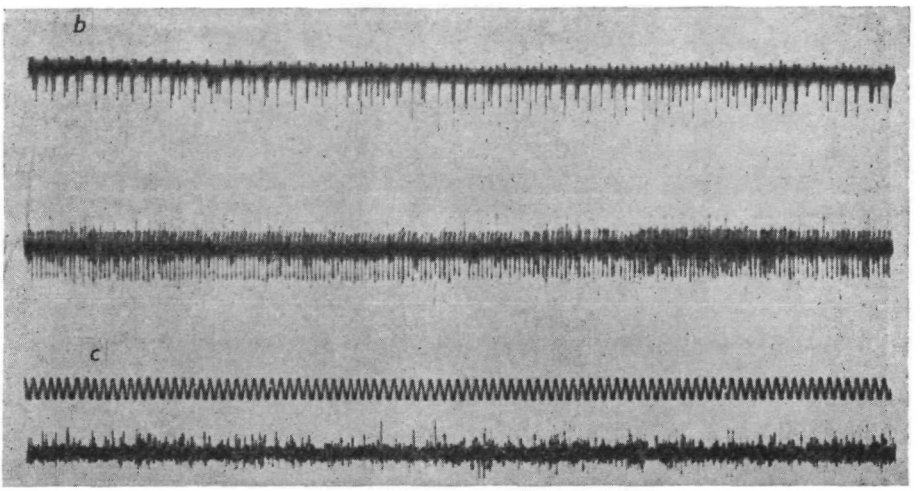


Fig. 1 (*b*). (i) and (ii) Typical oscillograph records of persistent repetitive discharge of *Periplaneta* nerve cord, induced by *Platymeris* saliva. (*c*) Action of bee venom on abdominal nerve cord of *Periplaneta*. For details see text. Time marker: 50 c.s.

Brief accelerating repetitive discharges comparable to those induced by *Platymeris* saliva were observed with a preparation of bee venom, (Text-fig. 1*c*) containing the contents of six poison sacs in 0·25 ml. saline.

Trypsin solution (1 % crystalline trypsin in saline) caused no increase in electrical activity within 30 min.

Action of saliva on muscle

Contraction of the tergal musculature in the heart-dorsum preparation has been noted above. The isolated Malpighian tubules of many insects exhibit rhythmic coiling and uncoiling effected by a spiral muscle band that is said to lack innervation (Palm, 1946). The action of *Platymeris* saliva on this muscle was examined using segments of the Malpighian tubules of *Schistocerca gregaria*. Segments were placed in a watch-glass containing 1 ml. saline (Belar, 1929, as used by Cameron, 1953). The addition of saliva solution was effected by briefly withdrawing the tubule with a small volume of saline into a wide-mouthed pipette. Saliva solution was added to the watch-glass and mixed to a known final concentration. The tubule segment was then returned. At concentrations down to 10^{-2} the immediate response was strong coiling followed by slow uncoiling with con-

current lysis of the tubule cells. At lower concentrations down to 10^{-6} the response was variable. In general the contractions became irregular and incomplete, movement eventually ceasing in either the coiled or extended state.

Lytic action of the saliva

Breakdown of fat body is the first visible effect of the saliva after paralysis. Sarcosomes of *Calliphora* flight muscle ruptured instantly when fragments of tissue mounted in saline under a coverslip were irrigated with a 0·1% solution. Muscle striation became diffuse within 5–10 min.

Gross changes in the appearance and mechanical properties of nervous tissue are observable within a short time of immersion in saliva solution. Segments of ventral nerve cord became opaque and lost rigidity. Previously clear outlines of giant fibres became diffuse and slight agitation reduced the contents to an homogeneous appearance. A remnant of the neural lamella which persisted after prolonged digestion appeared to consist largely of the collagenous components of the lamella (Smith & Wigglesworth, 1959). Histological examination of the action of the saliva on excised ganglia of *Oncopeltus fasciatus* using the osmic acid/ethyl gallate technique (Wigglesworth, 1957) revealed a rapid breakdown of cell membranes from the perineurium inwards, previously continuous lipid components of the cell walls becoming discontinuous and diffuse (Pl. 1, *a–c*). The neural lamella remained intact, although separation of laminae was observable after prolonged treatment.

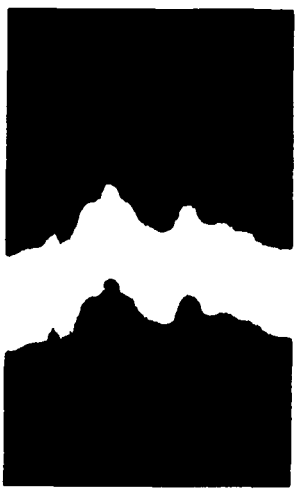
Composition of Platymerus *saliva*

The dried saliva is clear vitreous material, readily flaking to form a white powder. In the dried state it retains toxicity for at least 3 years, but declines slowly in potency in aqueous solution. Freshly secreted saliva has a pH of 6·6–6·8. Toxicity is retained after prolonged dialysis against distilled water in the cold and toxicity is not recoverable from concentrated dialysate. Toxicity is destroyed by papain and by performate oxidation by the method of Hirs (1956). Smears and sections gave negative tests for mucoid substances using the Bismark brown method of Leach (1947) as used by Day (1949) in examining the occurrence of mucoid substances in insects. Tests for mucoprotein using the Erlich direct reaction (Gottschalk, 1958) were also negative.

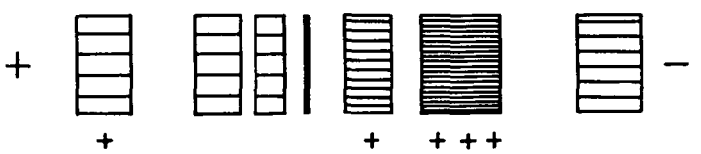
A single analysis of undialysed saliva gave the following percentage composition: C, 45·6%; H, 7·24%; N, 13·6%.

The ascending arm boundaries of Tiselius electrophoresis at pH 5·6 are shown in Text-fig. 2. The saliva is a complex mixture containing at least 6–8 proteins, the two major peaks having mobilities of $8{\cdot}4 \times 10^{-5}$ and 13×10^{-5}. It is of interest that the snake venoms studied electrophoretically by Poulsen, Joubert & Haig (1946) showed patterns of similar complexity. *Naja nigricollis*, the spitting cobra, had eight peaks, with mobilities between $11{\cdot}7 \times 10^{-5}$ and $0{\cdot}3 \times 10^{-5}$ at pH 6·2. Grasset, Brechnuhler, Schwartz & Pongratz (1956) found eight fractions in the venom of Russell's viper at pH 6.

Six fractions separated in starch gel at pH 8·5 (Text-fig. 3). Attempts were made to isolate a toxic fraction among the proteins separated by starch gel electrophoresis. Protein zones were located by staining a narrow strip from the margin of the run, replacing it as a marker so that the protein-bearing blocks could be cut from the gel. The contents were extracted by pressure after freezing and thawing the blocks. After dialysis against 0·2 M saline at pH 7 the extract was applied to a *Periplaneta* heart-dorsum preparation. Although the quantity of saliva used in the separation was sufficient to produce instantaneous effects had it been evenly distributed through the gel, none of the fractions had effects comparable to those of the whole saliva, nor had recombinations of the major bands.



Text-fig. 2. Tiselius electrophoresis of *Platymeris* saliva. Ascending arm, pH 5·6.



Text-fig. 3. Electrophoretic pattern of the saliva of *Platymeris rhadamanthus* in starch gel, pH 8·6. Horizontal hatching indicates intensity of staining. + indicates relative proteolytic activity.

Enzymes in Platymeris *saliva*

(*a*) *Protease*

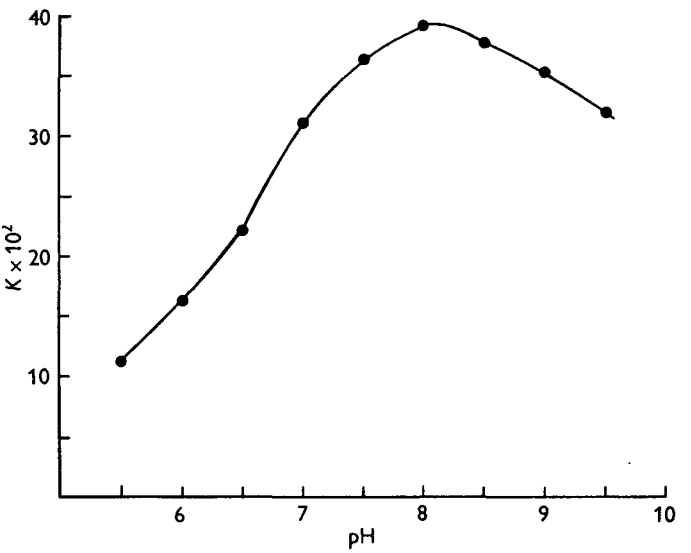
Three proteolytic zones were recognized in the electrophoretic pattern of *Platymeris* saliva using the method of Cuthbertson (personal communication) in which an agar gel containing casein, placed in contact with the starch gel, is cleared where it overlies a protease. The major protein zone proved to have strong proteolytic activity, while a neighbouring band and the extreme anode-migrating band, both containing less protein, showed much weaker activity (Text-fig. 3).

Both lobes of the principal salivary gland secrete protease as shown by the micro-method of Pickford & Doris (1934), but the contents of the accessory gland are not proteolytic.

Specificity. Beside casein and gelatin, *Platymeris* saliva digested blood fibrin and elastin. Release of colour from Congo red-stained substrate was maximal in the region of pH 8–8·5. Collagen, however, was not attacked; no evidence of activity was found using Hobson's (1931) method. The liberation of tyrosine from tendon estimated with Folin and Ciocalteau reagent was comparable to that of trypsin.

A preliminary examination of the major points of cleavage of the insulin B chain by *Platymeris* saliva indicates a similarity to a combination of trypsin and chymotrypsin.

pH *and activity.* Using the method of Charney & Tomarelli (1947) a broad pH optimum about pH 8·2 was found for a digestion period of 30 min. at 30° C. (Text-fig. 4).



Text-fig. 4. pH/activity for *Platymeris* salivary protease. Azocasein substrate.

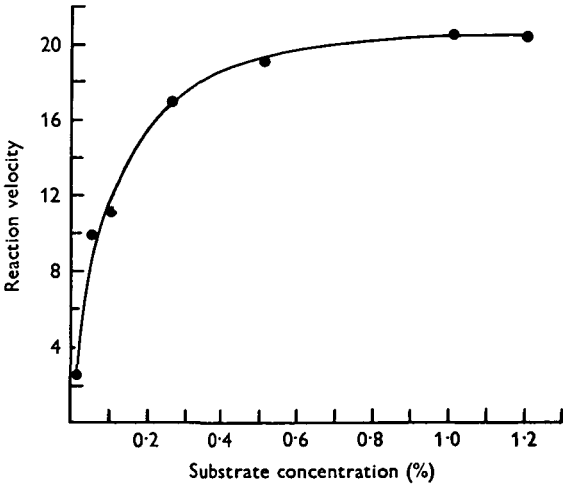
Substrate concentration. The substrate/activity curve for 30 min. digestion at 30° C. is shown in Text-fig. 5. A figure of 0·70% azocasein for K_m, calculated from the results shows close similarity to that of *Calliphora* larval gut protease determined by Evans (1958) using the same substrate.

Temperature and activity. For a 30 min. reaction period at pH 8·2 a temperature optimum of 40° C. was found (Text-fig. 6).

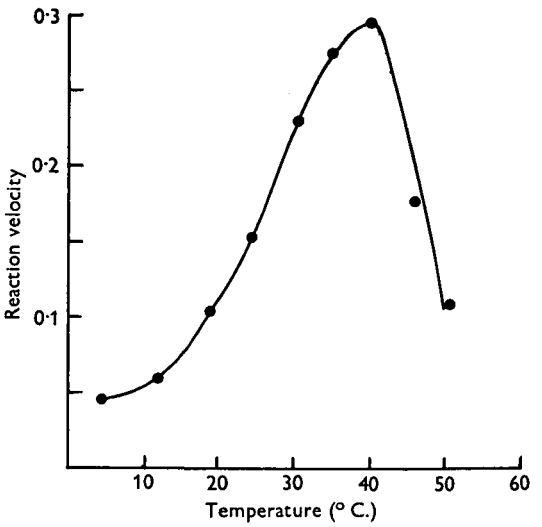
Inhibitors. (i) *Cyanide.* No inhibition was observed at pH 8·2 using 0·5% casein solution and saliva at $0{\cdot}5 \times 10^{-4}$ in cyanide solutions of 0·01, 0·05 and 0·1 M.

(ii) *Trypsin inhibitor.* The digestion of 0·5% azocasein solution was examined using crystalline trypsin and *Platymeris* saliva at concentrations of $0{\cdot}13 \times 10^{-3}$ with

and without equal quantities of soybean trypsin inhibitor. After 30 min. at 30° C., trypsin digestion was 91·5% inhibited and *Platymeris* salivary protease 15·5% inhibited.



Text-fig. 5. Substrate concentration/activity curve for *Platymeris* salivary protease. Azocasein substrate.

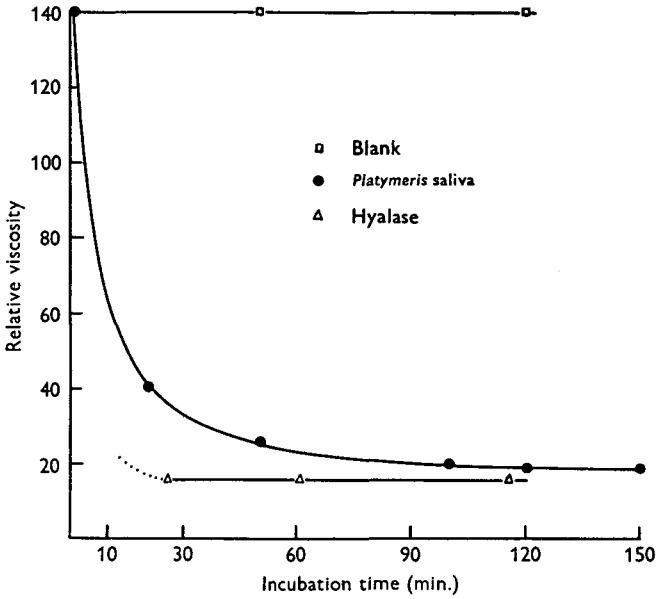


Text-fig. 6. Temperature/activity curve for *Platymeris* salivary protease. Azocasein substrate.

(*b*) *Hyaluronidase*

The reduction in viscosity of synovial fluid by *Platymeris* saliva shown by the 'ACRA' test was further examined by means of an Ostwald viscometer as follows. Synovial fluid was diluted 1:1 with isotonic saline. Hyaluronidase (Benger 'Hyalase') and *Platymeris* saliva were dissolved in saline to give solutions containing 0·5 mg./ml. Saliva solution heated to 98° C. for 10 min. provided a blank.

2 ml. synovial fluid was incubated at 30° C. with 0·25 ml. enzyme solution and the relative viscosity determined in an Ostwald viscometer in the same water-bath. Text-fig. 7 shows the fall in relative viscosity caused by hyaluronidase and by saliva.



Text-fig. 7. Reduction of viscosity of synovial fluid by *Platymeris* saliva.

(*c*) *Lipase and esterase*

Negative results were obtained in all experiments on *Platymeris* saliva. Lipase activity was found in the extracts of the mid-gut wall. Preliminary experiments indicated a tenfold increase in lipase activity in the gut wall 2 hr. after feeding compared with that of animals starved for 20 days.

(*d*) *Phospholipase*

The lytic activity of *Platymeris* saliva suggested the presence of a phospholipase, one of the most widespread constituents of animal venoms (Zeller, 1951). Phospholipase activity as measured by alkaline titration of an unbuffered emulsion of commercial lecithin (B.D.H.) was negligible. Through the generosity of Dr R. M. C. Dawson the action of *Platymeris* saliva on ^{32}P-labelled lecithin was examined. Saliva was incubated with an unbuffered emulsion containing ^{32}P-labelled lecithin for 30 min. at 37° C., and the acid-soluble phosphate released was compared with the total acid-soluble ^{32}P in the sample released by oxidation with $HClO_4$ (Table 3).

Table 3. *Release of acid-soluble* ^{32}P *from lecithin by* Platymeris *saliva*

	Blank	*Platymeris* saliva	Total ^{32}P
Activity counts/min.	64	1135	6478
% of total	1	17·5	—

(*e*) *Adenosine triphosphatase*

The quantity of acid-soluble phosphate liberated from 0·005 M-ATP solution plus 0·1 M-KCl with histidine buffer pH 7 did not exceed the blank value (Table 4).

Table 4. *Release of acid-soluble phosphate from ATP by* Platymeris *saliva*

	Blank no saliva	Without activator	Activator	
			0·005 M-Ca	0·005 M-Mg
P liberated	11·5	10·5	11	11

(*f*) *Serotonin*

Negative tests were obtained from spots of *Platymeris* saliva containing 1 mg. in a test for which a sensitivity of 10^{-4} μmoles cm.^{2} is reported (Jepson & Stevens, 1953).

DISCUSSION

Platymeris saliva, a mixture of at least six proteins, is comparable in complexity to snake venoms similarly studied. Three electrophoretic fractions, one of them the major component of the saliva, are proteolytic. Patterson & Fiske (1958) also reported the presence of three proteolytic fractions in gut homogenates from the stable fly *Stomoxys calcitrans*, though their electrophoretic behaviour differs from that of *Platymeris* saliva. The proteolytic activity of whole saliva resembles that of gut extracts from other insects examined with an azocasein substrate. The substrate affinity of 0·70% is closely similar to that of the gut protease of larval *Calliphora erythrocephala* (Evans, 1958). The pH optimum also resembles that of the *Calliphora* protease (7·8) and of *Blatella germanica* (8·2) (Day & Powning, 1949). Powning, Day & Irzykiewicz (1951) have examined the degree of inhibition of protease extracts from the gut of several species including Orthoptera, Diptera, Lepidoptera and Coleoptera, using an azocasein substrate. Only *Tenebrio* protease resembled that of *Platymeris* in being less inhibited than trypsin. The specificity of the whole saliva resembles that of trypsin plus chymotrypsin, although it also shows elastase activity.

The widespread presence of hyaluronidase as a 'spreading factor' in animal venoms has been reviewed by Favilli (1956). Romanini (1949) reported mucinolytic activity in whole insect extracts of several Heteroptera. The present study is the first demonstration of hyaluronidase activity in the pure saliva of an insect, although Stevens (1956) found activity in salivary gland and gut homogenates of *Periplaneta americana* using a turbidometric technique, confirmed in this study viscosimetrically using the contents of the *Periplaneta* salivary reservoir. Since Ogston & Sherman (1959) have demonstrated that trypsin and chymotrypsin together can remove 65% of the protein from the hyaluronate-protein complex of synovial fluid without loss of viscosity, *Platymeris* salivary protease is unlikely to be involved in the viscosity-reducing activity of the saliva. Hyaluronidase is said to cause the separation of the epithelial cells of the insect midgut (Day, 1949). In

the present study separation of dipteran fat body cells, except where they are in contact with oenocytes, was effected by immersion in saline containing 0·1% hyaluronidase (Benger Hyalase). It appears that in the insect as well as in the vertebrate hyaluronidase will facilitate the penetration of active substances by attacking the intercellular matrix, thus acting as a 'spreading factor'.

Platymeris *saliva as a venom*

The responses of innervated and non-innervated muscle, intact and isolated nerve, to treatment with *Platymeris* saliva indicate that the mechanism of paralysis does not involve a specific site of action. Rather it seems, the saliva attacks and disrupts the cell membranes on which the functioning of excitable tissue depends. It is suggested that the subsequent lysis is a gross extension of the initial membrane breakdown that causes paralysis, or to put the process in its adaptive context, that paralysis by assassin bug saliva is a special function of external digestion.

Knowledge of insect venoms is almost entirely restricted to those of the Hymenoptera. Pavan's (1958) review of insect venoms does not deal with the Heteroptera. The study of *Habrobracon* venom by Beard (1952) is the only work on the action of an insect venom on an insect, with the exception of a short note by Hartzell (1935) on ganglion lesions in a cicada caused by wasp venom. *Habrobracon* venom however is prey-specific, is not proteolytic and appears to act as an 'insect curare' and therefore stands in contrast to *Platymeris* saliva.

A parallel to *Platymeris* saliva outside the Insecta is to be found in the venom of snakes, also a salivary secretion. In both, proteins act as toxins, both have enzymic activity, and cobra and viper venoms show a similarity in the number of proteins present. The fate of the vertebrate and arthropod prey, however, share less ground, since only in the vertebrate can the haemolytic and histamine shock reactions play an important part in paralysis. Rapid loss of conduction following intense central nervous activity, and general contraction followed by relaxation and inexcitability of muscle are the responses of the arthropod for which the proteins of the assassin bug's saliva are responsible.

Tobias (1955) has demonstrated that hyaluronidase, papain, chymotrypsin and collagenase have no effect on the conduction and trans-surface potentials of isolated lobster axons. Nor does trypsin influence the activity of the insect central nervous system; and, unlike *Platymeris* saliva, proteases are slow to lyse erythrocytes (Ballentine & Parpart, 1940). Protease will, however, cause contraction and depolarization of frog sartorius muscle (Tobias, 1955) and bring the cockroach heart to systolic standstill.

Phospholipases cause both depolarization of nerve, and contraction and depolarization of muscle, and Tobias concludes that the integrity of the phospholipid layers is indispensable for their normal functioning. Now, Richards & Cutkomp (1945) state that cobra venom, which contains phospholipase A, is 'quite toxic to insects' causing paralysis of the nervous system, but that injections of 'maximal doses of lysolecithin into the haemocoel of insects was without effect'. They observe that the lysolecithin may not have penetrated to the nerves, but conclude that the

lysolecithin is not toxic to insects. Cobra venom contains protease as well as phospholipase and this will have effected muscular paralysis. Further, Tobias has shown that lysolecithin acts on lobster axons, although the ionic environment influences the degree of activity. The action of phospholipase C shows that the formation of lysophosphatides is not necessary for damage to occur. Bee venom contains phospholipase and its action on the insect central nervous system resembles that of *Platymeris* saliva, but it is a complex mixture (Neumann & Haberman, 1956) and can yield no precise comparison with *Platymeris* saliva.

The toxic action of the saliva cannot depend on hyaluronidase and protease alone, though these will facilitate its entry into tissue. The absence of the commonly occurring venom components cholinesterase, ATP-ase and serotonin and the rapid lysis (particularly of erythrocytes and mitochondria) effected by the saliva suggest that the action depends on a phospholipid-disrupting enzyme. The phospholipolytic activity of the saliva was found to be weak compared with snake venom, but as Lovern (1955) comments: 'If in the tissues lipids are lightly bound into macromolecules, their physical and chemical properties, including their behaviour as enzyme substrates, are likely to be different from those of free lipids, e.g. as a result of orientation and of changes of solubility'.

The role of Platymeris *saliva in digestion*

The saliva injected into the prey produces a viscid fluid with lipid droplets dispersed through it. Proteins are digested externally, but lipid is not hydrolysed until the ingested material reaches the midgut where lipase activity rises after feeding has taken place. The protease activity of the gut wall is relatively low and does not appear to fluctuate with feeding. Since only tryptic digestion seems essential for protein absorption (Fisher, 1954) it is possible that the protein breakdown effected by the saliva during external digestion and subsequent storage in the capacious midgut crop of the assassin bug is sufficient to yield assimilable products.

SUMMARY

1. The responses of whole insects, selected organs, and tissues to treatment with the saliva of an assassin bug *Platymeris rhadamanthus* are described. The excitability of muscle and nerve is rapidly abolished.

2. In the general lysis that follows immobilization only cuticular and collagenous structures are spared. The disruption of lipid layers in the walls of nervous tissue is histologically demonstrable at an early stage.

3. The saliva contains at least six proteins, and lacks mucoprotein or other mucoid substance. Three proteolytic fractions were recognized after starch-gel electrophoresis at pH 8·6, one of them forming the major component of the saliva. Attempts to locate a toxic fraction were unsuccessful.

4. The alkaline endopeptidase activity of whole saliva characterized with an azocasein substrate closely resembles gut proteases of other insects examined with the same substrate.

5. Hyaluronidase is present in the saliva and with protease acts as a spreading factor by breaking down the intercellular matrix.

6. Lipase and esterase activity were not detected in the saliva, but gut-wall extracts were lipolytic.

7. The saliva shows weak phospholipase activity. ATP-ase, and serotonin were not detected.

8. The mode of action of assassin bug saliva as a venom and in external digestion is discussed. It is suggested that its toxicity is due to the disruption of phospholipid layers of the cell wall and is the first manifestation of general lysis during external digestion.

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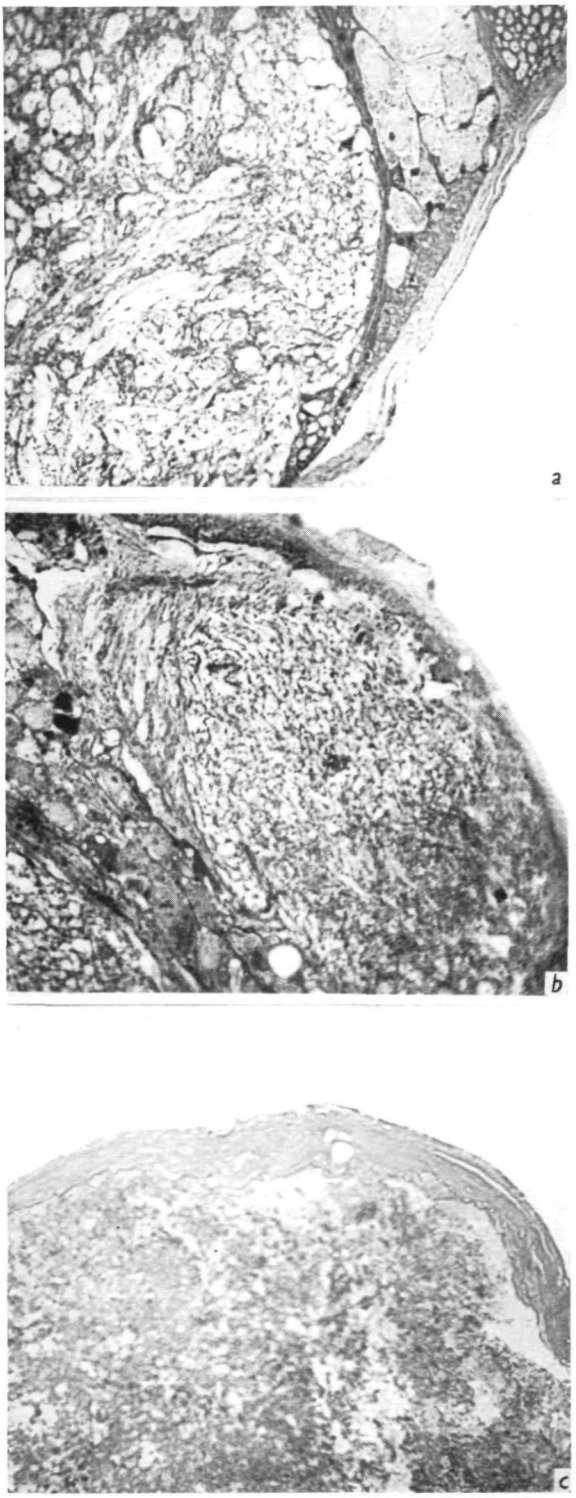

EDWARDS—THE ACTION AND COMPOSITION OF THE SALIVA OF AN ASSASSIN BUG *PLATYMERIS RHADAMANTHUS* GAERST. (HEMIPTERA, REDUVIIDAE)

(*Facing p.* 77)

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EXPLANATION OF PLATE

Stages in destruction of thoracic ganglion of *Oncopeltus fasciatus*. Transverse sections, 2 μ, osmic/gallate. *a*, 10 sec., histology normal. *b*, 10 min. lysis of outer axons visible, glial cells and inner regions intact. *c*, 30 min., lysis complete.