## Insect Venoms: Identification of Dopamine and Noradrenaline in Wasp and Bee Stings

To the biogenic amines which provide much of the pain and poisonousness of the venoms of diverse animals[1] can be added the presence of dopamine and noradrenaline in higher hymenopteran venoms.

The venom reservoirs from adult worker honey bees (*Apis mellifera*) were dissected, freeze dried and treated with formaldehyde vapour[2], using the modifications to this technique that have been made in this laboratory[3]. When whole mounts of venom reservoirs, prepared in this way, were examined by darkfield fluorescence microscopy, an extremely bright green fluorescence was observed within the reservoir (Figure). This colour is characteristic of catecholamines in tissues treated by the formaldehyde method. Venom reservoirs of vespid wasps were also examined by this technique. However, the heavy muscular layers of the venom sac walls make them too opaque for fluorophores in the lumen to be seen. The wall of the honey bee venom sac, by contrast, is non-muscular and translucent.

Since catecholamines have not been previously reported in hymenopteran venoms, I sought evidence for the identity of the compound, or compounds, responsible for the green fluorescence. Venom reservoirs of honey bees, a yellow jacket species (*Vespula arenaria*) and the bald faced hornet (*Vespula maculata*) were dissected out.

Reservoirs were placed in 5 ml of ice cold 0.4 *M* perchloric acid containing 10 mg of ascorbic acid. Reservoirs were punctured to allow the release of their contents, shaken gently for 5 min and then removed from the perchloric acid extract. This extract was then carried through an extraction procedure which is specific for catecholamines[4]. Extracts were chromatographed on precoated cellulose plates ('Analtech', Cellulose G MN-300, 500 µm thickness) in the following solvent systems: Butanol (4 parts): Acetic acid (1 part): Water (5 parts)[5]. Butanol saturated with 3 *N* HCl[6]. Chromatograms were developed with a potassium ferricyanide, ethylenediamine mixture[7] and examined under UV-light.

The results showed that, in each solvent system, the extracts of the venom reservoir contents of each of the 3 species, produced spots which had identical chromatographic characteristics to known samples of noradrenaline and dopamine. Extracts to which standards were added before application to the chromatogram produced superimposed spots. Estimates, based on the minimal detectable quantity of pure noradrenaline and dopamine, suggested that in each species there was roughly 10 times as much dopamine as noradrenaline. It appeared that honey bee reservoirs contained about 1 µg of total catecholamine per reservoir and that each of the *Vespula spp.* contained about 100 ng per reservoir.

To confirm the identification of these catecholamines, and to obtain accurate measurements of the amount of each amine present, the extraction method[4] was combined with a recent spectrophotofluorometric method[8]. The extracted catecholamine sample, eluted from alumina with 0.05 *N* HCl, was split into 2 equal portions. Each portion was titrated against 0.1 *N* NaOH, one to an end point of pH 6.5 and the other to pH 7.0. The first of these portions was then oxidized to produce the hydroxyindole fluorophore of noradrenaline and the other to produce the dopamine fluorophore[8]. The results (read on a 'Farrand' spectrophotofluorometer) showed fluorophores to be present which had the emission and excitation maxima characteristic of the hydroxyindole fluorophores of dopamine (excitation maximum 320 nm, emission maximum 375 nm) and noradrenaline (excitation maxima 380 and 285 nm, emission maximum 480 nm). Comparison of extracts of honey bee and *Vespula arenaria* reservoirs with standards, carried through the extraction and development procedure, gave the values in ng shown in the Table for the amounts of each catecholamine present in a single venom reservoir


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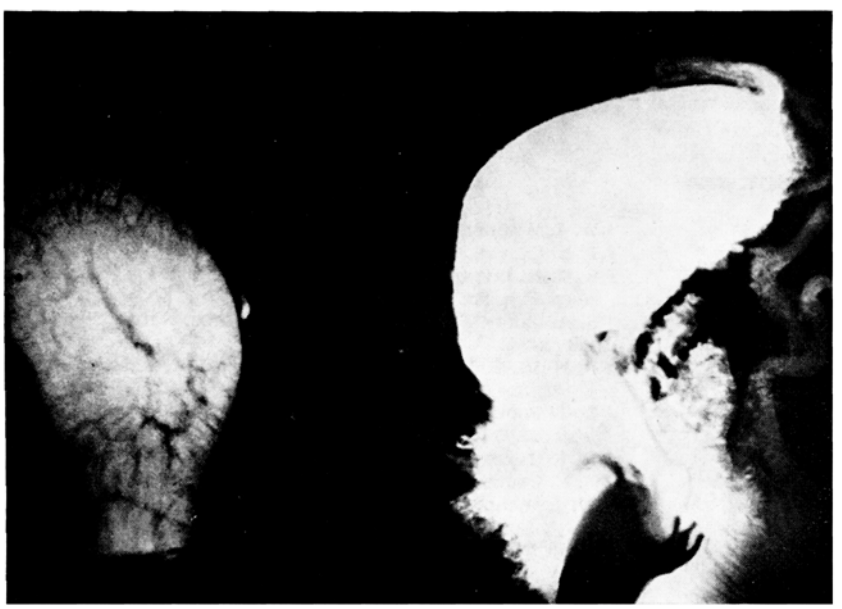

Fluorescence of a formaldehyde treated venom reservoir of *Apis mellifera* (right) compared to the autofluorescence of a control reservoir not exposed to formaldehyde (left). Photographed under darkfield fluorescent illumination (Leitz Ortholux microscope Heat Absorbing Filter KG2, Ultraviolet pass filter BG12, Barrier filter K510).

of each species. Although it is possible that some of the catecholamine extracted came from the tissue of the venom reservoir the extraction procedure was designed to minimize such extraction (reservoirs were opened by puncturing, rather than homogenization of whole reservoirs). It should also be noted that the catecholamine concentrations demonstrated represent a level of 1 mg/g in the honey bee and 100 µg/g in the wasp. These figures are even more significant when compared with the levels of catecholamines extractable from homogenized insect tissues which are of the order of 5–10 µg/g[9].

The failure of other investigators to note the presence of such significant amounts of catecholamines in these venoms probably lies in the utilization of dried, rather than fresh, venom extracts for chemical analysis and examination. This introduces the possibility of the decomposition of catecholamines to pharmacologically inactive derivatives. A commercial sample of crystalline bee venom (Calbiochem) was extracted and chromatographed with negative results.

In most hymenopterans the venom is used as a defense against other arthropods. It is therefore relevant to consider the physiological effect of 100 ng of dopamine injected into another insect. Dopamine has been reported to produce hyperactivity of the insect central nervous system[10] and to accelerate the heart beat rate[11]. I have found that the application of dopamine, to an isolated heart preparation of *Periplaneta americana*[12], in a concentration equivalent to the dilution of 100 ng in the haemolymph, produces a 2- to 3-fold increase in the rate of heart beat. Injection of 100 ng of dopamine in 1 ml of insect saline into intact cockroaches produced no hyperactivity, or other gross effect, but the heart beat rate (observed through the tergae) was markedly increased.

As an initial hypothesis it is therefore suggested that at least part of the significance of the dopamine content of vespid venoms lies in its ability to accelerate the circulation of the haemolymph, thus speeding the distribution of other chemical fractions of the venom to their sites of action[13].

*Zusammenfassung.* Die Anwesenheit von Dopamin und Noradrenalin im Bienen- und Wespengift wurde durch Fluoreszenzmikroskopie, Dünnschichtchromatographie und Spectrophotofluorimetrie nachgewiesen. Der Dopamin-Gehalt eines Stiches genügt, um die Herztätigkeit eines Insekts zu beschleunigen, was auch die Verteilung der giftigen Bestandteile zu den Wirkungsstellen beeinflussen kann.

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Amounts of catecholamines present in single honey bee and yellow jacket venom reservoirs

| | Dopamine (ng) | Noradrenaline (ng) |
|---|---|---|
| *Apis mellifera* | 775 ± 230 | 115 ± 20 |
| *Vespula arenaria* | 125 ± 10 | 4.0 ± 0.5 |

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## The Effect of Selective Cardiac Adrenergic β-Blockade on the Hypotensive Effect of Hydrallazine

Hydrallazine lowers the blood pressure most probably by a direct peripheral vasodilator action[1]. The fall in pressure causes a baroreceptor mediated sympathetic stimulation[2,3], which leads to an increase in cardiac output[4]. The increase in cardiac output must reduce the hypotensive effect. It might be expected that blockade of this reflex sympathetic stimulation would enhance the hypotensive effect of hydrallazine. However, a recent study showed that a combination of hydrallazine and propranolol was less effective in lowering blood pressure of the normal dog than hydrallazine alone. This led to the conclusion that peripheral β-adrenergic blockade antagonized the decrease in peripheral resistance caused by hydrallazine[4]. Thus, the hypothesis that a selective cardiac adrenergic β-receptor blockade might enhance the hypotensive effect of hydrallazine has been investigated. Practolol (Eraldin, ICI) was used to produce selective cardiac adrenergic β-receptor blockade[5].

*Materials and methods.* 9 mongrel dogs weighing 16 to 33 kg, were anaesthetized with thiopentone sodium (Pentothal, Abbott) 30 mg/kg i.v. supplemented with pentobarbitone sodium (Nembutal, Abbott) 2–4 mg/kg i.v. as needed during the experiment. A cuffed endotracheal tube was inserted and artificial ventilation with room air was maintained by a respirator (C. F. Palmer). Two polyethylene cannulas (O.D. 1 mm) filled with heparinized saline were inserted into the cephalic vein and advanced into the subclavian vein; one of these was used for anaesthetic administration and the second for hydrallazine and practolol administration. A third polyethylene cannula (O.D. 3 mm) inserted into the femoral artery and advanced into the abdominal aorta, and was connected to a transducer (Bell and Howell) and the blood pressure recorded on a 4-channel recorder (Devices). A constant electrocardiogram was obtained and the heart rate recorded by a ratemeter triggered by the

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