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THE PRESENCE OF HISTAMINE, 5-HYDROXYTRYPTAMINE AND A POTENT, SLOW CONTRACTING SUBSTANCE IN WASP VENOM

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The demonstration that an alcoholic extract of sea anemone tentacles contains a potent histamine liberator (Jaques and Schachter, 1954) prompted us to test the possibility that wasp venom might also possess such activity. Saline extracts of wasp venom were accordingly studied. We found that only some wasp glands contained venom capable of releasing histamine from perfused cat skin preparations. The venom itself, however, regularly contained large amounts of histamine and also an extremely potent, unidentified constituent which produced a delayed, slow contraction of the guinea-pig ileum. This latter activity resembled that which we detected in cat plasma following intravenous injection of the histamine releasing fraction of anemone extract.

5-Hydroxytryptamine could not be detected in crude venom by the guinea-pig ileum because of the presence of two other potent smooth muscle stimulants. It was, however, identified as a constituent of the venom by paper chromatographic analysis; its presence in the eluate was subsequently confirmed pharmacologically by its action on the guinea-pig ileum. Histamine was also identified by these procedures.

We are greatly indebted to Dr. T. S. Work for carrying out the chromatographic analyses.

METHODS

The histamine assay and the perfusion of cat skin preparations were performed as described in the preceding paper (Jaques and Schachter, 1954). Histamine was used as acid phosphate, 5-hydroxytryptamine as creatinine sulphate, tryptamine as hydrochloride, atropine as sulphate, and mepyramine as maleate. Values for histamine and 5-hydroxytryptamine are expressed as base throughout.

Isolated rat colon preparations for testing the smooth muscle stimulating action of wasp venom were suspended in modified Locke solution, containing half the usual amount of glucose and a quarter of the usual amount of calcium; this solution reduces spontaneous activity of the isolated muscle (Gaddum, Peart, and Vogt, 1949). As reported by Dalgliesh, Toh, and Work (1953), this preparation was insensitive to 100 μg. histamine added to the bath, thus enabling us to study the non-histamine smooth muscle action of wasp venom without having to antagonize the action of histamine present in the venom.

Wasp Venom.—The common wasp (*Vespa vulgaris*) was used as a source of venom. The wasp was first immobilized in the cold, then pinned on its back and the ventral part of the three last abdominal segments cut away. The sting was then grasped with fine forceps and the entire venom apparatus, consisting of the venom sac and glands plus Dufour's alkaline gland, was gently lifted out of its bed by pulling on the sting. Care was taken to free the venom apparatus from other parts, such as fat body, or rectum, which sometimes adhered to it. The material so obtained from a large number of wasps was dried *in vacuo* over P_2O_5 and stored at 0° C. for future testing. The percentage of soluble venom in the dried venom apparatus was determined by weighing 100 such structures and subsequently grinding them in distilled water. The insoluble residue was dried *in vacuo* over P_2O_5 for 48 hours and weighed. It was thus possible to calculate the percentage of soluble material in the venom apparatus, and in two such analyses 27.0 and 30.4% respectively of the dry weight of the structure was soluble. For convenience, we therefore considered 30% of the dry weight of the apparatus to consist of soluble venom; throughout the investigation expressed weights of venom refer to 30% of the weight of the entire dry venom apparatus. The mean weight of a single dry apparatus was 0.63 mg.

In other instances these structures were dissected out, weighed fresh, dissolved in saline and the supernatant fluid tested.

Chromatography of Wasp Venom.—Dried venom apparatus weighing 115 mg.—and consisting of approximately 35 mg. venom—was dissolved in distilled

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water, and 5 mg. venom was used for chromatography on Whatman No. 3 paper, 18 × 22 in. The venom solution was spread along a line 10 cm. from one edge of the paper and approximately 30 cm. in length, and markers (25.0 μg. histamine and 25.0 μg. 5-hydroxytryptamine) were placed on spots outside the main line of venom. The sheet was irrigated overnight with the butanol–acetic acid mixture of Campbell, Work, and Mellanby (1951), dried and cut into five strips, the two outer strips containing the marker samples of 5-hydroxytryptamine and histamine and the other three strips (two narrow and one wide) containing the venom. Ehrlich's reagent (Dalgliesh, 1952) was sprayed on one of the marker strips and on one of the narrow venom strips; 0.1% ninhydrin was sprayed on the other marker strip and on the second narrow venom strip.

RESULTS

Pharmacological Identification of Histamine in Wasp Venom

The venom sac and glands only, of 15 wasps, were dissected out; each was separately weighed, ground in a glass tissue grinder in a measured quantity of saline and the venom solution assayed for histamine. It was evident that venom produced a contraction of the guinea-pig ileum only partially due to histamine since the contraction was unusually protracted and the ileum failed to relax on washing out the bath as readily as it does after histamine. This was substantiated by the demonstration that wasp venom caused a delayed, slow contraction of the guinea-pig ileum in the presence of mepyramine. However, by assaying maximal dilutions of wasp venom which still elicited an immediate contraction of the guinea-pig ileum, it was possible to assay activity which could be abolished by mepyramine. It was possible in this way to assay the histamine content of untreated venom. The results of the 15 assays are shown in Table I. The mean histamine concentration was 4.3 mg./g. fresh venom sac plus its glands.

In view of the possible influence of the slow contracting effect of untreated wasp venom on the assay of its histamine content, the histamine concentration was also determined by Code's (1937) modification of the method of Barsoum and Gaddum, which involves prior removal of protein with trichloroacetic acid and subsequent boiling of the filtrate in concentrated hydrochloric acid. This treatment removed all traces of the venom material producing the delayed slow contraction of the ileum; the histamine assay then presented no difficulties. This analysis was performed on a pool of 160 venom apparatuses which had been dried *in vacuo* over phosphorus pentoxide and stored at 0° C. The mean histamine concentration by this method was 4.8 mg./g. of dry venom apparatus. Since only approximately 30% of the dry apparatus consists of soluble material, the mean concentration of histamine in the dry venom itself is of the order of 16 mg./g. This, to our knowledge, is a far higher histamine concentration than hitherto reported in animal tissues or secretions.

TABLE I

HISTAMINE CONTENT OF SINGLE WASP VENOM SACS PLUS THEIR GLANDS (MG./G. WET WEIGHT)

Weight of Venom Sac + Glands (mg.)	Histamine Content (Total; μg.)	Histamine Content (mg./g.)
0·6	3·6	6·0
0·6	1·8	3·0
0·5	1·5	3·0
0·7	0·6	0·9
1·1	3·0	2·7
0·9	2·4	2·7
0·8	1·6	2·0
0·8	2·5	3·1
0·7	5·0	7·1
0·7	8·5	12·1
0·7	6·2	8·9
1·0	1·9	1·9
0·9	2·5	2·8
0·8	2·8	3·5
0·7	3·4	4·9
Mean 0·8	3·2	4·3

Unidentified Substance in Venom Producing a Delayed, Slow Contraction of the Guinea-pig Ileum

Wasp venom regularly produced a characteristic delayed, slow contraction of the guinea-pig ileum in the presence of atropine and mepyramine; it also persisted after tryptamine desensitization (Gaddum, 1953). There was a delay of approximately 10 seconds before contraction began, and contraction reached its maximum in 45–60 seconds. Subsequent relaxation on washing out was somewhat more prolonged than after a histamine contraction. These facts readily eliminate the possibility that this contraction is due to known smooth muscle stimulants such as acetylcholine, histamine or 5-hydroxytryptamine. Marked contractions were obtained with 5.0 μg. of venom and as little as 0.25 μg. could be detected. Since the bath volume was about 18 ml., venom is highly effective in producing this contraction in concentrations of 1 in 3 million, or less. The effect was reproducible—though not so precisely as with histamine—and graded contractions were obtained. It was necessary, however, to lengthen the interval between contractions to about four minutes, otherwise some refractoriness developed. Fig. 1 shows the effect of graded doses of venom (0.25–5.0 μg.) on the guinea-pig ileum in the presence of

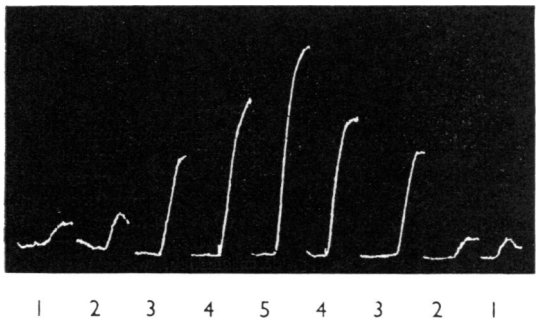

FIG. 1.—Effect of graded doses of wasp venom on the isolated guinea-pig ileum in an 18 ml. bath in the presence of atropine (0.2 μg.) and mepyramine (0·5 μg.). Contraction time, 50 seconds. Numbers 1 to 5 indicate contractions resulting from the addition to the bath of 0·25, 0·5, 1·25, 2·5 and 5·0 μg. wasp venom, respectively.

atropine and mepyramine. Fig. 2 shows the effect on a more sensitive ileum preparation of 5.0 μg. venom following tryptamine desensitization, in addition to the presence of atropine and mepyramine. Tryptamine desensitization greatly reduced the effect of 5-hydroxytryptamine whereas that of venom was unaffected. The delay in onset and the subsequent character of the contraction were similar to the slow contraction produced by cat plasma after intravenous injection of thalassine (Jaques and Schachter, 1954). The constituent of venom producing this contraction was little affected by heating venom at neutral *p*H in a boiling water bath for 5 minutes, but was destroyed after a few minutes of this procedure in 0.1N-NaOH. It was also completely eliminated by Code's method of histamine extraction. The venom regularly contracted the isolated rat colon though the sensitivity of this preparation was less than that of the guinea-pig ileum. Its action on this organ was little if at all affected by tryptamine desensitization indicating that this effect was not due to 5-hydroxytryptamine.

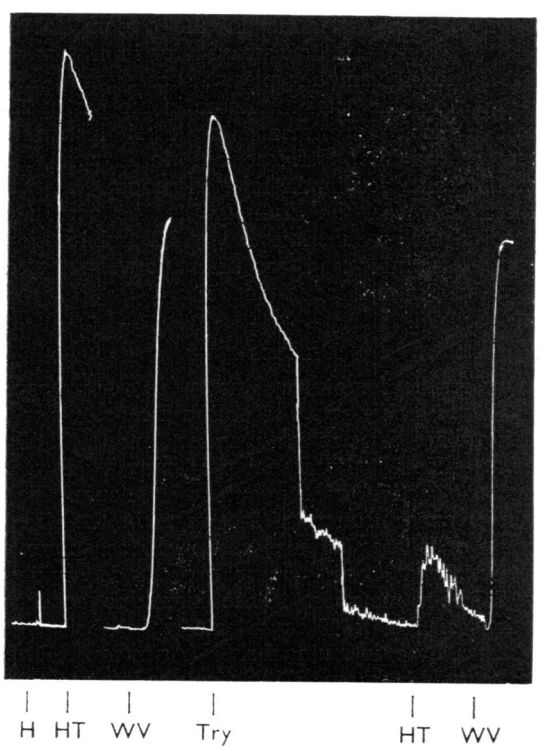

FIG. 2.—Effect of wasp venom on the isolated guinea-pig ileum after tryptamine desensitization. Atropine (0·2 μg.) and mepyramine (0·5 μg.) present in bath throughout. Continuous tracing indicates that solutions were not washed out between tests. Contraction time, 50 seconds. H, 1·0 μg. histamine; HT, 2·0 μg. 5-hydroxytryptamine; WV, 5·0 μg. wasp venom; Try, 200.0 μg. tryptamine.

Identification of Histamine and 5-Hydroxytryptamine in Wasp Venom by Paper Chromatography

The marker strip (see "Methods"), sprayed with Ehrlich's reagent (dimethylaminobenzaldehyde in hydrochloric acid) and dried in air, developed the single grey-blue spot characteristic of 5-hydroxytryptamine. The narrow strip from the chromatographed venom also developed a similar spot of the same Rf value and the same colour. No other coloured component was seen on this strip (for R_f values of 5-hydroxytryptamine and related compounds see Dalgliesh, Toh, and Work, 1953).

The marker strip sprayed with ninhydrin showed a single spot, close to the origin, corresponding to the known colour and R^f of histamine. The venom strip sprayed with ninhydrin showed a complex series of purple bands. The unsprayed paper containing the main bulk of the venom was cut into strips parallel to the origin and numbered from 1 to 5 so that, as far as possible, each strip corresponded to a single colour zone on the sprayed strips. Each strip was eluted with a mixture of 50% ethanol-water, the eluates taken to dryness and each redissolved in approximately 1 ml. of water.

The eluate from strip number 1, identified chromatographically as histamine, when tested on the guinea-pig ileum, contained a large amount of histamine so that 0.5 ml. of eluate diluted 1 in 1,000 was equivalent to 0.05 μg. histamine. The eluate activity was completely abolished by mepyramine. Since 5 mg. venom was used for the chromatogram this recovery gives a calculated value of approximately 20 mg. histamine per gram

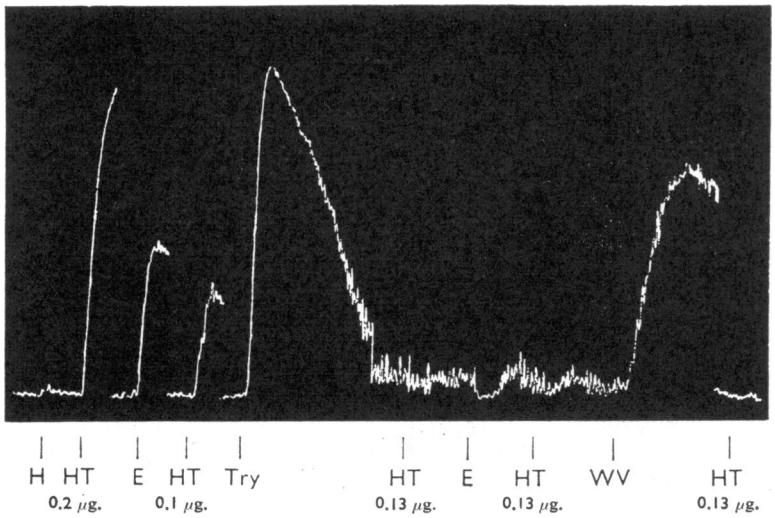

FIG. 3.—Effect on guinea-pig ileum of wasp venom paper chromatogram eluate with R_f value and colour reaction of 5-hydroxytryptamine. Atropine (0·2 μg.) and mepyramine (0·5 μg.) in bath throughout. The eluate is inactive after tryptamine desensitization, whereas untreated wasp venom is still effective. Continuous tracing indicates that solutions were not washed out between tests. H, 0·2 μg. histamine; HT, 5-hydroxytryptamine; E, 0·08 ml. eluate; Try, 200·0 μg. tryptamine; WV, 5·0 μg. wasp venom.

venom. This is in good agreement with the value of 16 mg./g. obtained by biological assay of the venom following Code's method of histamine extraction. It is greater than the histamine concentration (6 mg./g.) found in bee venom by Marcou and Derevici (1937).

The eluate of strip number 3, chromatographically identified as 5-hydroxytryptamine, possessed the pharmacological properties of this substance when tested on the guinea-pig ileum. Its activity was unaffected by mepyramine, but was abolished by tryptamine desensitization of the ileum (Fig. 3). The equivalent of 0.08 ml. of the eluate corresponded in activity to 0.13 μg. of 5-hydroxytryptamine, which yields a calculated value of approximately 0.32 mg. 5-hydroxytryptamine per gram venom. This value, like that of the histamine content of venom obtained from the chromatogram eluate, is a minimal one, since it is possible that some destruction occurs during the formation of the chromatogram and that elution is incomplete.

Histamine Liberating Activity of Wasp Venom

Four experiments were carried out on isolated perfused cat skin preparations to test the ability of wasp venom to release histamine. In each instance, several (2 to 8) venom sacs and their glands were weighed, ground in saline in a glass tissue grinder, centrifuged and the soluble material made up in saline so that 0.5 ml. contained the soluble material of the venom of one wasp. This volume of venom solution was injected intra-arterially into isolated skin in four experiments. Since the venom itself contained histamine, the histamine content of the perfusates was always increased; this was accompanied by a decreased rate of flow—probably largely due to the vascular action of the histamine in the venom. The injected venom was first assayed for its histamine content, and the amount injected subsequently subtracted from the total histamine in the perfusate. In two such experiments the total histamine recovered in the perfusate did not exceed the amount contained in the injected venom. In two others, however, the histamine in the perfusate exceeded the amount injected by 3.2 and 15.4 μg. respectively, indicating that histamine was released from the skin in these instances.

DISCUSSION

Our results demonstrate that wasp venom contains three highly active smooth muscle stimulants —histamine, 5-hydroxytryptamine, and a highly potent unidentified material producing a delayed, slow contraction of the guinea-pig ileum. The average concentration of histamine in the dried venom of a large number of wasps was approximately 16 and 20 mg./g. respectively, as determined by different methods. Since histamine concentrations of 1 in 1,000 cause pain when applied to a blister base in human skin (Armstrong, Dry, Keele, and Markham, 1953), it can be inferred that the injection of histamine by the wasp contributes to the pain and oedema following a wasp sting. Similarly, the minimal possible concentration of 5-hydroxytryptamine in venom was of the order of 0.32 mg./g., as determined by the assay of the eluate from a paper chromatogram. This concen-

tration would also be greater than the pain producing threshold concentration of this substance for human skin (Armstrong *et al.*, 1953). Although the high concentrations of histamine and 5-hydroxytryptamine can account for some features of the human skin reaction following a wasp sting, other factors are undoubtedly involved. For example, our experiments demonstrate the presence in venom of a highly potent substance which produces a delayed, slow contraction of the guinea-pig ileum; wasp venom is also able, occasionally, to release histamine. Furthermore, the venom contains large amounts of hyaluronidase (Jaques, R., unpublished) which would enhance the diffusion of toxic substances in the skin.

The widespread occurrence of histamine in animal tissues is well established. There is more recent evidence that 5-hydroxytryptamine may also be widely distributed in nature. It has been detected in the mucosa of mammalian gastrointestinal tract and in spleen (Erspamer, 1940; Erspamer and Asero, 1952); in blood serum (Rapport, Green, and Page, 1948; Rapport, 1949), where its source is the platelets (Rand and Reid, 1951); in the salivary glands of octopoda, and in the skin of amphibia (Erspamer and Boretti, 1951). Its presence in wasp venom further emphasizes its ubiquity.

In the preceding paper we have referred to descriptions, by previous workers and by ourselves, of the appearance in plasma of a smooth muscle stimulating substance which gives a characteristically slow contraction of the guinea-pig ileum; we suggested that different substances might produce this type of contraction. It is of interest, however, that the slow contractor in wasp venom has a similar action on the guinea-pig ileum to the substance which appears in cat plasma after injection of thalassine. Analysis of this constituent in wasp venom revealed several points of similarity to bradykinin (Rocha e Silva, Beraldo and Rosenfeld, 1949), the slow contracting substance obtained by incubating globulin with snake venom or trypsin, and which may be a polypeptide produced by proteolytic breakdown of globulin. The substance in wasp venom withstands boiling for long periods at neutral—though not at high—*p*H; contracts the rat colon—which is, however, less sensitive to it than the guinea-pig ileum—and is inactivated when the venom is treated by Code's method for histamine extraction. In this last, it is probably the boiling with concentrated hydrochloric acid that destroys the activity. All these properties have also been described by Rocha e Silva and his co-workers for bradykinin. The most effective purified bradykinin preparation they obtained produced strong contractions of the guinea-pig ileum in concentrations of 1 in 1–3,000,000. But crude, untreated wasp venom often produces a marked contraction in concentrations of 1 in 3,000,000 on a preparation rendered insensitive to acetylcholine, histamine and 5-hydroxytryptamine. Thus, crude wasp venom has the same order of potency as has a highly purified bradykinin preparation. The substance in crude venom producing this effect must be highly active. If, for example, it comprises 1% of the total wasp gland its potency must be as great as that of histamine.

It is well established that histamine is released in anaphylaxis. Recently, the release of bradykinin (Beraldo, 1950) and 5-hydroxytryptamine (Humphrey and Jaques, 1953) have also been demonstrated during this reaction. It is a curious fact that wasp venom contains large amounts of histamine, 5-hydroxytryptamine and a substance that resembles bradykinin.

Summary

1. Histamine, 5-hydroxytryptamine, and a potent unidentified smooth muscle stimulant were found in high concentrations in the venom of the common wasp.

2. Histamine was identified in untreated venom by tests on the isolated guinea-pig ileum; the histamine content of pooled wasp venom was approximately 16 mg./g. venom following Code's method of extraction. Histamine was also identified in venom by paper chromatography; bio-assay of the specific eluate gave a histamine content of approximately 20 mg./g.

3. 5-Hydroxytryptamine was identified in wasp venom by paper chromatography and the specific eluate found to be pharmacologically identical with this substance. Pooled wasp venom had a 5-hydroxytryptamine content of approximately 0.32 mg./g.

4. A potent, unidentified substance producing a delayed slow contraction of the guinea-pig ileum was also present in venom. The properties of this substance were similar to those described for bradykinin.

5. Wasp venom occasionally released histamine from perfused cat skin.

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