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PHARMACOLOGICALLY ACTIVE SUBSTANCES IN THE FAECES OF THE ORIENTAL WASP, *VESPA ORIENTALIS*, F.

BY

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During the last ten years a variety of pharmacologically active components has been found in the secretions of the poison glands of Hymenoptera (Jaques & Schachter, 1954; Schachter & Thain, 1954; Bhoola, Calle & Schachter, 1961). Similarly, much effort has been spent in elucidating the chemical constitution (Butler, 1961; Butler, Callow & Johnston, 1961), and the mechanism of action of the pheromones in queen secretions and in royal jelly, which can exert both growth-stimulating and growth-retarding effects (Verheijen-Voogd, 1959; Lüscher & Walker, 1963; Mitsui, Sagawa & Sano, 1964).

As far as we are aware, little attention has been paid to the possibility that glands other than the poison glands may secrete substances with specific pharmacological activities, although Butler *et al.* (1961) tested the pure pheromone from the maxillary gland of the queen bee on mammalian smooth muscle preparations and on whole animals. The substance was devoid of any activity in the systems examined. Nevertheless, it appeared to us that glands of the alimentary canal may produce active materials, resembling the active principles found in the corresponding organs of mammalians. In order to explore this possibility, we have started with social insects, which permit the collection of sufficient quantities of material. In this paper we report our observations on the faeces of *Vespa orientalis* F.

METHODS

Collection and extraction of faeces

Faeces of the oriental wasp were collected from vespiaries, fitted with sliding glass bottoms (Ishay, 1964). The bottom plates could be exchanged without interfering with the normal activities within the nest and without danger of attack by the wasps. The faeces had a sticky consistency and showed under the microscope the presence of amorphous material together with brownish, transparent plates. The faeces were kept in a vacuum desiccator and when dry were crushed in a mortar and subsequently stirred at room temperature with distilled water (4 ml./g) for 5 min. After centrifugation the brown supernatant fluid was decanted and extraction of the sediment was carried out twice. The second extract contained about one-half and the third about one-quarter of the active materials present in the first aqueous extract.

Dialysis against distilled water was carried out at 5° C in cellophane tubes of 1.2 cm diameter. Together with the active material, described below, a golden-yellow pigment diffused out. The dialysate showed an absorption maximum at 327 to 328 mμ.

Larval faeces were collected from the empty cells after emergence of the images. Wasp larvae have a closed midgut which opens towards the hindgut only shortly before pupation. The faeces, accumulating during larval life, are now ejected as a single lump that sticks to the roof of the cells, together with the

pupa. After the images had emerged, the dry blocks of faeces were collected, freed from adhering residues of the cocoon, crushed in a mortar, weighed and extracted as before. The weight of the faeces from a single larva was about 80 mg.

The queen may use the same cell two to three times for oviposition (Imms, 1957). In these circumstances a column of two to three lumps of faeces is found fixed to the roof of the cell (Fig. 1).

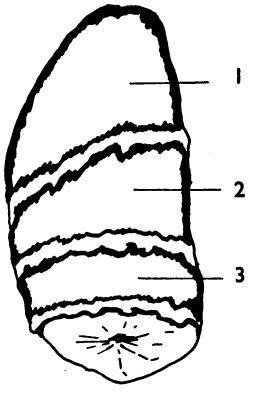

Fig. 1. Schematic drawing of a chain of three faecal blocks of larvae of *Vespa orientalis*. The upper face of each segment is convex and fits the concave lower surface of the foregoing block.

Activity tests

Guinea-pig ileum. Pieces of 3 cm length were suspended in 50 ml. of Ringer solution of the following composition (per l. of water): NaCl 9 g, KCl 0.42 g, and $CaCl_2$ 0.24 g. Before use, 0.5 g of sodium bicarbonate and 1 g of glucose were added. The organ-bath was kept at 37° C and was continuously aerated by a current of air. Contractions of the gut were magnified tenfold by a frontal lever and were recorded on a kymograph.

Blood pressure. A mercury manometer, connected to a cannula inserted in a carotid artery, served for registration of the arterial pressure of cats anaesthetized with sodium pentobarbitone (32 mg/kg, intraperitoneally).

Materials. Diphenhydramine (Benadryl) was a gift of Zori Pharmaceutical Laboratories, Tel-Aviv. Pronase, a *Streptomyces* peptidase, was purchased from the California Biochemical Corporation. Incubation with the enzyme was performed at 37° C, in 0.1 M-phosphate buffer of *p*H 7.0.

Paper chromatography. Whatman No. 1 paper sheets were employed. The chromatograms were developed by the descending method, using the following solvent: *n*-butanol: acetic acid: water (12 : 3 : 5, v/v). The spots were located by spraying with Pauly reagent (Block, Lestrange & Zweig, 1952).

RESULTS

Dual activity of the crude extract of wasp faeces

The aqueous extract of the wasp faeces produced a strong contraction of the guinea-pig ileum preparation. Usually the gut did not relax when exposed for 10 min or longer (Fig. 2,*b*). If acetylcholine was added at the end of the incubation period, its effect was strongly depressed (Fig. 2,*c*). After washing, the gut returned to its original length, but the antispasmodic effect was still present and full sensitivity to acetylcholine returned only after repeated changes of the bath fluid during 20 to 30 min (Fig. 2,*d* to *f*). The inhibitory effect increased slowly and reached its maximum after 7 to 8 min. Therefore, the antagonism against smooth muscle stimulants was always tested after 10 min contact.

The faecal extract was about as active against 5-hydroxytryptamine as against acetylcholine, but much less so against histamine (Fig. 3). Inhibition of histamine-induced contractions required at least four times more material than was needed for comparable depression of the acetylcholine- or 5-hydroxytryptamine-induced activity of the ileum.

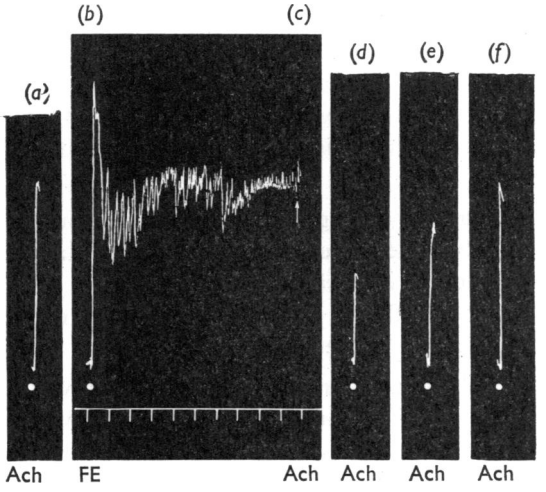

Fig. 2. Dual effect of the crude extract of wasp faeces on guinea-pig ileum. Acetylcholine (Ach), 4 ng/ml.; 0.5 ml. of faecal extract (FE), corresponding to 0.125 g of faeces. Time marks in minutes. Between sections, three washings. (*a*) Acetylcholine control. (*b*) Extract; note immediate contraction of the gut, followed by stabilization at a level of high muscular tone. After 10 min incubation, at (*c*) (arrow), addition of acetylcholine remains without effect. (*d*) After three washings, when the ileum has returned to its initial tone, again addition of acetylcholine. Note 50% reduction of the height of contraction, as compared to (*a*). (*e*) and (*f*) 6 and 12 min later, progressive recovery of original sensitivity of the gut to acetylcholine.

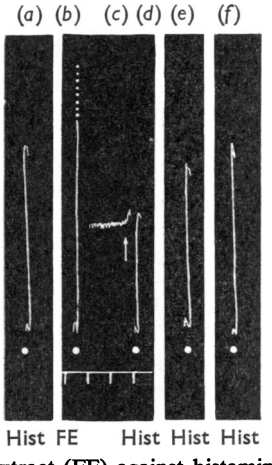

Fig. 3. Antagonistic effect of faecal extract (FE) against histamine-induced gut contraction. Histamine (Hist), 20 ng/ml. (*a*) Histamine control. (*b*) 2 ml. of faecal extract (four I50 doses for acetylcholine). The amplitude of the contraction was so large that the pointer came off the kymograph. After about 7 min incubation the lever returned to the height indicated. At (*c*) (arrow), without washing, addition of histamine remains without effect. (*d*) After three washings the histamine-induced contraction reaches only two-thirds of the control amplitude in (*a*). (*e*) and (*f*) Rapid recovery of sensitivity to histamine of the gut after 4 and 8 min, respectively. Time marks in minutes.

Separation of two active fractions from the faecal extract

Considering the possibility that the dual effect of the faecal extract might be due to the presence of two active substances, we first tried separation by solvent extraction. After the aqueous extract had been concentrated by lyophilization the residue was treated with cold methanol. After centrifugation the methanolic layer was brought to dryness by evaporation *in vacuo* and the solid was dissolved in water. The methanol-soluble portion induced only contraction of the gut and lacked any antagonistic effect against acetylcholine. The methanol-insoluble fraction was devoid of any kind of activity. The extraction procedure, therefore, had destroyed the antispasmodic substance completely. The same results were obtained with ethanol.

When the faecal extract was dialysed against distilled water, the gut-stimulating principle (E-factor) diffused out rapidly. The golden-yellow dialysate, obtained after 2 hr, caused the ileum to contract, but was devoid of inhibitory activity against acetylcholine. After 48 to 72 hr practically 100% of the E-factor was recovered in the dialysate. Thereafter, no further active material diffused out of the bag.

The brown liquid inside the bag retained the inhibitory activity (I-factor) of the crude extract, free of the excitatory compound. However, recovery was never complete. Usually only 50 to 70% of the original I-activity was found after dialysis.

Properties of the excitatory principle (E-factor) of the wasp faeces

The rate of contraction caused by the E-factor is comparable to that induced by acetylcholine or histamine, and much faster than the effect of bradykinin (Fig. 4). The contraction evoked by the E-factor was not modified by previous treatment with atropine, but was reduced by diphenhydramine in the same way as equiactive concentrations of histamine (Fig. 5). The similarity of the E-factor to histamine is further supported by tests on the anaesthetized cat (Fig. 6). Here, the E-factor produced an abrupt blood pressure fall similar to that of histamine, and in both instances the hypotensive action was abolished by

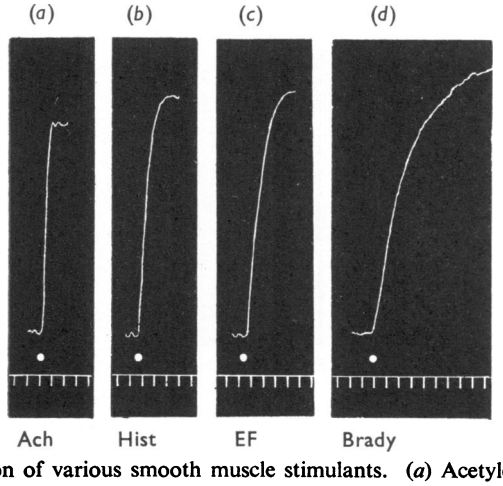

Fig. 4. Speed of contraction of various smooth muscle stimulants. (*a*) Acetylcholine (Ach), 10 ng/ml.; (*b*) histamine (Hist), 40 ng/ml.; (*c*) E-factor (EF), purified by dialysis, 0.5 ml.; (*d*) bradykinin (Brady), 20 ng/ml. Time marks 10 sec. Note that the speed of contraction in (*c*) is somewhat smaller than in (*b*), but considerably higher than in (*d*).

the same dose of diphenhydramine. The prompt responses to the E-factor made it improbable that an indirect action via a histamine-releasing mechanism was involved. The purified E-factor was subjected to paper chromatography. A spot, staining with the Pauly reagent and exhibiting the same R_F as histamine, was found. When this paper strip was

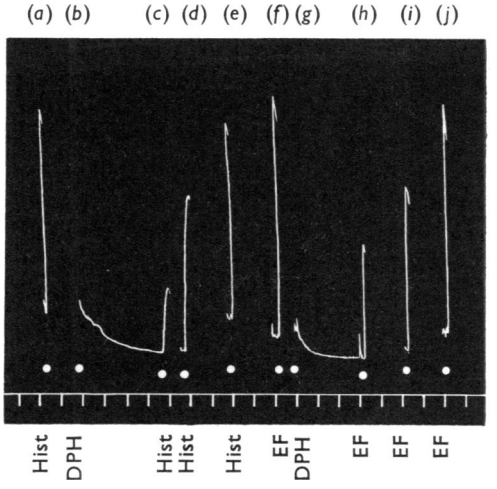

Fig. 5. Antagonism between diphenhydramine and E-factor of the wasp faeces. Histamine (Hist), 40 ng/ml.; diphenhydramine (DPH), 50 ng/ml.; E-factor (EF), 0.5 ml. in the organ-bath. Time marks in minutes. (*a*) Histamine control. (*b*) Diphenhydramine reduces the muscular tone ; after 4 min incubation, at (*c*), without washing, histamine. Height of contraction is reduced to 30%, as compared to (*a*). (*d*) and (*e*) Recovery of muscle tone and of sensitivity to histamine of the gut after 1 and 3 min respectively. (*f*) E-factor, control. (*g*) Diphenhydramine. (*h*) E-factor; the contractile effect is depressed by about 50%. (*i*) and (*j*) Recovery of muscular tone and of the susceptibility of the gut to the effect of the E-factor, after 2 and 4 min respectively.

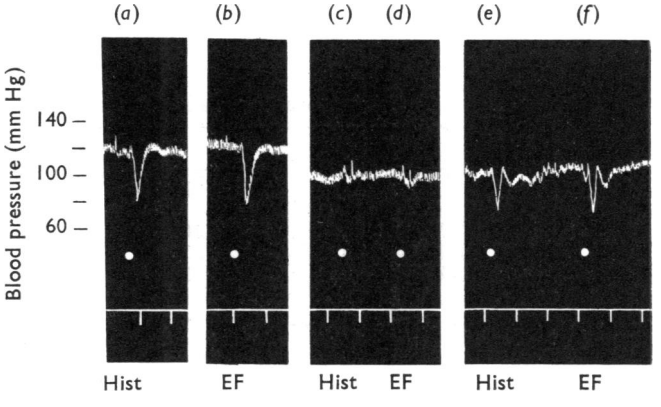

Fig. 6. Parallel effect of histamine and E-factor on the blood pressure of the anaesthetised cat. Female cat, 2.1 kg; narcosis was started with ether and maintained by intravenous injection of 20 mg/kg of pentobarbitone. At (*a*), (*c*) and (*e*), 0.4 μg/kg of histamine (Hist); at (*b*), (*d*) and (*f*), 0.1 ml. of purified E-factor (EF). (*a*) and (*b*) Controls. Between (*b*) and (*c*), injection of 0.5 μg/kg of diphenhydramine which lowered the blood pressure by about 20 mm Hg (not shown); 5 min later, at (*c*) and (*d*), repetition of the injection has practically no effect on blood pressure. Recovery of blood pressure response after 80 min at (*e*) and (*f*). Blood pressure at left in mm Hg; time marks in minutes.

extracted with water, the solution caused a strong contraction of the gut, an action that was abolished by diphenhydramine. Therefore, histamine is undoubtedly present in the E-factor. It was estimated that 1 g of faeces contained about 10 μg of histamine.

In the paper chromatogram two additional spots with a higher R_F were found, which reacted with the Pauly reagent but were ineffective on the guinea-pig ileum.

The inhibitory component (I-factor) of the faecal extract

The unit of the I-factor is defined by its I50-value, that is the amount, which when added to 50 ml. of bath fluid reduces to one-half the amplitude of an acetylcholine-induced contraction of about 7 cm. Like the crude extract, the purified I-fraction antagonized acetylcholine and 5-hydroxytryptamine to the same degree, but was only weakly active against histamine. We have also tested the antagonism of the isolated I-factor against the E-substance. In order to reduce the effect of the E-factor to one-half, about four I50-doses had to be added to the bath. Therefore, in this respect too, the E-factor resembles histamine.

The inhibitory component probably has a high molecular weight, since it does not diffuse through a cellophane membrane. This assumption is in accord with the following observations. (1) The faecal crude extract, when treated with pronase (50 mg/l.), lost its inhibitory activity almost entirely within 10 min. Therefore, the molecule of the I-factor is at least partly composed of peptides. (2) While the E-factor proved to be thermostable, the inhibitory component was slowly inactivated by heating to 50° C and quite rapidly at 80° C (Table 1). Activity was also easily lost by keeping the solution at room temperature or even in the refrigerator. Therefore, part of the decrease of the I-activity, encountered during dialysis in the cold room, may be ascribed to the marked thermal instability of the I-factor when in solution.

TABLE 1

EFFECT OF HEAT ON E- AND I-FACTORS OF THE FAECAL EXTRACT

The two factors were separated by dialysis and the outer solution, containing the E-factor, was concentrated until its activity was equivalent to about 1 μg/ml. of histamine. Both solutions were then kept in the same thermostat for the periods indicated and their activity was compared with that of the original solution

Incubation period (min)	Activity recovered at bath temperature (°C)					
	37		50		80	
	E	I	E	I	E	I
15	100	100	100	100	100	100
30	100	100	100	70	100	60
60	100	100	100	35	100	0

Active materials in larval faeces

When the larval faeces were collected soon after emergence of the images and extracted with water, the same two activities were detected as in adult faeces. Separation of the two components was achieved in the same way as described above. The E-factor was again identified with histamine. The I-factor of larval faeces proved to be thermolabile and was rapidly degraded by pronase. If the material proves to be chemically identical with the I-factor from adult faeces, an important additional source of this substance will be provided for further chemical and biochemical studies.

When collection of the larval faeces was delayed, only the E-factor was found in the aqueous extract, while the I-substance was missing. This accords with the greater sensitivity of the latter to a variety of external conditions, described above. It should, however, be noted that especially during the rainy period empty cells in the wasp nest are often heavily infested with fungi (*Trichotecium* and *Fusarium*) and bacteria (Ishay, 1965). This may be an additional reason for the occasional disappearance of the I-factor from larval faeces.

DISCUSSION

In the venom of *Vespa vulgaris*, a number of smooth muscle stimulants, such as histamine, 5-hydroxytryptamine and a slow-contracting substance, have been found (Jaques & Schachter, 1954). A similar array of active materials is present in the venom of the oriental wasp (Edery & Ishay, personal communication). However, antispasmodic substances have not been found in either secretion.

The faeces of *Vespa orientalis* contain two different components. One, the E-factor, has a low molecular weight, is thermostable, soluble in alcohol and not affected by this solvent, but its action is abolished by antihistamines. In all respects this factor is identical with histamine, while acetylcholine, 5-hydroxytryptamine and a kinin-like substance are absent.

The second component, the I-factor, has a high molecular weight and is inactivated by heat, denatured by alcohol and degraded by pronase. It cannot be excluded that this substance is a mixture of active materials. The I-factor has a rather specific spectrum of spasmolytic activity, as it antagonizes acetylcholine and 5-hydroxytryptamine about equally, but is much less effective against histamine and the E-factor.

The origin of the active materials of the wasp faeces is at present obscure. It is not impossible that the histamine is a product of the metabolism of the intestinal flora, since formation of this base by bacterial degradation of histidine is well-documented (Koslowski, Schneider & Heise, 1951; Nash, 1952). However, histamine is also present in the venom of the wasp, a clear indication that it may be produced by secretory cells of the insect itself.

The high molecular weight I-factor is presumably a specific product of the wasp organism. It may be secreted by the intestinal wall or by any of the secretory organs, associated with the alimentary canal, such as salivary glands or Malpighian tubes. In the vacuoles of the epithelial cells of the wasp Malpighian tubes, dark granules have been observed which are discharged into the lumen of the gut (Green, 1931).

In the larvae the Malpighian tubes open into the midgut. Therefore, the E- and I-factors in the larval faeces may originate from the same secretory organs as in adults, while production of the active materials by the hindgut is excluded. On the other hand, one cannot discard the possibility that they are not produced by the larvae themselves, but are ingested with the food furnished by the workers. In this case, the active materials may fulfil the function of a pheromone in the larval gut. If, in fact, the active substances in the larval faeces originate from the food supplied by the adults, one may be able to trace the origin of at least the I-factor to the salivary glands of the adult wasp. It should, however, be recalled that the reverse cycle, transfer of the I-factor from larvae to workers, is also possible, since wasps show the phenomenon of trophallaxis—that is, mutual food exchange (Imms, 1957). This problem is now being investigated.

It is unknown at present whether the pharmacologically active substances in the wasp faeces play any physiological role. We do not know whether they have any effect on the

intestinal wall of the wasp itself. In addition, the I-factor may exhibit other pharmacological activities, besides its influence on mammalian intestinal muscle. Clarification of the possible role of the I-factor in the insect organism will be possible when further supplies of the material become available.

SUMMARY

1. In the faeces of *Vespa orientalis*, two factors have been found which affect intestinal smooth muscle: an excitatory component, which has been identified as histamine, and an inhibitory substance.

2. The concentration of histamine was determined by biological assay as about 10 μg/g of faeces.

3. The inhibitory factor antagonizes acetylcholine and 5-hydroxytryptamine very effectively, but much less so histamine.

4. The two active components can be separated by dialysis, because the inhibitory factor does not diffuse through a cellophane membrane. The I-factor is inactivated by heat or by the enzyme pronase.

5. Likewise, in the larval faeces of the insect, histamine and an inhibitory substance are found. The origin of these materials and their possible role as physiological regulators is discussed.

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