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39. CONSTITUENTS OF THE VENOM OF THE AUSTRALIAN BULL ANT, *MYRMECIA PYRIFORMIS*

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Preliminary studies (1, 2), on the venom of an Australian bull ant, *Myrmecia pyriformis* (previously identified as *M. forficata*) revealed the presence of high concentrations of histamine (1-3% by weight of the dried venom) and another substance possessing smooth muscle stimulating properties. This latter component, separated from the histamine by means of ascending paper chromatography causes a slow, persistent contraction of the mepyramine-treated guinea pig ileum and of the virgin rat uterus, and produces a prolonged hypotensive response in the anaesthetized cat, distinct from the rapidly reversible depressor response to histamine. More recently Cavill *et al.* (3) have reported in the venom of a related species of ant, *M. gulosa*, the presence of hyaluronidase activity and a direct haemolytic component, in addition to histamine and a smooth muscle stimulant resembling that described for *M. pyriformis*. They achieved partial separation of the components by means of low voltage paper electrophoresis although this technique did not permit separation of the hyaluronidase from the smooth muscle stimulant.

Subsequently, we have shown that the crude venom of *M. pyriformis* possesses hyaluronidase activity (determined turbidimetrically by the method of Tolkesdorf *et al.*) (4) and the ability to lyse washed red blood cells using a modification of the method described by Neumann, Habermann & Hansen (5). I mg of the crude venom was found to be equivalent to 100 IU of hyaluronidase (Rondase) and is therefore 10 times as potent as commercial testicular hyaluronidase on a weight basis. Activity could be demonstrated in 10 μg crude venom.

On account of the prolonged nature of the local responses in man to sting by *M. pyriformis*, an investigation was made into the possibility that the venom contains components which might act indirectly through the endogenous release of pharmacologically active substances such as histamine and bradykinin. The crude venom was tested for its ability to release histamine from isolated rat mast cells (6) according to the technique described by Rothschild (7), and its potency in this respect was compared with 48/80. Saline extracts of venom or solutions of 48/80 were incubated with cells from the peritoneal fluid of rats and after incubation the residual histamine content of the cells was assayed on the guinea pig ileum. Activity was expressed in terms of % release. As little as 10 μg of venom was found capable of releasing appreciable amounts of histamine and this dose was roughly equivalent to 2.5 μg 48/80. In some experiments activity was demonstrated by allowing live ants to release venom directly into the incubating fluid containing the mast cells. Although the method was not sufficiently sensitive to show any response from 1 sting, the venom of 10 insertions of a sting resulted in considerable histamine release.

The method used for the detection of bradykinin-releasing activity was based on that described by Margolis *et al.* (8). Saline extracts of the venom were incubated with bradykinin-free rabbit plasma in the presence of EDTA and the bradykinin-released was assayed on the rat duodenum. In some experiments, suspensions of human bradykininogen replaced the samples of rabbit plasma and in all experiments comparisons were made with trypsin. Concentrations of venom 30 times those capable of producing histamine release showed no evidence of bradykinin-releasing activity.

Further characterization of these pharmacologically active components has been attempted by the application of fractionation procedures, boiling and digestion with proteolytic enzymes. By these means it has been possible to conclude that the various activities can be attributed to separate substances, with the exception of the haemolytic component which it has not been possible to separate from the smooth muscle stimulant.

Ascending paper chromatography was carried out in water saturated butanol acetic acid (5:4:1), these conditions having been used to separate the histamine from the smooth muscle stimulant in the preliminary studies. Chromatograms were run for 24 hrs after which 1/2″ strips were eluted in saline and eluates submitted to tests for pharmacological and enzymic activity. Hyaluronidase activity disappeared altogether under these conditions, histamine was clearly separated from all other activities, but there was considerable overlap of the histamine releasing, smooth muscle stimulant and haemolytic components. However, a high percentage of the histamine releasing activity had a Rf value close to zero and it appears that a separate component is responsible for at least part of the histamine releasing activity of the venom. The partial separation of this factor from the other active components by means of low voltage paper electrophoresis at pH 6.24 again suggests that there may be two fractions contributing to histamine release, one of which is a distinct component and the other indistinguishable from the smooth muscle stimulant.

Electrophoresis was carried out at 4°C and a pH of 6.24 as described by Cavill *et al.* (3). A current of 0.3 mamps/cm was applied for 16 hours after which the paper was divided into 1″ strips, eluted in saline and tested for the various activities. Hyaluronidase and the smooth muscle stimulant could not be separated and in our experiments the haemolytic activity moved towards the cathode parallel to the smooth muscle stimulant. This is in contrast to the haemolytic component of *M. gulosa* which is reported to move towards the anode.

Dialysis through Visking cellophane membranes was carried out for 24 hours at 4°C with the ratio of inner to outer volume being 1:10. After dialysis, the volume of the dialysate was reduced 10 fold by freeze drying and redissolving in the appropriate amount of distilled water.

With the exception of histamine which was always 100% dialysable, the components were only slowly dialysable and their rates of dialysis were somewhat variable. However histamine releasing activity and the smooth muscle stimulant were always in detectable concentrations whereas hyaluronidase and haemolytic activities were sometimes less than 10% dialysed, and could not be detected. The behaviour of each component on chromatography, electrophoresis and dialysis is summarized in the Table I. Table II shows the percentage activity of each component remaining after digestion with trypsin, chymotrypsin and boiling for 10 minutes at pH 6.0.

TABLE I *

COMPONENT	PAPER CHROMATO-GRAPHY Rf value	ELECTRO-PHORESIS Rate of movement to Cathode: ins/16 hours	DIALYSIS % Dialysed in 24 hours
Histamine	0.22 (0.18 — 0.26)	5.25 (4.5 — 6.5)	90% (3)
Smooth muscle stimulant	0.14 (0.13 — 0.15)	1.3 (0.5 — 2.5)	13 — 30% (3)
Histamine release	0 — 0.15	2.25 (1.5 — 3.5)	36% (1)
Hyaluronidase	—	1.25 (0.5 — 2.5)	Undetectable < 8% (2)
Haemolytic	0.14 (0.11 — 0.17)	2.0 (1.5 — 2.5)	Undetectable < 12% (1)

* Figures in brackets refer to number of separate determinations.

TABLE II *

COMPONENT	BOILING pH 6.0 % Activity remaining	TRYPSIN DIGESTION % Activity remaining	CHYMOTRYPSIN DIGESTION % Activity remaining
Histamine	100% (3)	100% (3)	100% (1)
Smooth muscle stimulant	37% (3)	10% (4)	50% (2)
Histamine release	100% (2)	10 — 20% (4)	30 — 40% (2)
Hyaluronidase	3% (3)	20 — 67% (3)	—
Haemolytic	31% (2)	10% (1)	—

* Figures in brackets refer to number of separate determinations.

The various procedures outlined above shed light on some characteristics of the various components of the venom of *Myrmecia sp.* although detailed characterization of the constituents requires more satisfactory methods of fractionization. In many respects the venom resembles that of the bee and has constituents belonging to each of the three classes described by Habermann (9) for **HYMENOPTERA** venoms — namely biogenic amines, peptides and non-enzymic proteins, and enzymes, — typified by histamine, the smooth muscle stimulant and hyaluronidase respectively.

The smooth muscle stimulant fraction is slowly dialysable through Visking cellophane membranes and is only partially destroyed by boiling. However, almost all the activity is lost by incubation with trypsin. It contracts the smooth muscle of the guinea pig ileum, the virgin rat uterus and the blood vessels of the isolated rabbit ear. Tachyphylaxis occurs, but least markedly in the perfused rabbit ear and consequently this preparation, treated with mepyramine, has been used most frequently, doses being administered at intervals of 20 minutes. An example of this is shown in Fig. 1. Activity has been demonstrated with 10 μg of crude venom. There has been no clear-cut distinction between this factor and the haemolytic component and it is possible that the smooth muscle stimulant possesses haemolytic properties. It may also account for some of the histamine releasing activity.

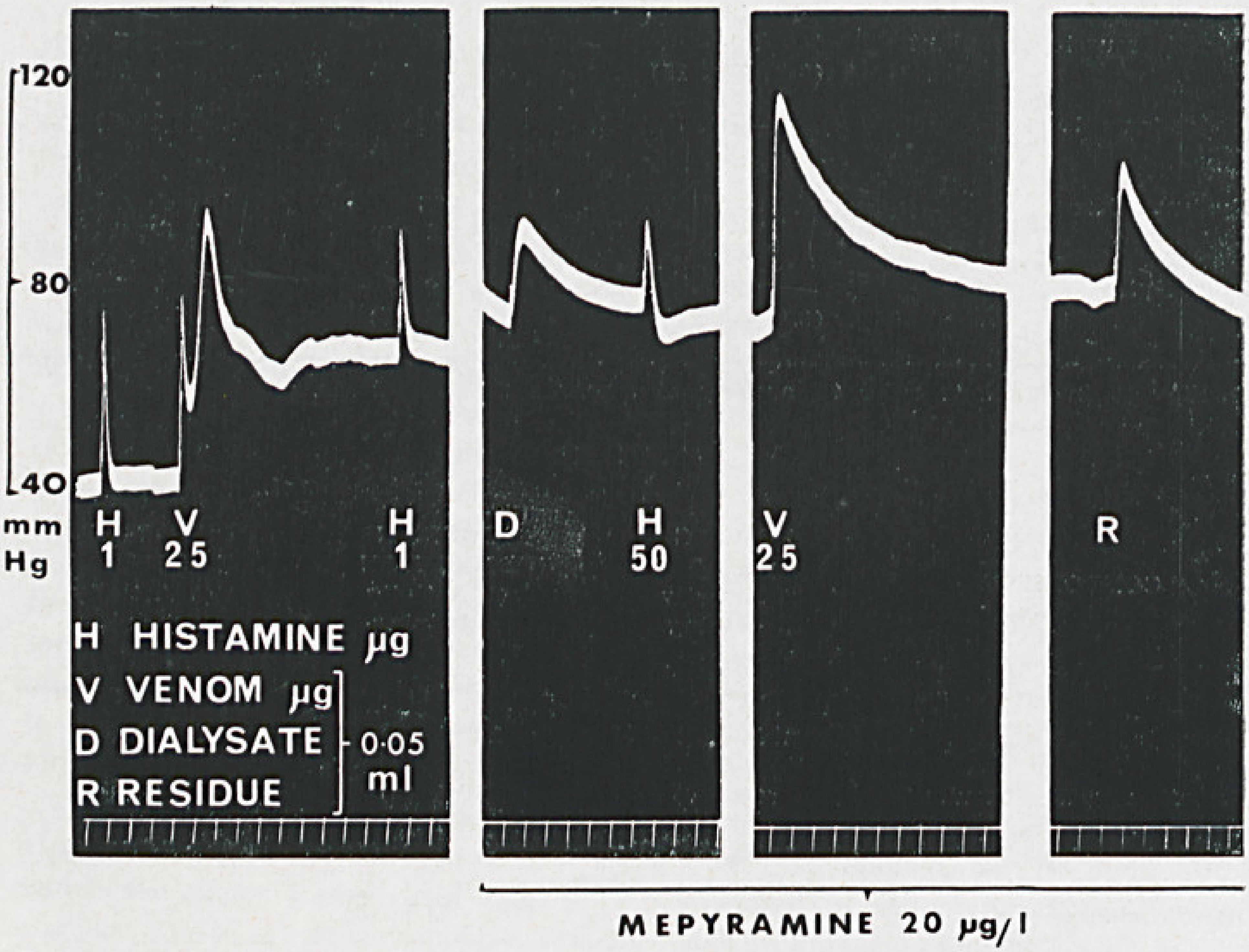

Fig. 1 — Isolated Rabbit Ear perfused with Kreb's solution at 37°C. Perfusion rate 7 mls/min. Constriction recorded by rise in perfusion pressure. Dialysate and residue derived from a 25μg equivalent of venom dialysed for 24 hours. Time scale — 1 minute.

Phospholipase A is widely distributed among insect and animal venoms and it is conceivable that the venom of *Myrmecia sp.* also contains this enzyme, and if this is so, it could contribute to the histamine releasing activity. In this conection attention is drawn to two properties of Phospholipase A — namely its heat stability and its ability to dialyse slowly, both of which apply to the histamine releasing activity of the ant venom. Phospholipase A and lysolecithin themselves possess various pharmacological activities including smooth muscle stimulation, a delayed and prolonged hypotensive effect and increased capillary permeability — all of which are properties possessed by the venom. Larger scale separation and attempts at preparation of relatively pure fractions are in progress, and it is hoped to embark on a more detailed analysis of the components, particularly the smooth muscle stimulant and the histamine releasing factor as soon as purer fractions become available.

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