# 3

# Methods for the Collection of Venoms

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## I. INTRODUCTION

Despite the early recognition (see Chapter 1) of the dangerous nature of the venom of bees, wasps and ants, it was the end of the nineteenth century before venoms started to attract the attention of medical research workers. A prerequisite to studies on the nature of the venoms was the development of methods for their collection. Before collection of venom could be possible, the Hymenoptera must be collected in the field or reared in or near the laboratory.

Social Hymenoptera usually can be collected in the field in large quantities or reared near the laboratory (honey-bees). Solitary wasps must be one by one collected in the field or reared in the laboratory. A number of solitary wasps cannot be reared in the laboratory in large quantities, often because of complicated mating behaviour. For example, Simonthomas and Poorter (1972) studied the mating behaviour of the digger wasp *Philanthus triangulum* (Fig. 1) and found that the male must have a large territory, which could hardly be built in the laboratory. Since *P. triangulum* is used for the study of its venom (see Chapter 5) and mating failed in the laboratory, a large number of wasps have been collected in the field. This is possible when a population of this wasp reaches a large density, as has happened several times in the twentieth century in Europe, and recently also in Egypt (Simonthomas and Simonthomas, 1980).

**Fig. 1** Copulation in the male territory of *Philanthus triangulum* in the vicinity of Naboude, France. From Simonthomas and Simonthomas-Heymans (1973).

Some solitary wasp species can be reared in the laboratory. The first requisite is a large number of well-developed prey insects. Rearing is relatively easy for parasites of common insect pests of stored food products. Species of the genus *Microbracon*, wasps the venoms of which have been studied extensively (see Chapter 5), can be reared on several microlepidopteran larvae. The most successful method is mass rearing using the sandwich method, in which caterpillars are locked up between two pieces of lace, the cover of the wasp chamber (Subba Rao, 1955). A modified breeding apparatus is shown in Fig. 2. At the Department of Pharmacology of the University of Amsterdam, *Mircobracon hebetor* was reared for 20 years; during much of that time, production was 25,000 ± 1600 female wasps per 3 months (mean ± SEM $n = 46$) (R. T. Simonthomas, personal communication).

The first collection of honey-bee worker venom was performed by Langer

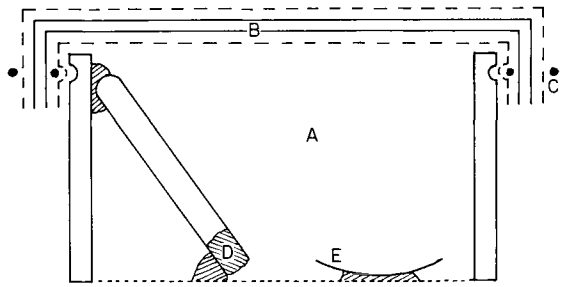


**Fig. 2** Rearing chamber for small wasps parasitizing microlepidopteran larvae. (A) Wasps' chamber, Perspex tube 60 mm long and 80–90 mm in diameter, with a stainless steel gauze bottom; (B) two layers of lace with two layers of cellulose in between (sandwich); the larvae are placed between the tissue sheets; the pieces of lace are fixed by rubber bands (C); a tube with water is closed by a piece of cotton-wool (D); the tube as well as a disk for honey (E) are fixed with plasticine. Modified after Subba Rao (1955).

(1897). Langer held a bee between two fingers and collected the clear drop of venom that appeared at the tip of the extended sting. He referred to the venom he collected in this way as 'genuine bee venom' and proceeded to describe its properties. At the beginning of the twentieth century this method was used for mass collection of venom. Langer (1897) also described a method for extracting venom reservoirs by manually pulling them out of the abdomen.

In addition to the 'milking' method and the manual removal of venom reservoirs, a number of newer methods for the mass production of hymenopteran venoms have been described.

These methods are based on a number of different appearances.

A. Extraction methods:
   1. Extraction from homogenates of whole insects.
   2. Extraction from homogenates of sting apparatuses which have been removed from
      a. Ethanol-fixed insects
      b. Deeply frozen insects
      c. Recently killed insects or from immobilized living insects

B. Collection of venom ejected through the sting as a result of:
   1. Mechanical stimulation
   2. Electrical stimulation
   3. Chemical stimulation
      a. With anaesthetics
      b. With alarm releasers

## II. BRIEF DESCRIPTIONS OF THE METHODS OF VENOM COLLECTION

### A. Extraction Methods

All extraction methods result in preparations in which the venom is contaminated with other compounds. Extracts of whole insect bodies are obviously contaminated with materials from all other body regions. Extraction of the venom containing parts of hymenopteran bodies produces solutions which contain venom from the venom reservoirs contaminated by extracts of venom reservoir tissues, venom glands, other accessory glands and possibly parts of the reproductive organs, the intestinal tract or the nerve cord (Fig. 3). Such impurities may not significantly affect the activity of the more potent venoms (e.g. *Microbracon* venom, which will induce deep paralysis when applied as very dilute solutions) (see Chapter 5), but in less potent venoms the activity may be masked by the presence of impurities. Purification with chemical methods is here indicated.

#### *1. Extraction of Homogenates of Whole Insects*

Homogenization of batches of whole insects has been used as a first step in the mass production of ant venom. The advantage of this method of collecting these venoms is that ant venoms contain volatile components (e.g. monocarboxylates) and odorous products (e.g. limoline) (Quilico *et al.*, 1960) which can be retained in extracts of whole bodies. The dolichoderine ants also produce terpenoid compounds (e.g. iridomyrmecine) (see Chapter 8) which are sprayed by the ants from the abdominal gland. These volatile substances can also be retained in distillates of whole ants (Cavill and Hinterberger, 1960). Extraction of whole female wasps has also been used in the mass production of venom from the wasp *Microbracon hebetor* (Visser *et al.*, 1976). The initial extract is very impure but provides a starting point for the purification and isolation of active principles (see Chapter 5).

Extracts of whole venom apparatuses of bees and wasps (*Apis mellifera, Polistes apachus, Vespula arenaria, V. pennsylvania*) contain several proteins not found in venoms obtained by electrical 'milking' (O'Connor *et al.*, 1964; Hsiang and Elliott, 1975). Unpurified extracts of homogenates of whole insects are obviously of very limited value in pharmacological studies.

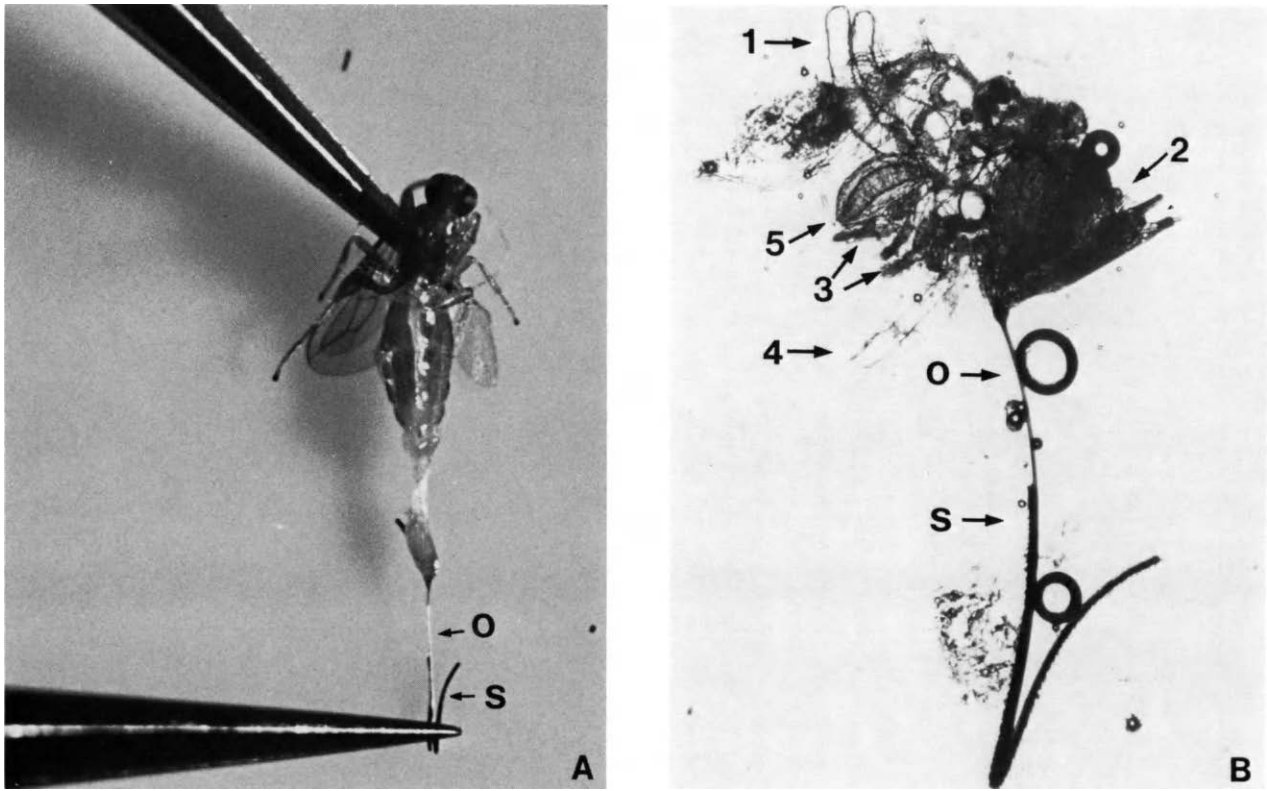


**Fig. 3** Removal of the venom apparatus of *Microbracon kirkpatricki* from specimens killed and stored at −20°C. (A) The extraction is performed by pulling the ovipositor (o) and its sheath (s). The venom apparatus is thus separated from the rest of the body. From Piek *et al.* (1982). (B) The venom organ immersed in water, seen under low magnification. (1) Accessory glands; (2) chitinous parts; (3) venom glands; (4) part of the ventral nerve; (5) venom reservoir. Photography by the courtesy of Dr. Jan van Marle.

**Table I**

Activities of the Solutions of Venom of *Philanthus triangulum*, Expressed in Bee Units[a] per 100 Wasps[b,c]

| Treatment of wasps and of venom solutions | B.U. per 100 wasps |
|---|---|
| Transported alive by car (~1000 km) | |
| On arrival killed at −25°C; extracted | |
| Venom solution tested immediately | 65,63 |
| Venom solution stored at −25°C and tested after 14 months | 25 |
| Venom solution stored at −25°C and tested after 23 months | 15 |
| Wasps kept in the laboratory for 1 week; extracted venom solutions tested immediately afterwards | 45,45 |
| Wasps kept for 3 weeks; extracted; tested immediately | 100,95 |
| Wasps stored on arrival at −25°C; extracted in the course of 3 months (tested immediately after extraction) | 55,40,40,60,35 |
| Transported alive by plane; on arrival killed at −25°C; extracted; tested immediately | 30,31 |
| Killed and transported at a temperature between −5 and −10°C; on arrival extracted (tested immediately) | 40,35 |
| Killed and transported at −196°C (liquid $N_2$); on arrival stored at −25°C; extracted in the course of 3 months (tested immediately) | 50,40,125,40,25,40 |

[a]The Bee Unit (B.U.) is defined as the activity of a *P. triangulum* venom preparation, injected in equal amounts into 10 honey-bee workers, that causes five of these bees to remain paralysed for at least 1 hr.

[b]See also Chapter 5.

[c]After Piek *et al.* (1971).

### 2. *Extraction of Homogenates of Sting Apparatuses Removed from Ethanol-fixed Insects*

Flury (1920) removed the venom apparatus from honey-bee workers fixed in ethanol. He dried and pulverized the venom apparatus before preparing an aqueous extract. The active principles from the extract were precipitated with ethanol. This method gave a low yield of active material.

### 3. *Extraction of Homogenates of Sting Apparatuses Removed from Deeply Frozen Insects*

The activity of venom from the digger wasp *Philanthus triangulum*, prepared by extracting homogenized sting apparatuses dissected from deeply

frozen specimens, has been compared with the activity of the venom collected by other methods (Piek *et al.*, 1971). Table I shows that the activity varies considerably between different samples. Despite this variation apparently little or no loss of the venom's paralysing activity occurs when wasps are immersed and transported in liquid nitrogen (Table I). This method may therefore be appropriate when Hymenoptera must be collected and killed in locations away from the laboratory. Liquid nitrogen refrigerators (for example, Type LR-10, Union Carbide Corp., Indianapolis, United States of America), are now available, which, at normal use, lose about 0.2 litre of nitrogen per day; that is, the lifetime of a 10-litre refrigerator is about 50 days. In the laboratory the insects can be stored in liquid nitrogen or can be transferred into an electrical refrigerator. However, storage of wasps (*P. triangulum*) at $-25°C$ resulted in a decrease in venom activity with increasing storage times (Table I).

After thawing, the venom apparatuses or the venom reservoirs can be removed from the body, as, for example, has been described for *Philanthus triangulum* (Piek *et al.*, 1971) and for *Paravespula* species (Ishay *et al.*, 1973; Klein *et al.*, 1981).

## 4. Extraction of Homogenates of Sting Apparatuses or Venom Reservoirs from Living Immobilized Insects or from Recently Killed Insects

Insects are easily immobilized by carbon dioxide or a mixture of carbon dioxide and oxygen (5–15%). Carbon dioxide sometimes caused wasps to eject some of their venom. This problem can be avoided by chilling the wasps to 2 to 4°C in a refrigerator before exposing them to the carbon dioxide. Some caution is needed in the use of carbon dioxide to immobilize insects which are to be used in long-term experiments. Ribbands (1953) has reviewed, for example, the long-term consequences of carbon dioxide immobilization of honey-bees (*Apis mellifera*). One of the effects he discussed is that bees immobilized with carbon dioxide soon after eclosion failed to collect pollen later in their lives. Thus carbon dioxide can induce an abnormal behaviour, which might affect the composition of the venom produced.

Hymenoptera are easily killed, without loss of venom, by placing them in a refrigerator at or below $-20°C$. Venom apparatuses may then be removed from thawed bodies.

Langer's (1897) preparation of bee venom was an extract made from freshly removed venom reservoirs. The venom apparatuses of honey-bees and wasps can be easily removed from the abdomen by holding the sting with forceps and gently pulling it, with the other parts of the venom system attached, out of the abdomen. The venom reservoir can then be cut free and transferred to an appropriate medium for extraction.

Starting with bees or wasps frozen at −20°C, Shulman *et al.* (1964) pulled the stings, along with the stinging apparatus, with a pair of fine forceps. The stinging apparatuses were quickly frozen on a glass slide placed on dry ice. The venom reservoir was removed from the stinging apparatus; contamination with other parts was avoided as far as possible.

Cavill *et al.* (1964) have described the preparation of isolated venom reservoirs of the bull ant (*Myrmecia gulosa*). They obtained a yield of ~0.3 mg of dry venom per reservoir, which is ~0.35 percent of the body weight of the insect. Lewis and de la Lande (1967) prepared isolated venom reservoirs of *M. pyriformis*. They stored living ants at 4°C and cut the abdomen from the body at a later time. They then carefully dissected the sting apparatus away from adjacent tissues and separated the reservoirs from the rest of the stinging apparatuses. They obtained ~0.4 mg of dry venom reservoir preparation from each ant and prepared aqueous or saline extracts of this dried crude venom.

A special problem is the collection of venom from those Hymenoptera which do not have a venom reservoir. We were not able to find such a reservoir in the sphecid wasp *Ampulex compressa* (see Chapter 5, Figs. 22–25). The absence of the reservoir has been confirmed by histological investigation (see Chapter 2). In such cases pure venom can only be collected from the tip of the sting (Section II,B).

## B. Collection of Venom Ejected through the Sting

The extraction of complete or incomplete venom apparatuses produces venom which is contaminated with constituents originating from the tissues and not belonging to the normal venom. Native venom (where native is defined as a venom identical with that normally injected into an enemy or prey) may be collected from the tip of the sting. Langer (1897) called the bee venom produced in this way 'genuine bee venom'. However, venom collected in this way may differ from the native venom by contamination with enteric discharge or other products released by the insect.

### 1. *Collection of Venom Droplets that Appear at the Tip of the Sting of Killed Hymenoptera*

After Hymenoptera stored at low temperature are thawed, in some cases small droplets of venom appear at the tip of the sting at thawing. Piek *et al.* (1983) collected from the tip of the stings of *Bombus terrestris*, just thawed from liquid nitrogen store, droplets of venom that showed a biological activity of about 1.5% of that of the whole venom reservoir. Although the yield was

poor, these authors argued that the fact that both a cholinergic and a slow-contracting factor were present in extracts of venom reservoirs, as well as in the above-described droplets, indicates that both factors are components of the venom itself.

All subsequently described methods were carried out with living Hymenoptera.

## 2. Collection of Venom Ejected through the Sting following Mechanical Stimulation

Langer's technique, described in the introduction to this chapter, is not difficult. The method has been applied to the collection of venom from wasps and ants. Blum *et al.* (1958) collected venom from major workers of the fire ant, *Solenopsis saevissima*, in the field. They held the ants by the petiole with forceps and stroked the tip of the abdomen with a fine capillary until the sting was protruded. Droplets of venom, issuing from the tip of the sting, were collected in the capillary. This procedure is most easily performed with the aid of a dissecting microscope at low magnification.

## 3. Collection of Venom Ejected through the Sting following Electrical Stimulation

Markovič and Molnár (1954) were the first to use electric shocks to obtain venom from honey-bees, although this method had been used earlier to collect spider venom (Baerg, 1938). Weide (1958) described a device, consisting of a small beehive fitted with a number of parallel wires alternatively coupled to an alternating current source, for the collection of honey-bee venom. The wires were covered with wet filter paper. When the current was switched on, the bees stopped walking, contracted their abdomens, protruded their stings and ejected venom. This method was also used by Palmer (1961), who removed three frames with bees from a hive, stimulated the bees to sting and then returned the bees without notably damaging the colony.

The electric 'milking' equipment has been modified in various ways. The stimulating voltage has been made variable; agar gel sheets have been tested in place of filter paper over the wires. As these gels are aqueous, current was carried by stainless steel wires into the gel itself. Bees standing on the gel reacted to applied current by stinging and injecting venom into the gel. A later modification is the replacement of the paper or gel by a thin sheet of inert silicone. Bees sting through the sheet and venom appears on the lower side of the sheet as globules which dry in the atmosphere, usually within 20 min (Palmer, 1961).

Benton *et al.* (1963) and Benton (1965) described a device for shocking bees electrically which fits in a standard hive, making it possible to collect venom from several thousand honey-bees. The device consists of a wooden frame with copper or steel wires stretched across it at $\frac{1}{8}$-inch (3.18-mm) intervals. Alternate wires carry an electric charge, compared with the ground ones. The circuit is completed when a bee comes into contact with two adjacent wires. The bees sting through a piece of nylon sheet which is stretched over a glass plate and is fitted under the wires. The collecting apparatus is placed underneath the brood chamber of a colony of bees and may be moved from hive to hive. Each colony is 'milked' for 5 minutes using a 12-V wet-cell battery in conjunction with a converter (12 V dc to 115 V ac). An electrical timer is used to break the circuit for 4 sec at 3-sec intervals. An average of 20 beehives must be milked in this way to obtain 1 g of venom. The method introduced an unexpected problem. Benton *et al.* (1963) reported that they had to dry the nylon sheet for a few minutes before inserting it into the next hive. If this was not done the bees became extremely irritable and the operator might be severely stung. Morse and Benton (1964) encountered unusually aggressive bees as far as several hundreds yards from recently 'milked' colonies. Apparently, the large quantity of alarm pheromone released in the area stimulates bees to attack intruders in the area of the apiary (see Section II,B,5).

Morse *et al.* (1967) tried to modify the method to collect venom from *Apis dorsata*, which cannot be kept in beehives. The collecting apparatus was placed under the nest and the bees brushed on to it. Only a few bees responded by stinging. Gillaspy and Grant (1979) described an apparatus for collecting venom from *Polistes* wasps by electrical stimulation. The venom is collected from a glass plate covered with thin plastic through which stings must penetrate to deposit venom on the glass, thus protected for contamination. About 8000 wasps had been 'milked' and 0.7 g of venom collected.

Piek (1966) described a modification of the mass-collecting device of Benton *et al.* (1963) to 'milk' smaller wasps electrically. It consists of a plastic box with the dimensions 40 × 40 × 10 mm (Fig. 4). The bottom of the box is covered with filter paper. Directly above the paper, a number of stainless-steel wires are placed about 1 mm apart. The wires are connected alternatively to the positive and negative poles of a stimulator. In the practical application of this apparatus about 300 female wasps (*Microbracon hebetor*) were placed in the box. The wasps were stimulated by a current of up to 0.3 mA, applied for 1 sec at 3-sec intervals, which caused them to eject venom. Most of the 'released' venom was absorbed by the filter paper. The pieces of filter paper, containing the venom contaminated with faeces, were extracted with either water or an insect saline. However, this protein venom in particular was

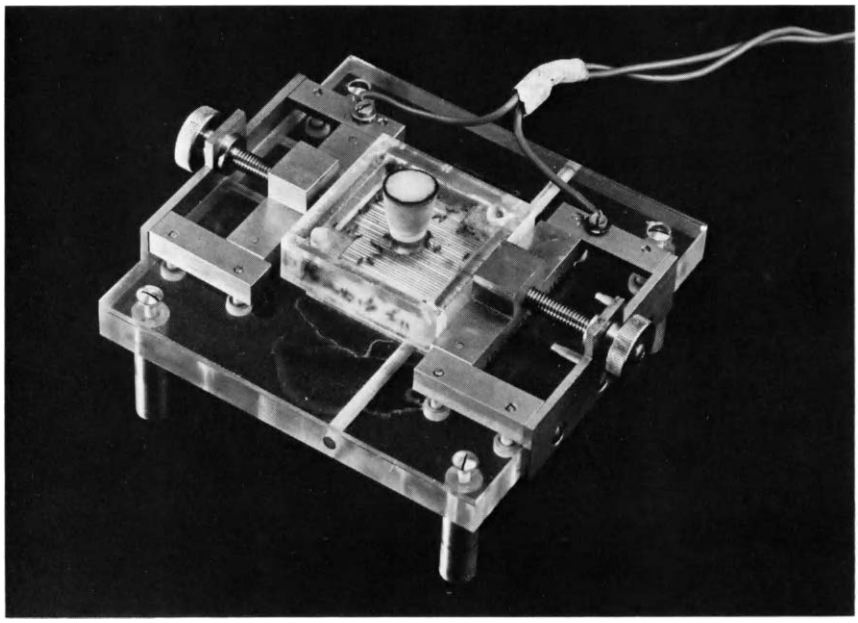

**Fig. 4** Apparatus for the collection of venom from the wasp *Microbracon hebetor* by electrical stimulation. From Piek (1966).

absorbed with such a high affinity that extraction resulted in a considerable loss of activity.

The mass production methods described for venom collection from bees and wasps do not seem to be applicable to the ant *Myrmecia gulosa* (Cavill and Robertson, 1965).

Mitchell *et al.* (1961) developed a 'milking' method for individual honeybees. Bees were held in small, bottomless, platinum cups so that the tip of the abdomen projected through the bottom of the cup and the wings were spread over the upper lips. A platinum halo fits over each bee's head to hold the insect in place. Bees were immobilized with carbon dioxide, placed in the cups and allowed to revive. 'Milking' was achieved by an electric current applied through connections to the bee cups and the halo assemblies. Each shock (one per minute) produced a droplet of venom at the tip of the bee's sting. A maximum of three droplets per bee were collected in water, dried and then kept under refrigeration. O'Connor *et al.* (1963) modified Mitchell's method for venom collection from bees and wasps. A mounted wasp was supported by a clamp directly beneath a nichrome wire lead from a spark

coil (10,000 V). When the insect began to revive from the $CO_2$ treatment, it was stimulated until it secreted venom. The collected venom was dried *in vacuo* over $P_2O_5$ and could then be stored at 0°C for several months without loss of activity. Gałuszka (1972) measured the diameter of venom reservoirs of honey-bee workers which were electrically stimulated. After 20 shocks the diameter was decreased to about 20% of the control value (Fig. 5). The content may be decreased roughly to the square of this percentage. Gałuszka (1972) also measured the production of venom by one bee after 10 shocks and found that the dry weight was 0.011 mg.

### 4. *Collection of Venom Ejected through the Sting by Chemical Stimulation with Anaesthetics*

General anaesthetics usually cause an initial excitation in vertebrates. Excitation can also be observed in insects treated with these anaesthetics. The term anaesthetic may not be strictly appropriate in this context but will be used for substances with a common application in vertebrate anaesthesia.

Flury (1920) collected a swarm of bees in a 10–20 litre bottle and introduced some diethyl ether vapour. Afterwards, the inside of the bottle and the bees were washed with water. The aqueous extract was dried, leaving a syrupy residue. This crude venom preparation retained a high level of activity for at least 2 months.

This method has been modified for the collection of venom from the wasp *Microbracon hebetor* by Drenth (1974). In this modification liquid trilene (trichloroethylene) is used as an anaesthetic. Trilene (10 ml, bp 87°C) is placed in a 500-ml flask in a water bath at 72°C (Fig. 6). Air (10 litres/hr) is passed over the fluid and then through a tube (50 mm long, 25 mm diameter) fitted with a glass filter (Micro glass filter G3) and containing several hundred female wasps. The wasps are excited by the trilene vapour and eject venom, probably mixed with faeces. Stimulation was continued for 5 min; the wasps were then exposed to a stream of air for 30 to 60 min before being stimulated again for a further 5 min with trilene vapour. The 'milked' wasps were stored at −20°C, the empty tube was filled again with wasps, and the procedure was repeated until a total of about 5000 wasps has been 'milked' in the same tube. The frozen wasps were then suspended in 10 ml of ice-cold distilled water and shaken gently for 1 hr. The water was then drawn through the filter and the tube, still containing the wasps, was rinsed three times with 2 ml ice-cold distilled water. The filtrate was dried *in vacuo* over $P_2O_5$ at 4°C and stored at −20°C. Drenth (1974) found that filtration through a millipore filter resulted in a loss of about 50% of the activity of this extract. The stablility of the remaining filtrate, however, seemed to be improved.

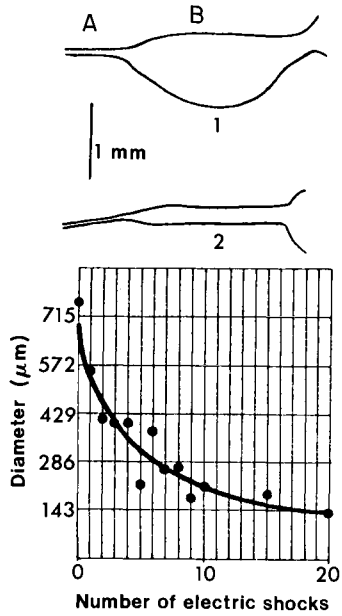


**Fig. 5** Effect of electrically milking honey-bee workers on the size of the venom gland (A) and the venom reservoir (B). Top: glands and reservoirs of 12-day-old bees; 1, control; 2, after 20 electric shocks. Bottom: diameter (in μm) of reservoirs as a function of the number of electric shocks applied to the bees. From Gałuszka (1972).

## 5. *Collection of Venom Ejected through the Sting as a Result of Stimulation with Alarm Releaser Chemicals*

Butler (1609) in his *Feminine Monarchie* wrote, 'When you are stung, or any in the company, yea though a Bee have striké but your clothes, specially in hot wether, you were best be packing as fast as you can; for the other Bees smelling the rancke savour of the poison cast out with the sting will come about you as thicke as haile.'

Huber (1814) added to Butler's observation when he commented on the way in which bees were irritated by the smell of an isolated sting. Beekeepers can confirm that the odour around a recent sting stimulates further attacks. This response of the honey-bee is a fundamental part of the defence of the bee community against its larger enemies (Ribbands, 1953).

In recent years a number of workers have identified chemicals that communicate alarm among members of hymenopteran colonies. Social hymenopteran alarm substances are always associated with defencive organs or glands. The originate either from the venom gland or from distinct alarm

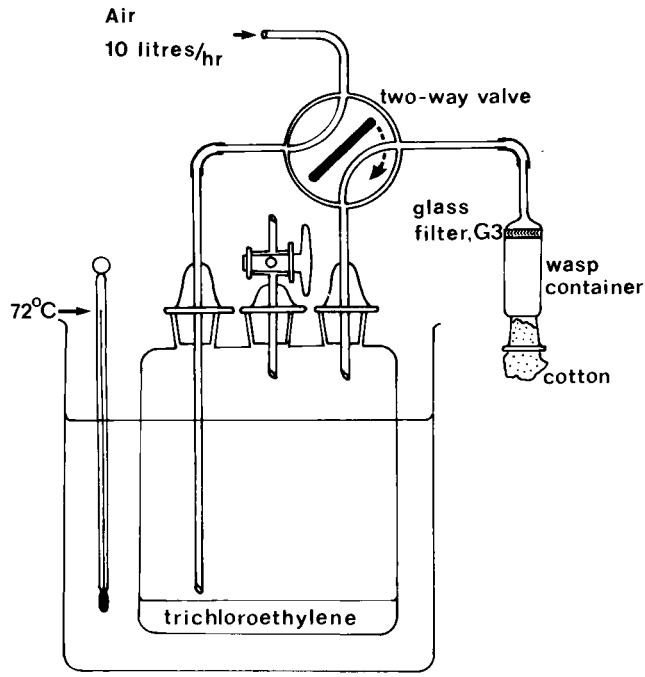


**Fig. 6** Equipment for the collection of venom from the wasp *Microbracon hebetor*, using trilene, according to the method described by Drenth (1974).

substance-secreting glands (e.g. *Apis mellifera*) (Ghent and Gary, 1962). In the former case they may be identical with the venom (formic acid in *Formica* sp., ketones in Dolichoderinae), or may be volatile materials mixed with the venom (in *Vespa* and in Myrmicinae) (Maschwitz, 1964a,b). Multicomponent alarm pheromones from the African weaver ant (*Oecophylla longinoda*) have been described by Bradshaw *et al.* (1975). Excited male workers give out two alarm releasers, one originating from the mandibular glands, the other being a mixture of formic acid from the venom reservoir and *n*-undecane from Dufour's gland (see Chapter 2). (For alarm pheromones of ants see also Chapter 9, Section V,A,6.)

Blum (1969) lists 20 identified alarm pheromones, of which 19 come from the Hymenoptera. Such alarm substances can be considered to be pheromones. In the Apidae the alarm pheromones may be iso-amyl acetate or iso-pentyl acetate. Citral, 2-heptanone and 2-nonanone have also been identified in bees (see the reviews by Blum, 1969; Pain, 1971). Boch *et al.*

(1962) identified the active alarm material of *Apis mellifera* as iso-amyl acetate. Morse and Benton (1964) and Morse *et al.* (1967) found that species of *A. mellifera*, *A. indica*, *A. dorsata* and *A. florea* secrete iso-pentyl acetate as an alarm substance. Even the docile bee species (*A. indica*) respond to iso-amyl acetate in the same way as *A. mellifera*. In these experiments an 'artificial mouse' of black felt stuffed with cotton was placed on a hive entrance board. The bees did not react to the 'mouse' until two drops of iso-amyl acetate had been put on it. About 20 sec later the bees attacked the 'mouse' in force. A minute later the 'mouse' was so covered with bees, which appeared to be stinging, that it could not be seen any more. The bees also attacked several people standing 8–10 m away (Morse and Benton, 1964; Morse *et al.*, 1967).

Although alarm releasers probably play a role in exciting social Hymenoptera during mass collection of venoms, the alarm releasers have not been extensively used for this purpose.

## III. CONCLUSIONS

A variety of stimulation techniques are available for the collection of venom from individual Hymenoptera as well as from large numbers of insects simultaneously. Mass-collected venoms and extracts prepared from venom apparatuses are contaminated with soluble substances originating from the faeces or tissues. These venom preparations can only be used in physiological studies if the venoms are very potent and are applied in extremely dilute solutions. Purification of the active principles of the venom is normally required.

Venom most closely resembling that injected in nature may be obtained by collecting venom droplets from the sting. Venom preparations made from the extracted contents of venom reservoirs may also be native. However, it is not certain that the venom produced by the insect when stinging normally is completely identical with the contents of the venom reservoir. Our present knowledge indicates that the active principles of hymenopteran venom are secreted by the acid gland and are stored in the reservoir. The accessory glands (see Chapter 2) do not seem to contribute to the toxic activity of the venoms.

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