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Chemistry and Pharmacology of Solitary Wasp Venoms

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I. INTRODUCTION

This chapter reviews our present knowledge on the pharmacology and chemistry of venoms produced by solitary wasps. Among the Hymenoptera that produce a venom the social wasps (Chapter 6), bees (Chapter 7) and ants (Chapter 9) are most familiar to men. The venoms of these social Hymenoptera are used by these insects for defending themselves and their colonies. These venoms produce pain and local damage in large vertebrates and are lethal to insects and small vertebrates. Multiple stings of bees, ants or wasps, as well as allergic reactions, may be responsible for killing larger vertebrates. Of all solitary wasps, only some bethylid species may attack humans (see Bethylioidea, Section II,B,3).

In this discussion the term social wasps refers to members of the Vespinae, despite the fact that some wasps, while taxonomically included in solitary wasp families, display social or semi-social life. If, for example, hymenopteran social behaviour is simply defined by the activity of an individual benefiting the larvae of another individual of the same species, then the sphecid wasp *Microstigmus comes* must be qualified as a social wasp (Matthews, 1968). If sociality is the condition shown by species in which there is division of labour, then the guarding behaviour of males of the sphecid *Tachytes*

distinctus qualifies this wasp as being semi-social (Lin and Michener, 1972). Nevertheless the family Sphecidae, in its totality, is considered to be a family of solitary wasps.

The solitary wasps are much less well known as 'venom producers' than the social wasps, bees and ants. Yet, of the roughly 250,000 described species of Hymenoptera (Malyshev, 1966) the vast majority are solitary wasps (Evans and Eberhard, 1970). According to Akre and Davis (1978), there are about 15,000 species of aculeate wasps (see Chapter 1 and the next section), ~95% of which are solitary.

Solitary wasps are often identified with wasps that do not kill but paralyse their prey. However, the term 'solitary wasp' includes more of the Hymenoptera than only those wasps that produce paralysing venoms. The solitary, plant-feeding wasps (Symphyta, or sawflies) probably do not produce a paralysing venom. Moreover, a number of parasitic Terebrantia have a 'venom apparatus' which produces secretions that do not paralyse prey, but may change their metabolism and/or endocrine control.

II. DIVERSITY OF SOLITARY WASP VENOMS

In Chapter 1 the order of Hymenoptera is subdivided into two major groups: the Symphyta (or sawflies), most of which are phytophagous, and the Apocrita, most of which are entomophagous. The sawflies do not have a 'wasp waist', such as is characteristic for the Apocrita. The latter group is subdivided into two sections: the Terebrantia, which have an ovipositor (terebra or drill), which is also used as a ductus venatus, and the Aculeata, having an aculeus or sting, which is considered to be a fully modified ovipositor, no longer used for oviposition (see also Chapter 2). The section of Terebrantia includes three important superfamilies: the Ichneumonoidea with the large families Ichneumonidae and Braconidae, the Cynipoidea and the Chalcidoidea. The section of Aculeata includes seven superfamilies: Bethyloidea, Scolioidea, Pompiloidea, Sphecoidea, Vespoidea, Apoidea and Formicoidea. The venoms of the social part of the Vespoidea are described in Chapter 6, those of the Apoidea in Chapters 7 and 8 and those of the Formicoidea in Chapter 9.

A. Symphyta

Although members of one superfamily of the Symphyta (the Orussoidea) are ectoparasites of buprestid larvae (Cooper, 1963), the other Symphyta are plant-feeders, the females of which deposit their eggs in plant tissues. The secretions or excretions of their larvae cause the formation of galls, within

which the larva feeds. Galls and similar deformations are also caused by insects of other orders (e.g., Hemiptera, Diptera, Lepidoptera). Haviland (1922) suggested that the evolutionary ancestors of wasps that paralyse insects might have laid eggs in the galls produced by other insects and at a later time may have become parasitic on the insects which formed the gall. This would then represent the first evolutionary step in the development of hymenopteran parasitism of other insects.

If the plant feeding symphytans are to be regarded as the evolutionary ancestors of the parasitic hymenopterans, then it becomes interesting to study the chemical nature and biological activity of the secretion produced by the living members of the Symphyta.

In the abdomen of the female wood wasp *Sirex noctilio* F., paired glands secrete a colourless mucus into a large reservoir which is connected, by a duct, to the base of the ovipositor. Boros (1968) has characterized this substance as a protein–mucopolysaccharide complex. A fungal symbiont (*Amylostereum areolatum* Boidin) is associated with *S. noctilio* (Gaut, 1969). Development and survival of the immature stages of *S. noctilio* are dependent on active growth of the symbiotic fungus (Madden, 1981). Massive attack of *S. noctilio* on *Pinus radiata*, combined with the simultaneous inoculation with its fungal symbiont, rapidly causes physiological changes in the stems and leaves of the trees which often result in their death (Coutts, 1969a). These effects were originally thought to be caused by the fungal toxins. However, both attack by *S. noctilio* and injection of *S. noctilio* extracts into logs resulted in the accumulation of starch and the concomitant increase in the dry weight of leaves, which could not be duplicated by inoculation with the fungus or by extracts from logs inoculated with this fungus (Coutts, 1969a). Neither the fungus alone nor wasp mucus along could normally kill a tree but the combination of the fungus and mucus was lethal. The injection of mucus from *Sirex noctilio* caused accumulation of starch in *Pinus radiata* needles and in some cases checked the growth of the tree. These changes were reversible. In some trees mucus caused a continued accumulation of photosynthetic products in the needles and a concomitant reduction in the chlorophyll content (Coutts, 1969a,b).

Fong and Crowden (1973) have shown a progressive series of changes in 1-year-old *Pinus radiata* needles after the injection of mucus from glands dissected from *Sirex noctilio*. The immediate effect of mucus on the needles appears to involve an impairment of the tree's normal water regulation with resulting tissue desiccation, distortion and eventual collapse of the translocation cells of the phloem. These effects are accompanied by dramatic changes in the rates of certain metabolic processes (e.g. respiration), as well as changes in the levels of peroxidases and amylases (Fong and Crowden, 1973).

Spradbery (1973) has bioassayed dilute solutions of mucus from different species of wood wasps, on detached shoots. The mucus of only one species, *Sirex noctilio*, induced rapid changes in the radial growth of the stem, quantity of starch in the needles, needle pressure and the colour of the foliage of living trees. The other species (*S. juvencus, Urocerus gigas, U. augur, U. sah* and *Xeris spectrum*) produced no phytotoxic symptoms (Spradbery, 1973).

The major component of *S. noctilio* mucus appears to be a protein–polysaccharide complex with probable molecular weight in the range 60,000–100,000 (Wong and Crowden, 1976).

B. Apocrita

The venoms of the solitary Apocrita, with the exception of solitary bees, have quite a different mode of action. A sting of these wasps seldom produce more than momentary pain in man. These venoms are, however, very active in insects and in spiders. In the Arthropoda, paralysis is the common consequence of apocritan stings.

A very early account of the pompilid wasp *Batozonellus lacerticida* describes them making aggressive attacks on small lizards, which were killed and brought into the ground (Pallas, 1771). Olberg (1959) did not understand what inspired Pallas to write 'such a nonsense', and the observation has never been confirmed.

Table I summarizes some aspects of host paralysis caused by solitary wasps. A survey of the table suggests that the normal effect of stings of solitary Apocrita is locomotor paralysis. However, within the Terebrantia a number of wasp species do not paralyse their prey at all. Descriptions of parasitism by hymenopterans that do not paralyse their prey seldom comment on this negative phenomenon. Therefore, data in Table I concerning the absence of paralysis may not reflect the proper proportion of the absence of paralysis.

As a rule ectoparasitic wasps paralyse the host prior to the oviposition. Exceptions are known, for example, *Trichomalus fasciatus* (Chalcidoidea), an external parasite of *Ceuthorrhynchus assimilis* (Coleoptera), does not paralyse its prey (Sweetman, 1958). Species that are internal parasites usually do not paralyse the prey or produce a paralysis of very short duration. Aphids are often not paralysed. Aculeate hunters of aphids seem to kill prey (see Section II,B,3, Sphecoidea), some braconid parasites of aphids lay an egg internally, and the aphids show very little reaction. The aphid prey of *Praon palitans* (Braconidae), for example, walks away rapidly when released from the oviposition (Schlinger and Hall, 1960). The morphology described by these authors shows that the single venom gland is extremely small, and a reservoir may be absent. This may be an example of the reduction of both the paralysing capacity and venom producing system.

Solitary Hymenoptera which deposit their eggs in a host egg or in a pupa do not need a paralysing venom, but this does not necessarily imply that their venoms are not active at all. The pteromalid wasp *Nasonia* (= *Melittobia*) *vitripennis* (superfamily Chalcidoidea) is a parasite of the pupa of several muscoid dipteran species. Evans (1933) described its behaviour and suggested that it had no need to render its host quiescent, this being reflected in the small size of its venom glands. However, King (1959) and Beard (1964) have demonstrated that something associated with the stings of *Nasonia vitripennis* proves fatal to the host. Ratcliffe and King (1967) exposed 9-day-old pupae of *Calliphora* spp. to females of *N. vitripennis*. Adult flies would normally emerge from these pupae within the next 24 hr, while the eggs of the parasite take 24–36 hr to hatch and would not have done so by the time the adult flies would have emerged. Therefore, host death could not be a result of the feeding activity of the parasite larvae or the results of factors released as the parasite eggs hatched. The mortality of the pupae stung by *N. vitripennis* (100% mortality) was compared with pin-pricked pupae (30% mortality) and with control puparia (10% mortality). Ratcliffe and King (1967) also showed that the active principles of the venom are present in the acid gland and not in the alkaline gland of *N. vitripennis* (for the morphology of these glands see Chapter 2). Pupae pricked with fine pins previously dipped in a saline containing the content of the acid gland showed a mortality of 70% compared with 40% mortality in pin-pricked controls and 16% mortality in untreated pupae.

Gordh and Hawkins (1981) reported that if the host (larvae of Microlepidoptera) of the bethilid wasp *Goniozus emigratus* is paralysed immediately prior to the period of pupation, the paralysed host larva will pupate. Otherwise host paralysis results in death (see also Section III,A).

The original idea (see Chapter 1) that the alkaline gland is part of the venom system seems not to be justified. This gland (Dufours gland) probably plays a part in oviposition. Guillot and Vinson (1972b) prepared an acetone extract from the alkaline gland of the ichneumonid wasp *Campoletis perdistinctus* and treated larvae of *Heliothis virescens* with this extract. They found that larvae treated in this way were rarely attacked by the wasp. These authors considered the material extracted from the alkaline gland to be a pheromone.

From these experiments it is clear that the 'venoms' of certain solitary wasps may have quite different functions from the induction of paralysis, which is generally considered to be the common effect of solitary wasp venoms.

Table I summarizes data, culled from about 600 references, on the effects of solitary wasp stings, grouped by the superfamilies to which the species belong. Some caution is appropriate in interpreting this information since most of the citations represent single observations, and some authors omit the reasons that have led them to conclude that prey is paralysed, killed, or

Table I
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Suborder Terebrantia				
Superfamily Ichneumonoidea				
Family Ichneumonidae				
Aenoplex carpocapsae Cush.	*Carpocapsa pomonella*	Lep.	+I	McClure (1933)
Calliephialtes grapholitica (Cress.)	*Acrobasis caryae*	Lep.	+C	Nickels *et al.* (1950)
Ephialtes extensor Tasch.	*Cydia pomonella*	Lep.	+PC	Rosenberg (1934)
Ephialtes ruficollis (Grav.)	*Galleria mellonella*	Lep.	+C	Juillet (1959)
	Rhyacionia buoliana	Lep.	+C	
Hemiteles hemipterus F.	*Cydia pomonella*	Lep.	+PI	Rosenberg (1934)
Nemeritis canescens (Grav.)	*Ephestia kuehniella*	Lep.	–	Richards and Thomson (1932)
	Ephestia kuehniella	Lep.	+TI	Piek and Simon Thomas (1969)
	Galleria mellonella	Lep.	+TI	
Paniscus cristatus Thoms.	Caterpillars	Lep.	–	Shevyrev (1912), see Malyshev (1966)
Paniscus geminatus Say	*Heliothis obsoleta*	Lep.	+T	Vance (1927)
Paniscus ocellaris Thoms.	*Agrotis segetum*	Lep.	+T	Shevyrev (1912), see Malyshev (1966)
Paniscus spinipes Cush.	*Heliothis obsoleta*	Lep.	+T	Vance (1927)
Pimpla pomorum Ratz.	*Anthonomus pomorum*	Col.	+P	Speyer (1925)
Polysphincta eximia Schm.	Spiders	Ara.	+T	Clausen (1940)
Rhyssa persuasoria L. and R.	Siricidae	Hym.	+	Spradbery (1968)
Sericopimpla sagrae sauteri Cushman	Japanese bagworms	Col.	+P	Iwata (1976)
Spilocryptus incubitor Ström	*Cydia pomonella*	Lep.	+	Rosenberg (1934)
Tersilochus conotracheli (Riley)	*Conotrachelus* sp.	Col.	+T	Sweetman (1958)
Zaglyptus iwata Uch.	*Clubiona japonica*	Ara.	+P	Iwata (1976)
Family Braconidae				
Alysia manducator Panz.	Sheepfly maggots	Dip.	+TC	Myers (1927)
Apanteles epinotiae Vier.	*Acrobasis caryae*	Lep.	+T	Nickels *et al.* (1950)

	Laspeyresia caryana	Lep.	+T	Piek and Simon Thomas (1969)
Apanteles glomeratus L.	*Pieris brassicae*	Lep.	+T	Piek and Simon Thomas (1969)
Apanteles medicaginis Mues.	*Colius eurytheme*	Lep.	–	Allen (1958)
Aphidius testaceipes (Cress.)	Aphidae	Hem.	–	Sweetman (1958)
Bassus acrobasidis Cush.	*Acrobais caryae*	Lep.	+T	Nickels *et al.* (1950)
Cedria paradoxa (Wekn.)	*Hapalia machaeralis*	Lep.	+CD	Beeson and Chatterjee (1935)
Clinocentrus gracilipes	*Anthophila fabriciana*	Lep.	+CT	Shaw (1981, 1983)
Coeloides brunneri Vier.	*Dendroctonus pseudotsugae*	Col.	+CD	Ryan and Rudensky (1962)
Coeloides dendroctoni Cush.	*Dendroctonus monticollae*	Col.	+DI	De Leon (1935)
Colastes braconius (Hal.)	Curculionid larvae	Col.	+(T)C	Shaw (1983)
Diachasma tryoni Cam.	*Ceratitis capitata*	Dip.	+TI	Pemberton (1918)
Heterospilus cephi Roh.	*Cephus pygmaeus*	Hym.	+	Sweetman (1958)
Iphiaulax kimballi Kirkl.	*Diatraea grandiosella*	Lep.	+CD	Kirkland (1982)
Iprobracon rimae Walcot	*Diatraea saccharalis*	Lep.	+PI	Jaynes (1933)
Leiophron apicalis Cutis	Mirid nymphs	Hem.	+T	Waloff (1976)
Macrocentus ancyclivorus	*Grapholita molesta*	Lep.	+	Newman (1965)
Microbracon brevicornis	Gelechiidae	Lep.	+PC	Génieys (1922, 1925); Taylor (1932)
(= *Habrobracon*, = *Bracon*)	Tortricidae, Pyralidae	Lep.	+PC	
	Pyraustidae	Lep.	+PC	
	Arctiidae	Lep.	+PC	
	Nymphalidae	Lep.	+PC	
	Noctuidae spp.	Lep.	+PC	
Microbracon cephi Gahan	*Cephus. cinctus*	Hym.	–	Seamans (1929)
(= *Bracon* F.)	*Cephus cinctus*	Hym.	+P	Nelson and Farstad (1953)
Microbracon chinensis (Szépl.)	*Chilo zonellus*	Lep.	+PC	Cherian and Narayanaswami (1942); Subba Rao (1955)
Microbracon gelechiae (Ashm.)	*Phthorimaea operculella*	Lep.	+PC	Trouvelot (1921); Génieys (1925); Piek *et al.* (1974)
(= *Habrobracon johansenni* Vier.)	*Ostrinia nubilalis*	Lep.	+PC	
	Galleria mellonella	Lep.	+PC	
	Ephestia kuehniella	Lep.	+PC	
	Corcyra cephalonica	Lep.	+PC	

(continued)

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Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Microbracon greenii (Ashm.)	*Phatyedra gossypiella*	Lep.	+	Glover (1934); Angalet (1964)
	Ostrinia nubilalis	Lep.	+	
	Earias fabia	Lep.	+	
	Eublemma amabilis	Lep.	+	
	Laccifer lacca	Hem.	+	
	Microlarinus laeynii	Col.	+	
Microbracon hebetor (Say)	Gelechiidae	Lep.	+PC	Hase (1922, 1924); Donohoe, see Clausen (1940);
(= *Habrobracon*, = *Bracon*)	Pyralididae	Lep.	+PC	Morrill (1942); Ullyett (1943); Beard (1952); Apanna
	Ephesita figulilella	Lep.	+T	(1952); Piek *et al.* (1974)
	Ostrinia nubilalis	Lep.	−	
	Noctuidae	Lep.	+PC	
Microbracon hylobii (Ratz.)	*Hylobius abietis*	Col.	−	Munro (1916)
Microbracon kirkpatricki (Wilkins)	*Chilo simplex*	Lep.	+	Azab *et al.* (1968); Cross *et al.* (1969)
(= *Bracon*)	*Corcyra cephalonica*	Lep.	+PC	
	Ephestia kuehniella	Lep.	+PC	
	Galleria mellonella	Lep.	+C	
	Pyrausta (= *Ostrinia*) *nubilalis*	Lep.	+	
	Sesamia cretica	Lep.	+	
Microbracon lineatellae (Fischer)	*Anarsia lineatella*	Lep.	+PC	Laing and Caltagirone (1969)
(= *Habrobracon*)	*Paramyelois transitella*	Lep.	+PC	
Microbracon lutus (Prov.)	*Achatodes zeae*	Lep.	+PC	Balduf (1929)
	Lixus scrobicollis	Col.	+PC	
Microbracon mellitor (Say)	*Anthonomus fulvus*	Col.	+PC	Willard (1927); Folsom (1936); Adams *et al.* (1969)
(= *Bracon*)	*Anthonomus grandis*	Col.	+PC	
	Pectinophora gossypiella	Lep.	+PC	

	Rhynchitis bicolor	Col.	+PC	
Microbracon pectoralis (Wesm.)	*Etiella zinckenella*	Lep.	+	Parker (1951)
	Orneodes hexadactyla	Lep.	+	
Microbracon piger (Wesm.)	*Cnephasia longana*	Lep.	+	Parker (1951); Clausen (1956)
	Etiella zinckenella	Lep.	+P	
Microbracon pini Mues.	*Pissodus strobi*	Col.	+T	Taylor (1929)
Microbracon pygmaeus (Prov.)	*Coleophora pruniella*	Lep.	+PC	Doner (1934)
	Haploptila innotabilis	Lep.	+PC	
Microbracon terebella (Wesm.)	*Cephus pygmaeus*	Hym.	–	Salt (1931)
Microbracon thurberiphagae Mues. (= *Bracon*)	*Ancylostomia stercorea*	Lep.	+	Bennet (1959)
Microbracon variabilis (Prov.)	*Aerobasis caryae*	Lep.	+PC	Nickels *et al.* (1950)
Microgaster tibialis Nees	*Pyrausta* (= *Ostrinia*) *nubilalis*	Lep.	–	Vance (1935)
Monoctonus paulensis (Ashm.)	Dactynotine aphids	Hem.	+TC	Calvert and van den Bosch (1972)
Oncophanes laevigatus (Ratz.)	Tortricidae	Lep.	+P	Shaw (1983)
Petalodes unicolor Wesm.	Leafspring larvae	Lep.	+T	Shaw (1983)
Phanomeris catenator (Hal.)	Leaf-mining sawflies	Hym.	+TC	Shaw (1983)
Phanomeris phyllotomae Mues	*Heterarthrus nemoratus*	Hym.	+PI	Clausen (1956)
Praon palitans Mues.	*Therioaphis maculata*	Hem.	–	Schlinger and Hall (1960)
Rhaconotus scirpophagae Wkl.	*Scirpophaga auriflua*	Lep.	+PD	Cherian and Israel (1938a)
Rhogas plecopterae Chatt.	*Plecoptera reflexa*	Lep.	~	Chatterjee (1943)
Rhogas testaceus (Spin.)	*Earias fabia*	Lep.	+T	Amad (1943)
	Earias insulana	Lep.	+T	
Rhoptocentrus piceus Marsh.	*Hyalotrupes bajulus*	Col.	+C	Kühne and Becker (1974); Mashood Alam (1952)
Rhysipolis decorator (Hal.)	*Caloptila* larvae	Lep.	+T	Shaw (1983)
Stenobracon deesae (Cam.)	*Chilo zonellus*	Lep.	+	Narayanan and Subba Rao (1955); Chaudhuri (1954)
	Corcyra cephalonica	Lep.	+	
	Scirpophaga nivella	Lep.	+	
Stenobracon nicevillei Bingh.	*Chilo* sp.	Lep.	+	Cherian and Israel (1938b)
	Scirpophaga auriflua	Lep.	+	
	Sesamia sp.	Lep.	+	
Xenarcha lustrator (Hal.)	Leaf-mining sawflies	Hym.	+TI	Shaw (1983)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Superfamily Cynipoidea				
Figites anthomyiarum Bouché	*Ephestia kuehniella*	Lep.	+T	Doutt (1963)
	Larvae of flies	Dip.	+T	Sweetman (1958)
Superfamily Chalcidoidea				
Aphelinus jucundus Gahan	*Macrosiphon cornelli*	Hem.	+PT	Clausen (1940)
Aphelinus semiflavus (Howard)	*Macrosiphon solani*	Hem.	+	Wilbert (1964)
	Myzus persicae	Hem.	−	Hartley (1922)
Brachyneria fouscolombei (Dufour)	Blowfly larvae	Dip.	−	Roberts (1933)
Cirrosphilus vittatus Wlk.	*Stigmella malella*	Lep.	+(→†)	Eveleens and Evenhuis (1968)
Coccophagus gurneyi Comp.	*Pseudococcus gehani*	Hem.	+T	Sweetman (1958)
Dahlbominus fuscipennis (Zett.)	Sawflies	Hym.	+I	Clausen (1956)
Dicladocerus sp.	*Archips fumiferana*	Lep.	+	Dowden and Carolin (1950)
Elachertus cacoeciae How.	*Archips fumiferana*	Lep.	+	Dowden and Carolin (1950)
Elasmus claripennis Cam.	*Eublemma amabilis*	Lep.	+I	Mahdihassan (1934)
Elasmus hispidarum Ferr.	Promecotheca bicolor	Col.	+I	Taylor (1937)
	Promecotheca reichi	Col.	+I	
Eulophus longulus Zett.	*Rhynchaenus* larvae	Lep.	+P	Sunby (1957)
Eulophus viridulus Thoms.	*Ostrinia nubilalis*	Lep.	+P	Parker and Smith (1933)
Euplectrus agaristae Craw.	*Phalaenoides glycine*	Lep.	−	Noble (1938)
Eupteromalus nidulans (Thom.)	Caterpillars	Lep.	+P	Clausen (1956)
Eurytoma arctica Thom.	Curculionidae larvae	Col.	+?	Nuorteva (1957)
Habrocytus cerealellae (Ashm.)	*Sitotroga cerealella*	Lep.	+PC	Noble (1932); Fulton (1933); Clausen (1940)
Lariophagus distinguendus Först.	*Calandra granaria*	Col.	+PC	Hase (1924); T. Piek (personal observation)
	Sitophilus granarius	Col.	+PC	
Melittobia acasta	Larvae of Hymenoptera	Hym.	+P	Balfour Browne (1922); Malyshev (1966)

Melittobia chalybii Ashm.	*Emenes fraterna*	Lep.	+D	Buckell (1929)
Microplectron fuscipennis Zett.	*Diprion sertifer*	Hym.	+I	See Clausen (1940)
Pleurotropis panei Ferr.	*Promeocotheca niciferea*	Col.	+	Taylor (1937)
	Gonophora taylori	Col.	+	
	Gonophora xanthomelana	Col.	+	
Rhopalicus pulchripennis Gahan	*Eupelmus pini*	Col.	+P	Taylor (1929)
Schizonotus sieboldi (Ratz.)	*Plagiodera versicola*	Col.	+I	Dowden (1939)
Solenotus begini (Ashm.)	*Phytomyza atricornis*	Dip.	+D	Doutt (1957)
Tetrastichus taylory (Ferr.)	*Elasmus hispidarum*	Hym.	–	Taylor (1937)
Tetrastichus sp.	*Archips fumerferana*	Lep.	+	Dowden and Carolin (1950)
Tetrastichus flavigaster B. and M.	Psyllid nymphs	Hem.	+T	Moran *et al.* (1969)
Trichomalus fascitatus (Thom.)	*Ceuthorrhynchus assimilis*	Col.	–	Sweetman (1958)
Suborder Aculeata				
Superfamily Bethyloidea				
Family Dryinidae				
unknown species	Insects	—	+T	Newman (1965)
Aphelopus sp.	Cicadellidae	Hem.	+T	Sweetman (1958)
Haplogonatopus japonicus E. and H.	Insects	—	+T	Clausen (1940)
Pseudogonatopus hospes Perk.	*Perkinsiella saccharicida*	Hem.	+	Swezey (1919), see Sweetman (1958)
Family Bethylidae				
Allepyris microneurus Kief.	*Anthrenus verbasci*	Col.	+C	Iwata (1942); Yamada (1955), see Iwata (1976)
Cephalonomia tarsalis (Ash.)	*Oryzaephilus surinamensis*	Col.	+C	Powell (1938)
	Sitophilus oryza	Col.	+	
Cephalonomia waterstoni Gahan	*Laemopholoeus* sp.	Col.	+	Finslayson (1950)
Epyris extraneus Bridwell	*Conocephalum seriatum*	Col.	+I	Williams (1919a)
Goniozus claripennis Först.	*Oenophthira pilleriana*	Lep.	+T	Voukassovitch (1924)
Goniozus emigratus (Rohwer)	*Paramyelois transitella*	Lep.	+(→†)	Gordh and Hawkins (1981)
Goniozus gallicola Fouts.	*Deoclona yuccasella*	Lep.	+IP	Gordh (1976)
Goniozus legneri Gordh	*Amyelois transitella*	Lep.	+PI	Gordh *et al.* (1983)
Holepyris hawaiiensis (Ashm.)	*Plodia* sp., *Corcyra* spp.	Lep.	+C	Bridwell (1920b)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Laelius anthrenivorus Trani.	*Anthrhenus verbasci*	Col.	+PC	Vance and Parker (1932), see Clausen (1940)
Laelius trogodermatis Ash.	Dermestidae	Col.	+	Sweetman (1958)
Parasclerodermus berlandi Maneval	*Thanasimus formicarius*	Col.	+I	Maneval (1930)
Parisierola cellularis (Kieffer)	*Acrobasis caryae*	Lep.	+PC	Nickels *et al.* (1950)
Parisierola emigrata Rohn.	*Pectinophora gossypiella*	Lep.	+(T)C	Sweetman (1958); Iwata (1976)
Parisierola nephantidis Mues.	*Nephantis serinopa*	Lep.	+P	Jayaratnam (1941); Remadevi *et al.* (1981)
Sclerodermus domesticum Latr.	Cerambycidae	Col.	+	Kühne and Becker (1974)
Sclerodermus immigrans Bridwell	Lepidoptera larvae	Lep.	+	Bridwell (1920a)
	Coleoptera larvae	Col.	+	
Family Sclerogibhidae				
Sclerogibba embiidarum Kieff.	*Oligotoma greeniana*	Emb.	+T	Ananthasubramanian and Ananthakrishnan (1959)
Family Chrysididae				
Chrysis shanghaiensis Smith	*Monema flavescens*	Lep.	+	Du Buysson (1898)
	Monema flavescens	Lep.	+I	Piel (1933); Parker (1936)
Superfamily Scolioidea				
Campsomeris annulata Fabr.	*Popillia japonica*	Col.	+PC	Clausen *et al.* (1927); Sweetman (1958)
Campsomeris aureicollis Lep.	Cane grubs	Col.	+	Jarvis (1931)
Campsomeris dorsata (Fab.)	*Ligyrus* grubs	Col.	+PI	Nowell (1916)
Campsomeris formosana Guér.	White grubs	Col.	+T	Tryon (1902); Illingworth (1919)
Campsomeris janava Lep.	Grubs	Col.	+CP	Corbett, see Clausen (1940)
Campsomeris marginella Sm.	Scarabaeidae	Col.	+P	Sweetman (1958)
Campsomeris pulchrivesta Cam.	Grubs	Col.	+CP	Corbett, see Clausen (1940)
Campsomeris tasmaniensis	White grubs	Col.	+	Illingworth (1919)
Cosila chilensis Guér.	*Pseudodelphus cilatus*	Col.	+	Janvier (1933)
Diamma bicolor Westwood	*Gryllotalpa coarctata*	Ort.	+TI	Hardy (1911)
Dielis annulata F.	*Holotricha helleri*	Col.	+	Leefmans (1915)

Dielis luctuosa Smith	*Leucopholis rorida*	Col.	+P	Leefmans (1915)
Dielis thoracica F.	Grubs	Col.	+	Leefmans (1921)
Elaphroptera atra Guér.	*Maipa* sp.	Col.	+PC	Janvier (1933)
Elaphroptera dimidiata Guér.	*Oryctomorphus* spp.	Col.	+I	Janvier (1933)
	Liogenys spp.	Col.	+I	
	Amblypterus variabilis	Col.	+I	
Elaphroptera erythryra Spin.	*Maipa concexa*	Col.	+	Janvier (1933)
Elaphroptera herbsti André	*Athlia rustica*	Col.	+	Janvier (1933)
Elis annulata Fabr.	*Popillia janponica*	Col.	+I	Clausen *et al.* (1927, 1932)
Epartiothynnus opaciventris Turner	Lamellicorn grubs	Col.	+I	Williams (1919b)
Megascolia flavifrons Fab.	*Oryctes nasicornis*	Col.	+PI	Passerini (1840, 1841)
	Oryctes nasicornis	Col.	+PC	Piek and Simon Thomas (1969)
Megascolia procer	*Chalcosoma atlas*	Col.	+	Leefmans (1933)
Methoca ichneumonides	*Cicindela* spp.	Col.	+	Adlerz (1903); cf. Malyshev (1966)
Methoca japonica Yasum.	Beetle larvae	Col.	+TC	Iwata (1942)
Methoca stygia (Say)	*Cicindela repanda*	Col.	+	Palmer (1976)
Methoca punctata Williams	*Cicindela* larvae	Col.	+TID	Williams (1919c)
Methoca striatella Williams	*Cicindela* larvae	Col.	+PC	Williams (1919c)
Pterombrus cicindelicidus Williams	*Cicindela* larvae	Col.	+C	Williams (1928a)
Pterombrus iheringi Ducke	*Megacephala affinis*	Col.	+PC	Williams (1928a)
Pterombrus piceres Krombein	*Pseudoxychila tarsalis*	Col.	+	Palmer (1976)
Scolia bifasciata (v. d. Lind.)	*Cetonia aurata*	Col.	+PC	Fabre (1879–1910)
Scolia manilla Ashm.	Lamellicorn grubs	Col.	+PC(I)	Williams (1919c)
Tiphia femorata F.	*Rhizotrogus salstitialis*	Col.	+T	Adlerz (1916), see Malyshev (1966)
Tiphia lucida Ashm.	*Adoretus* grubs	Col.	+T	Williams (1919c)
Tiphia morio F.	*Amphimallon majalis*	Col.	+TC	Janvier (1956)
Tiphia parallela Smith	*Ligyrus* grubs	Col.	+TI	Nowell (1916)
Tiphia popilliavora Rohw.	*Popillia japonica*	Col.	+TC	Clausen *et al.* (1927)
Tiphia segregata Crawf.	*Anomala* grubs	Col.	+TC	Swesey, see Williams (1919c)
Triscolia rubiginosa L.	*Xylotrupes gideon*	Col.	+	Leefmans (1921)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Superfamily Pompiloidea				
Agenia subcorticalis (Walsh.)	Attidae spp.	Ara.	+PI	Hartman (1905)
Agenia variegata L.	*Xysticus* sp.	Ara.	+I	Maneval (1939)
Agenioideus cinctellus Priesner	*Phlega fasciata*	Ara.	+T	Gros (1982b)
Agenioideus humilis Cress.	*Araneus cornutus*	Ara.	+PC	Evans and Yoshimoto (1962)
	Neoscona sp.	Ara.	+P(I)	Eberhard (1970)
Amblyellus rubusta Gussakowsky	*Chiracanthium* sp.	Ara.	+PC	Gros (1982b)
Anoplius americanus	*Arctosa littoralis*	Ara.	+TC	Evans and Yoshimoto (1962)
Anoplius amethystinus Fabr.	*Lycosa rubida*	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius apiculatus (Smith)	*Lycosa helluo*	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius carolinus (Banks)	*Wadotes hybridus*	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius chalybeatus Schröd.	Lycosidae	Ara.	+TI	Molitor (1939a,b); Soyer (1953)
Anoplius cleora (Banks)	*Arctosa littoralis*	Ara.	TC	Krombein (1952); Evans and Yoshimoto (1962)
Anoplius concinnus Dahlb.	Spiders	Ara.	+TI	Soyer (1953)
Anoplius dispar Dahlb.	*Lycosa cinera*	Ara.	+C	Maneval (1939)
	Spiders	Ara.	+TI	Soyer (1953)
Anoplius illinoensis (Roberts.)	*Lycosa* sp.	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius imbellis Banks	*Pardosa ramulosa*	Ara.	+T	Wasbauer (1957)
Anoplius ithaca (Banks)	*Lycosa* sp.	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius fraternus Banks	Lycosidae	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius krombeini Evans	*Schizocosa* sp.	Ara.	+T	Krombein (1953)
Anoplius marginalis Banks	Spider	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius marginatus (Say)	Spiders (7 fam., 22 sp.)	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius nigerrimus Scop.	*Trochosa robusta*	Ara.	+T	Soyer (1953)
Anoplius papago Banks	*Lycosa* sp.	Ara.	+	Evans (1964a)

Anoplius relativus	*Geolycosa dimifex*	Ara.	+TC	McQueen (1979)
Anoplius samariensis Pal.	Spiders	Ara.	+T	Soyer (1953)
Anoplius semirufus (Cress.)	Lycosidae	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius subcylindricus Banks	Thomisidae	Ara.	+T	Evans and Yoshimoto (1962)
Anoplius ventralis tarasus (Banks)	*Alopecosa gertschi*	Ara.	+T	Powell (1958)
Anoplius viaticus L.	*Trochosa terricola*	Ara.	+(P)TC	Thijsse (1907); Soyer (1953); Piek (1978)
Anoplius virginiensis (Cresson)	*Agelenopsis pennsylavanica*	Ara.	+TC	Evans and Yoshimoto (1962)
Anosplius barbilabris Wolf.	*Nemesia* sp.	Ara.	+T	Gros (1982c)
Aporus faciatus Smith	Attidae	Ara.	+TI	Peckham and Peckham (1898)
Aporinellus faciatus	Spiders	Ara.	+T	Evans and Yoshimoto (1962)
Aporinellus sexmaculatus Spin.	*Saitis barbipes*	Ara.	+P	Gros (1982b)
Arachnophoctonus ferrugineus Say	*Lycosa* sp.	Ara.	+C	Rau and Rau (1918)
Auplopus architectus Say	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Auplopus nigrellus Banks	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Auplopus caerulescens Dahlb.	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Batozonellus annulatus (F.)	*Argiope amoena*	Ara.	+C	Tsuneki (1968b)
Batozonellus lacerticida (Pallas)	*Argiope* spp.	Ara.	+	Olberg (1959)
Batozonellus maculifrons (Smith)	*Neoscona scylla*	Ara.	+T	Tsuneki (1968b)
Calicurgus annulatus	Spiders	Ara.	+	Fabre (1879–1910); Evans and Matthews (1973a)
Calicurgus hyalinatus Fabr.	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Ceropalus maculata Fab.	Lycosidae	Ara.	+P(T)	Ferton (1879)
Cryptocheilus affinis (v. d. Lind.)	*Agelena labyrinthica*	Ara.	+TC	Minkiewicz (1934), see Richards and Hamm (1939)
Cryptocheilus comparatus Richards	*Lycosa narbonensis*	Ara.	+PC	Gros (1982a)
Cryptocheilus decemguttatus Costa	*Nomisia aussereri*	Ara.	+PC	Gros (1982a)
Cryptocheilus distinctus (Smith)	*Eriophora biapicata*	Ara.	+C	Evans *et al.* (1981)
Cryptocheilus rubellus	Black-bellied tarantula	Ara.	+C	Hingston (1928)
Ctenostegus murrumbidgee Evans	Web spider	Ara.	+	Evans *et al.* (1981)
Cyphononyx costaneus Klug.	*Lycosa bedeli*	Ara.	+P	Gros (1982a)
Deuteragenia hircana	*Epiblemum* sp.	Ara.	+	Nielsen (1932a)
Dicyrtomellus luctuosus Mocsary	Lycosid spiders	Ara.	+T	Gros (1982b)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Dipogon brevis Cress.	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Dipogon papago Banks	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Dipogon sayi Banks	Thomisidae	Ara.	+P	Evans and Yoshimoto (1962)
Dipogon variegata L.	Xysticus bifasciatus	Ara.	+P	Gros (1982a)
Elaphrosyron socius Evans	Spiders	Ara.	+	Evans and Matthews (1973a)
Episyron biguttatus Fabr.	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Episyron quinquenotatus (Say)	Aranidae	Ara.	+TC	Evans and Yoshimoto (1962)
Episyron rufipes (L.)	*Araneus cornutus*	Ara.	+TI	Veenendaal (personal communication)
Fabrogenia fusiformis (Saussure)	*Lycosa pictiventris*	Ara.	+	Evans and Matthews (1973a)
Haploneurion apogonum Kohl	*Araneus adiantus*	Ara.	+I	Janvier (1930)
Haploneurion minus Kohl	*Dolomedes pullatus*	Ara.	+I	Janvier (1930)
Hemipepsis sp.	*Lycosa* sp. (trapdoor spider)	Ara.	+	MacNulty (1961)
Hemipepsis capensis	*Palytes* sp.	Ara.	+TC	Skaife (1954)
Hemipepsis ustulata Stål	*Brachythele longitarsis*	Ara.	+T	Williams (1956)
Homonotus iwatai Yasum.	*Cheiracanthium rufulum*	Ara.	+TC	Iwata (1932)
Homonotus sanguinolentus Fabr.	*Cheiracanthium cornifex*	Ara.	+PC	Nielsen (1936)
Icazus khargenensis Priesner	*Aelurillus* spec.	Ara.	+T	Gros (1982b)
Notocyphus tyrannicus Sm.	Avicularidae	Ara.	+TI	Williams (1928a); cf. Sweetman (1958)
Pepsis dubiata Cress.	Spider	Ara.	+I	Rau and Rau (1918)
Pepsis limbata Guér.	*Phryxotrichus roseus*	Ara.	+	Janvier (1930)
Pepsis marginata Palissot de B.	*Cryptopholis portoricae*	Ara.	+T	Petrunkevitch (1926)
Pepsis mildei Stål.	*Aphonopelma* sp.	Ara.	+T	Williams (1956)
Pepsis thisbe Lucas	*Aphonopelma* sp.	Ara.	+	Williams (1956)
Phanagenia bombycina Creys.	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Planiceps hirsutus Banks	*Aptostichus ?stanfordianus*	Ara.	+	Williams (1928a)
Planiceps saussurei Spin.	*Calathotarsus coronatus*	Ara.	+I	Janvier (1930)

Poecilopompilus interruptus Say	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Pompiloides tropicus	*Lycosa carolinensis*	Ara.	+I	Rau and Rau (1918)
Pompilus acrobatus	*Lithyphantes corrolatus*	Ara.	+	Ferton (1902)
Pompilus arctus Cress.	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Pompilus belardoo Evans	*Lycosa* sp.	Ara.	+	Evans and Matthews (1973a)
Pompilus biguttatus Fabr.	*Epeira strix*	Ara.	+TC	Peckham and Peckham (1898)
Pompilus calipterus Say	*Xysticus ferox*	Ara.	+C(I)	Peckham and Peckham (1898)
Pompilus cinctellus Sp.	*Tertrix coarctata*	Ara.	+TI	Ferton (1897, 1905)
Pompilus cinerus (Fabricius)	*Lycosa speciosa*	Ara.	+	Evans *et al.* (1981)
Pompilus cingulatus Rossi	*Lycosa* spp.	Ara.	+T	Ferton (1897, 1910)
Pompilus fuscipennis St. Farg.	*Thomisus* spp.	Ara.	+(C)	Peckham and Peckham (1898)
Pompilus gibbus Fabr.	*Xysticus* sp.	Ara.	+C	Maneval (1939)
Pompilus luctuosus Mocs.	*Lycos bi-impressa*	Ara.	+TC	Ferton (1891)
Pompilus marginatus Say	*Phidippus tripunctatus*	Ara.	+TC(I)	Peckham and Peckham (1898)
Pompilus misturatus Kohl.	*Lycosa albata*	Ara.	+I	Janvier (1930)
Pompilus nubecula Costa	Spiders	Ara.	+I	Ferton (1905)
Pompilus petinipes (L.)	*Larinia lineata*	Ara.	+	Ferton (1902)
	Epeira dalmatica	Ara.	+	Ferton (1905)
Pompilus plicatus Costa	*Ctenizia sauvagei*	Ara.	+	Ferton (1905)
Pompilus proximus Dahlb.	*Lycosa inquilina*	Ara.	T(I?)	Maneval (1939)
Pompilus quinquenotatus Say	*Epeira strix*	Ara.	+TC	Peckham and Peckham (1898)
Pompilus republicanus Kohl	*Tertrix coarctata*	Ara.	+T	Ferton (1910, 1923); Gros (1982b)
Pompilus rytiphorsu Kohl	*Latrodectus 13-guttatus*	Ara.	+	Ferton (1910)
Pompilus scelestus Cress.	*Lycosa gulosa*	Ara.	+TC	Peckham and Peckham (1898)
	Dolomedes sp.	Ara.	+TC	Rau and Rau (1918); Evans and Yoshimoto (1962)
	Phidippus sp.	Ara.	+TC	
Pompilus sericeus v.d. Lind.	*Linyphia triangularis*	Ara.	+C	Maneval (1939)
Pompilus sexmaculatus	Thomisidae	Ara.	+	Ferton (1897)
Pompilus spinolae Kohl.	*Lycosa aspersa*	Ara.	+	Janvier (1930)
Pompilus wesmaeli (Thoms.)	*Oxyptila albimana*	Ara.	+	Ferton (1923)
Pompilus triviatus Dahlb.	*Arctosa perita*	Ara.	+	Bristowe (1948)
	Tarentula accentuata	Ara.	+T	Ferton (1908, 1910)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Pompilus vagans Costa	*Nemesia badia*	Ara.	+TI	Ferton (1897, 1908, 1910)
Priocnemis cornica Say	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Priocnemis hestia (Banks)	*Agroeca* sp.	Ara.	+P	Evans and Yoshimoto (1962)
Priocnemis leucocoelius	*Nemesia badia*	Ara.	+P	Ferton (1897)
Priocnemis hyalinatus	*Meta segmentata*	Ara.	+CT	Ferton (1897)
Priocnemis minorata Banks	Agelenidae, Anyphaenidae	Ara.	+P	Evans and Yoshimoto (1962)
	Clubionidae, Lycosidae	Ara.	+P	
Priocnemis notha Cress.	Spiders	Ara.	+P	Evans and Yoshimoto (1962)
Priocnemis perturbator (Harris)	*Trochosa terricola*	Ara.	+	Bristowe (1948)
Priocnemis vachali	*Nemesia badia*	Ara.	+	Ferton (1901)
Priocnemioides unifasciatus (Banks)	*Lycosa antelucana*	Ara.	+T	Evans and Yoshimoto (1962)
Pseudagenia carbonaria Scop.	*Chiracanthium siedlitzi*	Ara.	+I	Grandi (1926)
(= *Auplopus*)	*Chiracanthium pelasgicum*	Ara.	+I	
	Clubiona brevipes	Ara.	+C	Maneval (1939)
Pseudagenia argenteosignata Spin.	Drassidae	Ara.	+C	Janvier (1930)
Pseudagenia gayi Spin.	*Araneus cinaberinus*	Ara.	+C	Janvier (1930)
Pseudagenia mellipes (Say)	*Phidippus tripunctatus*	Ara.	+I	Rau (1928); Evans and Yoshimoto (1962)
Salius dispertitus Kohl.	*Lycosa aspersa*	Ara.	+	Janvier (1930)
Salius dumosus Guér.	*Lycosa aspersa*	Ara.	+	Janvier (1930)
Salius flavipes Guér.	*Lycosa* spec.	Ara.	+	Janvier (1930)
Salius hirticeps Guér.	Young tarantulas	Ara.	+	Janvier (1930)
Sericopompilus apicalis (Say)	Anyphaenidae	Ara.	+C	Evans and Yoshimoto (1962)
	Thomisidae	Ara.	+C	
	Salticidae	Ara.	+C	
	Oxyopidae	Ara.	+C	
	Araneidae	Ara.	+C	

Tachyachetes filicornis Tournier	Salticid spiders	Ara.	+T	Gros (1982c)
Tachypompilus ferrugineus (Say)	*Lycosa antelucana*	Ara.	+	Evans and Yoshimoto (1962)
	Lycosa rabid	Ara.	+	
	Lycosa helluo	Ara.	+	
	Dolomedes sp.	Ara.	+	
Turneromylia melangolicus (Smith)	*Olios punctatus*	Ara.	+	Evans *et al.* (1981)
Superfamily Sphecoidea				
Alysson melleus Say	Cicadellidae	Hem.	+PC (?)	Evans (1966a)
Ammatomus moneduloides (Packart)	*Rhynchomita microrhina*	Hem.	+	Krombein (1959)
Ammobia ichneumoneta (L.)	*Neoconocephalus ensiger*	Lep.	+PI	Frisch (1937)
Ammobia pensylvanica (L.)	*Microcentrum rhombifolium*	Orth.	+TI	Frisch (1938)
Ammophila aemulans Kohl	*Gonoclostera timonides*	Lep.	+C	Tsuneki (1963)
Ammophila azteca Cam.	*Smerinthus* sp.	Lep.	+	Evans (1965)
	Nematus sp.	Hym.	+	
	Amauronematus sp.	Hym.	+	
Ammophila campestris Latr.	*Salandriino* sp.	Hym.	+(I)	Grandi (1926)
Ammophila dives Brullé	Crickets	Ort.	+	Smirnov (1915), see Roth (1928)
Ammophila haimatosoma Kohl	Noctuidae	Lep.	+I	Roth (1928)
Ammophila heydeni (Dahlb.)	Geometridae	Lep.	+I	Ferton (1908); Molitor (1939a,b)
	Noctuidae	Lep.	+I	Krombein (1972)
Ammophila hirsuta Scop.	Caterpillars	Lep.	+	Fabre (1879–1910)
Ammophila laevicollis Ed. André	Caterpillar	Lep.	+	Ferton (1920)
Ammophila parkeri Menke	Geometridae	Lep.	+PC	Powell (1964)
Ammophila polita	Caterpillar	Lep.	+I	Peckham and Peckham (1898)
Ammophila procera (Dahlb.)	*Nadata gibbora*	Lep.	+I	Hartman (1905); Evans (1959a)
Ammophila rufipes Guér.	Caterpillars	Lep.	+I	Janvier (1928)
Ammophila sabulosa L.	Caterpillars	Lep.	+	Maneval (1932); Malyshev (1966)
	Geotrupidae	Lep.	+TI	T. Piek (personal observation)
Ammophila sabulosa nipponica Tsun.	Noctuidae	Lep.	+I	Tsuneki (1968a)
Ammophila tydei Guill.	*Agrostis* sp.	Lep.	+	Roth (1928)
Ammophila urnaria Cress.	Caterpillars	Lep.	+I	Peckham and Peckham (1898)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Ampulex assimilis Kohl.	*Shelfordella tartara*	Dic.	+I(T)	Hingston (1925a, 1928)
Ampulex canaliculatus Say	*Poreoblatta virginia*	Dic.	+(TC)I	Williams (1929)
Ampulex compressa (Fabr.)	*Periplaneta americana*	Dic.	+TI	Ferchauld (1742); Maxwell-Lefroy (1909); Williams (1942)
Ampulex sibirica (= *compressiventris* Guér.)	Cockroaches	Dic.	+I	Sharp (1901)
Ampulex sonnerati Kohl	Cockroaches	Dic.	+I	Sonnerat (1776)
Aphilantrops frigidus	Queen ant	Hym.	+C(I)	Peckham and Peckham (1898)
	Formica fusca	Hym.	+D	Wheeler (1928)
Astata bicolor Say	Bugs	Hem.	+C(I)	Peckham and Peckham (1898)
Astata boops Schrank	Pentatomidae	Hem.	+I	Tsuneki (1969b)
Astata boops graeca de Beaumont	*Lygaeus pandurus*	Hem.	+	Krombein (1972)
Astata occidentalis Cress.	Pentatomidae	Hem.	+I	Evans (1957)
Astata picea A. Costa	*Dolychoris baccarum*	Hem	+	Ferton (1901)
Astata unicolor Say	Bugs	Hem.	+C(I)	Peckham and Peckham (1898)
Bembecinus neglectus (Cress.)	*Gyponana octolineata*	Hem.	+C	Evans (1955)
Bembecinus tridens (Fabr.)	*Idiocerus taeniops*	Hem.	+I	Ferton (1902); Lüps (1973)
(= *Stizus*)	*Homotoma ficus*	Hem.	+I	
Bembix belfragei Cress.	Lygaeidae	Hem.	+P	Hartman (1905)
Bembix brullei Spinola	*Toxomerus* sp.	Dip.	+C(†)	Janvier (1928)
	Sarcophaga flavifrons	Dip.	+C(†)	
	Syrphus gayi	Dip.	+C(†)	
	Syrphus pyrastri	Dip.	+C(†)	
	Melanostoma fenestratum	Dip.	+C(†)	
	Sarconesiopsis caerulea	Dip.	+C(†)	
	Eristalis tenax	Dip.	+I	

Bembix (= Monedula) chilensis (Spin.)	Diptera	Dip.	+CI	Janvier (1928)
Bembix integra Panz.	Diptera (5 families)	Dip.	+	Grandi (1926)
Bembix musca Handl.	*Trigonia carbonaria*	Hym.	+P(I)	Evans and Matthews (1973b)
Bembix oculata Latr.	*Musca corvine*	Dip.	+C	Ferton (1899)
	Melithreptus strigosus	Dip.	+C	
	Eristalis tenax	Dip.	+C	
	Lucilia sericata	Dip.	+C	
	Merodon spinnipes	Dip.	+C	
	Flies	Dip.	+PC	T. Piek (personal observation)
Bembix rostrata L.	Flies	Dip.	+PC	Fabre (1879–1910); Wesenberg Lund (1891); Marchal (1893); Ferton (1899)
Bembix spinola Lep.	*Lucilia ceasar*	Dip.	+I	Krombein (1936)
Bembix sienebdoma Parker	Lacewings	Neu.	+	Evans (1978)
Bembix tuberculiventris Turn.	Colletidae, Halictidae	Hym.	+PC	Evans and Matthews (1973b)
Bembix zonata Klug	*Lathyrophthalmus aenus*	Dip.	+	Bernard (1935)
	Flies	Dip.	+PC	T. Piek (personal observation)
Bembidula variegata Spinola	Hemiptera	Hem.	+I	Janvier (1928)
Bicyrtes fodiens (Handl.)	*Mormidea lugens*	Hem.	+PC(I)	Evans (1966a)
Bicyrtes quadrifasciata Say	Stinkbugs	Hem.	+PC(I)	Evans (1966a)
Bicyrtes ventralis	Pentatomidae	Hem.	+P	Parker (1917)
Bothynostethus distinctus Fox	Chrysomelidae	Col.	+I	Kurczewski and Evans (1972)
Brachymeris curvitarsis Latr.	*Liometopum microcephalum*	Hym.	+I	Emery (1893)
Brachymerus luteicollis (Lep. and Br.)	*Tapinoma erraticum*	Hym.	+I	Ferton (1890)
Cerceris arenaria L.	*Brachyderes incanus*	Col.	+CI(PC)	Grandi (1926); Hamm and Richards (1930); T. Piek (personal observation)
	Brachyrhinus sulcatus	Col.	+PC	Smith (1932)
Cerceris auritus	Rhinophoridae	Col.	+	Bosc, see Walckenaer (1817)
Cerceris blakei Cress.	*Derelomus basalis*	Col.	+	Krombein (1963)
Cerceris bupresticida Duf.	Curculionidae	Col.	+PC	Fabre (1879–1910); Dufour (1841)
Cerceris californica Cress.	*Acmaeodera* spp.	Col.	+D?	Linsley and MacSwain (1956)
Cerceris chilensis Spinola	Curculionidae	Col.	+	Janvier (1928)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Cerceris clypeata Dahlb.	Beetles	Col.	+P(I)	Peckham and Peckham (1898)
Cerceris deserta Say	Beetles	Col.	+P(I)	Peckham and Peckham (1898)
Cerceris emarginata Panz.	*Halictus* spp.	Hym.	+CI	Ferton (1910)
Cerceris finitima Cress.	*Chaetocnema pulicaria*	Col.	+	Strandtmann (1945)
Cerceris fumipennis Say	Bupresiticidae	Col.	+	Cartwright (1931)
Cerceris gayi Spinola	Coccinellidae	Col.	+CI	Janvier (1928)
Cerceris hortivaga Kohl	*Halicuts* spp.	Hym.	+I	Tsuneki (1965a)
Cerceris nigrescens Smith	*Hyperodes delumbis*	Col.	+I	Krombein (1936)
	Beetles	Col.	+PC	Peckham and Peckham (1898)
Cerceris ornata Latr.	*Halicuts* spp.	Hym.	+TI	Marchal (1887)
Cerceris quadrifasciata Panz.	*Strophosomus capitatus*	Col.	+C	T. Piek (personal observation)
Cerceris quinquefasciata Rossi	*Crioceris* sp.	Col.	+I	Hamm and Richards (1930)
Cerceris raui Roh.	*Thecesternus humeralis*	Col.	+T(I)	Rau (1928)
Cerceris rubida Jur.	Curculionidae	Col.	+I	Grandi (1926)
Cerceris rufinoda Cress.	*Tychius picirostris* Col.	Col.	+I	Strandtmann (1945)
Cerceris rybyensis (L.)	*Halictus* spp.	Hym.	+T	Marchall (1887); Hamm and Richards (1930)
Cerceris serripes Fabr.	*Calandra*	Col.	+P	Strandtmann (1945)
Cerceris simplex J. Smith	*Metapoloba pruinosa*	Col.	+I	Alcock (1974)
(= *C. ornata* Latr.)	*Halictus* spp.	Hym.	+D	
	Halicuts laevigatus	Hym.	+I	Molitor (1939b); Bristowe (1948)
Cerceris tuberculata v.d. Lind.	*Cleonus ophtalmicus*	Col.	+	Fabre (1855, 1879–1910)
Chlorion atratum (Lepel.)	Grasshoppers	Ort.	+	Strandtmann (1945)
Chlorion aurentulus Fabr.	*Gryllacris brevispina*	Ort.	+I	Williams (1919c)
Chlorion auripes Fern.	Orthoptera	Ort.	+TI	Rau (1928)
Chlorion caeruleum L.	*Gryllulus abbreviatus*	Ort.	+I	Peckham and Peckham (1898)

Chlorion ichneumoneum (L.)	*Orchelium vulgare*	Ort.	+I	Rau and Rau (1918)
	Microcentrum sp.	Ort.	+PI	Woodbury (1930); Frisch (1937)
	Longhorned grasshoppers	Ort.	+I	Ristich (1953)
Chlorion laeviventrus (Cress.)	*Anaburs simplex*	Ort.	+I	La Rivers (1945)
Clypeadon evansi Bohart	*Pogonomyrmex rugosus*	Hym.	+C	Alcock and Gamboa (1975)
Clypeadon latynctus (Cress.)	*Pogonomyrmex occidentalis*	Hym.	+	Evans and Eberhard (1970)
Coelocrabro ambiguus (Dahlb.)	Jassidae	Ort.	+I	Bristowe (1948)
Crabro brevinodus Spinola	Aphididae	Hem.	+I	Janvier (1928)
Crabro brevis v.d. Lind.	*Crepidodera ferruginea*	Col.	+C(I)	Benoist (1915); Grandi (1927); Maneval (1928)
(= *Entomognatus*)	*Thyamis succineus*	Col.	+C(I)	
	Longitarsus luridus	Col.	+C(I)	
Crabro claudii Herbst.	Aphididae	Hem.	+	Janvier (1928)
Crabro crassinodus Spin.	Aphididae	Hem.	+I	Janvier (1928)
Crabro davidsoni Sandh.	*Empoasca* spp.	Hem.	+	Davidson and Landis (1938)
	Typhlocyba spp.	Hem.	+	
	Erythroneura spp.	Hem.	+	
Crabro gayi Spinola	*Taxomerus* sp.	Dip.	+C(I)	Janvier (1928)
	Melanostoma sp.	Dip.	+C(I)	
	Hilemya cilicrura	Dip.	+C(I)	
	Helomyza sp.	Dip.	+C(I)	
	Acinia sp.	Dip.	+C(I)	
Crabro interruptus	Moths	Lep.	+	Peckham and Peckham (1898)
Crabro longinodus Spinola	*Sarcophaga* spp.	Dip.	+I	Claude-Joseph (1928)
	Helomyza spp.	Dip.	+I	
	Ortalis spp.	Dip.	+I	
Crabro peltarius (Schreber)	*Musca domestica*	Dip.	+I	Simon Thomas and Veenendaal (1974)
Crabro rufotaeniatus Kohl	*Anopheles annuliventris*	Dip.	+?(C?)	Janvier (1928)
	Chironomus pallidulus	Dip.	+?(C?)	
	Phthyria sp.	Dip.	+?(C?)	
	Microlepidoptera	Lep.	+?(C?)	
	Ants	Hym.	+?(C?)	

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Crabro sexmaculatus	Flies and gnats	Dip.	−?	Peckham and Peckham (1905)
Cratolarra pitamawa Rohw.	Gryllid crickets	Ort.	+I	Williams (1919c)
Crossocerus elongatulus (v.d. L.)	Flies	Dip.	+I	Bristowe (1948)
Diploplectron peglowi Krombein	*Sphaerobius insignis*	Hem.	+I	Kurczewski (1972a)
	Lygaeus sp.	Hem.	+I	
Dolichurus bicolor (Lep.)	*Ectobia livida*	Dic.	+I	Maneval (1932)
Dolichurus corniculus Spin.	*Ectobius lapponicus*	Dic.	+I	Ferton (1923); Maneval (1928)
	Blatta germanica	Dic.	+CT	Benoist (1927)
Dolichurus haemorrhous Costa	*Loboptera decipiens*	Dic.	+I	Ferton (1895b)
Dolichurus stantoni Asm.	Cockroaches	Dic.	+I	Williams (1919c)
Diodontus americanus Packard	Aphids	Hem.	+PC(I)	Peckham and Peckham (1898)
Feronius formicarius Ferton	*Tapinoma erraticum*	Hym.	+I	Ferton (1895a)
Glenostictia scitula Fox.	*Perdita* bees	Hym.	+D( )	Evans (1966a)
Gorytes caniculatus (Pack.)	Cicadellidae	Hem.	+PCD	Evans (1966a)
Gorytes chilensis Sauss.	*Dictiophora chilensis*	Hem.	+	Janvier (1928)
Gorytes gayi Spinola	Homoptera	Hem.	+C(I)	Janvier (1928)
Gorytes planifrons (Wesm.)	*Issus coleoptratus*	Hem.	+C	Maneval (1939)
Harpactopus (= Sphex) abdominalis Say	*Disosteria carolina*	Ort.	+TI	Peckham and Peckham (1898)
Harpactus laevis Latr.	*Athysanus* sp.	Hem.	+I	Maneval (1928)
Hoplocrabro quadrimaculatus F.	Flies	Dip.	+	Maneval (1928)
Hoplisoides costalis (Cress.)	Membracidae	Hem.	+PC	Reinhard (1925)
Hoplisoides tricolor (Cress.) (= *Psammaecius*)	*Parabolocatus bunneus*	Hem.	+C	Evans *et al.* (1954)
Isodontia mexicana Sauss.	*Oecanthus niveus*	Ort.	+I	Lin (1966)
Larra americana Saussure	*Sexpteriscus didactylus*	Ort.	+TI	Williams (1928a)

Larra analis Fabr.	*Gryllotalpa hexadactyla*	Ort.	+T	Smith (1935)
Larra anathema Rossi	*Gryllotalpa gryllotalpa*	Ort.	+TC	Malyshev (1941), see Malyshev (1966)
Larra argentata	Crickets	Ort.	+C	Ashmead (1894)
Larra femorata (Sauss.)	*Grylloptalpa coarctata*	Ort.	+T	Williams (1928a)
Larra lusonensis Rohwer	*Nemobius histro*	Ort.	+(T)I	Williams (1919c)
Larra scelesta Turner	*Gryllotalpa nitidula*	Ort.	+T	Williams (1928a)
Larropsis distincta Sm.	Crickets	Ort.	+I	Bohart and Bohart (1962)
Lindenius albilabris (F.)	Chloropidae	Dip.	+I	Bristowe (1948)
Lindenius panzeri (v.d. Lind.)	Flies	Dip.	+I	Abrahamsen, see Miller and Kurczewski (1975)
Liris aequalis (Fox)	Cricket nymphs	Ort.	+T	
Liris argentata (Beauvois)	Cricket nymphs	Ort.	+T	
Liris haemorrhoides Fabricius	Crickets	Ort.	+I	Williams (1928a)
Liris nigra v.d. Lind.	*Gryllulus domesticus*	Ort.	+T	Steiner (1958, 1962, 1976)
Lyroda subita Say	Crickets	Ort.	+I	Peckham and Peckham (1898)
Mellinus arvensis (L.)	Syrphidae, Stratiomyidae	Dip.	+P	Rabaud (1917); Spooner (1928); Molitor (1939a); Bristowe (1948)
	Flies of different genera	Dip.	+P(I)	Huber (1961)
Mellinus bimaculatus Pack.	*Pegomya* sp.	Dip.	+C	O'Brien (1983)
Mellinus sabulosus F.	Flies	Dip.	+PI	Adlerz (1903); see Hamm and Richards (1930)
Microbembex californica Bohart	Col., Dip., Hym., Hem., Der.	—	—	Goodman (1970)
	Spiders	Ara.		
Microbembex monodonta (Say)	Dead arthropods	—	—	Evans (1966a)
Microbembex sulfurea Spinola	*Galeodes variegata*	Ara.	+C(I)	Janvier (1928)
[= *M. ciliata* (Fabricius)]	Fly larvae	Dip.	+C(I)	
	Beetle larvae	Col.	+C(I)	
	Ants	Hym.	+C(I)	
Microbembex uruguayensis Holmberg	Carabid beetles	Col.	+	Llano (1959), see Evans (1966a)
Memesa bicolor Shuchard	*Grypotes puncticollis*	Hem.	+	Janvier (1955)
Miscophus bicolor Dahlb.	Spiders	Ara.	+P	Ferton (1895a)
Miscophus bonifaciensis Ferton	*Lycosa perita*	Ara.	+P	Ferton (1895a)
	Epeira sp.	Ara.	+P	

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Monedula chilensis Spin [= *Zyrzyx chilensis* (Eschschola)?]	Flies	Dip.	+I	Janvier (1928)
Nitelopterus slossonae (Ashmead)	Spiders	Ara.	+	Krombein and Kurczewski (1963)
Notogonidea williamsi Rohw.	*Nemobius histro*	Ort.	+(T)I	Williams (1919c)
Oxybelus ambiguus Gerst.	*Asilus atricapillus*	Dip.	+TC	Ferton (1902)
Oxybelus emarginatum Say	*Atherigona orientalis*	Dip.	+I	Krombein and Kurczewski (1963)
Oxybelus exclamans Viereck	*Senatainia* sp. (Sarcophagidae)	Dip.	+I	Kurczewski (1972b)
Oxybelus melancholicus Chevrier	Flies	Dip.	+C	Ferton (1923)
Oxybelus quatuordecimnotatus Oliv.	*Sarcophaga arorum*	Dip.	+C	Ferton (1902)
	Rhinophora decepticola	Dip.	+C	
	Lauxania aena	Dip.	+C	
	Chortophila striolata	Dip.	+C	
Oxybelus sericeum Robert	*Ephydra riparia*	Dip.	+	Bohart and Marsh (1960)
Oxybelus uniglumis L.	Flies	Dip.	+	Wesenberg Lund (1891)
	Flies	Dip.	+I	Adlerz, see Hamm (1930)
	Flies	Dip.	+C	Steiner (1979)
	Flies	Dip.	+C	T. Piek (personal observation)
Palarus latifrons Kohl	*Apis mellifera*	Hym.	+PC	Skaife (1954)
	Ichneumonidae	Hym.	+	
	Apidae	Hym.	+	
Palarus orientalis Kohl	*Apis indica*	Hym.	+C	Cherian (1937), see Clausen (1940)
Palarus saishiuensis Okamoto	*Thiphia* spp.	Hym.	+	
	Sphecidae	Hym.	+	
Paranysson melanopyrus (Smith)	*Natalicola pallidus*	Hem.	+I	Bequaert (1933)
Passaloecus eremita Kohl	Aphids	Hem.	–	Lombolt (1973)

Passaloecus gracilis	Aphids	Hem.	–	Corbet and Blackhouse (1975)
Passalocecus isignis	Aphids	Hem.	–	Corbet and Blackhouse (1975)
Pemphredon lugubris (Fabr.)	*Pterochlorus roboris*	Hem.	+PI	Janvier (1961)
Philanthus bilunatus Cress.	Colletidae	Hym.	+P	Evans (1959□)
	Halictidae	Hym.	+P	
Philanthus denticollis Spinola	*Halictus* spp.	Hym.	+TI	Janvier (1928)
	Apis mellifera	Hym.	+TI	
	Melitoma chilensis	Hym.	+TI	
Philanthus flavifrons Cress.	*Apis mellifera*	Hym.	+I	Bohart (1954)
Philanthus gibbosus Fabr.	*Halictus* spp.	Hym.	+C(I)	Reinhard (1924)
	Crossocercus sulcus	Hym.	+	Evans (1959b)
Philanthus lepidus Cress.	Andrenidae	Hym.	+	Evans (1959b)
	Halictidae	Hym.	+	
Philanthus politus Say	Colletidae	Hym.	+P	Evans (1959b)
	Andrenidae	Hym.	+P	
	Halictidae	Hym.	+P	
Philanthus politus pacificus Cress.	Bees and wasps	Hym.	+	Powell and Chemsak (1959)
Philanthus punctatus	*Halictus* spp.	Hym.	†(=PC?)	Peckham and Peckham (1898)
Philanthus solivagus Say	Eumenidae, Andrenidae	Hym.	+P	Evans (1959b)
	Halictidae, Colletidae	Hym.	+P	
Philanthus triangulum (F.)	*Apis mellifera*	Hym.	+T(I)	Fabre (1879); Roth (1917); Rathmayer (1962a,b)
Philanthus venustus (Rossi)	*Halictus* Spp.	Hym.	+	Ferton (1905)
(= *raptor* Lep.)	*Andrena schinkenella*	Hym.	+	
	Evylaeus interruptus	Hym.	+	Krombein (1972)
Pison areolatus Spinola	Spiders	Ara.	+C	Janvier (1928)
Pison chilense Spinola	Spiders	Ara.	+C	Janvier (1928)
Podalonia luctuosa F. Smith	Lepidoptera	Lep.	+I	Newcomer (1938)
	Noctuid larvae	Lep.	+PI	Steiner (1983b)
Podalonia hirsuta Sopoli	*Mamestra brassicae*	Lep.	+PI	Gervet and Fulcrand (1970)
				Truc and Gervet (1974)
	Agrostis nigrum	Lep.	+P	

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Podalonia valida (Cress.)	*Estigmene ?acraea*	Lep.	+	Steiner (1974)
Podalonia violaceipennis Lep.	*Malacosoma* sp.	Lep.	+	Williams (1928b); Hicks (1932b); Krombein (1936)
	Lycophota saucia	Lep.	+	
	Chorizagrostis agrestis	Lep.	+	
Podium flavipenne Latr.	*Epilampa abdomen-nigrum*	Dic.	+TI	Williams (1928a)
Prionyx astratus Lep.	*Disosteria carolina*	Ort.	+TI	Peckham and Peckham (1898)
	Melanoplus differentalis	Ort.	+I	Rau and Rau (1918)
Prionyx parkeri Bohart & Menke	Oedipodinae	Ort.	+	Steiner (1981a,b)
Prionyx subfuscatus (Dahlb.)	Grasshoppers	Ort.	+I	Evans (1958)
Prionyx striatus Smith	*Xyleus ?centralis*	Ort.	+	Evans (1958)
Prionyx thomae (F.)	Locusts	Ort.	+I	Hartman (1905)
	Arphia xanthoptera	Ort.	+D	Rau and Rau (1918)
Psammophila tydei Le Guillon	Caterpillars	Lep.	+	Hingston (1928)
Psen ater (F.) [*Dahlbomia atra* (F.)]	*Issus coleoptratus*	Hem.	+P(T)	Janvier (1955)
	Jassus lanio	Hem.	+	
	Cixius simplex	Hem.	+	
Rubrica surinamensis (De Geer)	Diptera	Dip.	−(?)	Evans *et al.* (1974)
	Perthemis moona Odo.	Odo.	−(?)	
	Monea sp.	Lep.	−(?)	
Salius conicus Say	Lycosidae	Ara.	+(?)	Peckham and Peckham (1898)
Salius opacus (Pérez)	*Nemesia badia*	Ara.	+P(T)	Ferton (1910)
Seciphon caementarium (Drury) (= *Pelopaeus caementarius*)	*Epeira strix* (= *Araneus cornutus*)	Ara.	+T(C)	Peckham and Peckham (1898)
	Epeira juniperi	Ara.	+	Rau and Rau (1918); Rau (1928)
	Aegiope cophenaria	Ara.	+	

Sceliphron spirifex L.	Spiders	Ara.	+	Aptel (1929)
Sceliphron vindex Lep.	*Thomisus graciosus*	Ara.	+(I)	Janvier (1928)
	Attus elegans	Ara.	+(I)	
	Attus comatus	Ara.	+(I)	
	Araneus cinaberinus	Ara.	+(I)	
	Araneus labyrinthea	Ara.	+(I)	
	Philodromus funebris	Ara.	+(I)	
	Metargiope trifasciata	Ara.	+(I)	
Sericophorus sculpturatus Rayment	Blowflies	Dip.	+C	Rayment (1955)
Solenius nigrifrons (Cress.)	*Sirphus ribesii*	Dip.	+	Krombein (1936)
Sphecius speciosus (Drury)	*Tibicen pruinosa*	Hem.	+PC	Riley (1892); Harzell (1935); Evans (1966a)
	Tibicen species	Hem.	+	Lin (1979)
Sphex aberti (Hald.)	Geometridae	Lep.	+T	Hicks (1932a)
Sphex aegyptius Lep.	Locusts	Ort.	+	Newman (1965)
Sphex albisecta Lep.	Locusts	Ort.	+	Fabre (1879–1910)
Sphex argyrius Brullé	*Pholidoptera* sp.	Ort.	+I	Berland (1958)
Sphex chilensis Spinola	*Acridium* sp.	Ort.	+	Janvier (1928)
Sphex cognatus Smith	*Conocephalus*	Ort.	+PI	Ribi and Ribi (1979)
Sphex cyaniventris Guérin	*Mantis crenaticollis*	Dic.	+I	Janvier (1928)
Sphex ichneumonea L.	Meadow grasshopper	Ort.	+TC	Peckham and Peckham (1898)
Sphex latreilli Lep.	Orthoptera	Ort.	+I	Janvier (1928)
Sphex lobatus F.	*Brachytrypes portensus*	Ort.	+TC	Hingston (1928)
Sphex maxillosus F. (= *flavipennis*)	Tettigoniidae	Ort.	+ID	Fabre (1879–1910); Molitor (1939a)
	Locusts	Ort.	+	Picard (1903)
Sphex nigellus Smith	*Conocephalus melas*	Ort.	+C(I)	Piel (1933); Tsuneki (1964)
	Conocephalus gladiatus	Ort.	+	
	Hexacentrus japonicus	Ort.	+	
	Ducretia japonica	Ort.	+	
Sphex nigricans (Dahlb.)	*Catocala* sp.	Lep.	+I	Strandtmann (1945)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Sphex occitanicus L. and S.	*Locusta viridissima*	Ort.	+	Fabre (1879–1910); Ferton (1909); Berland (1938)
	Ephippigera terrestris	Ort.	+	
	Ephippigera vitrum	Ort.	+	
Sphex paludosus Rossi	*Platycleis tesselata*	Ort.	+	Berland (1959)
Sphex subfuscatus Dahlb.	*Chorthippus fuscipennis*	Ort.	+I	Piel (1935)
	Calopterus italicus	Ort.	+C(I)	Ferton (1902)
Sphex umbrosus Christ.	*Homorocoryphus fuscipes*	Ort.	+I	Piel (1935)
Steniola duplicata (Prov.)	Flies	Dip.	?	Evans (1966a)
Steniola obliqua (Cress.)	Flies	Dip.	?	Evans (1966a)
Stictia carolina (Fabr.)	Horse flies	Dip.	+TC	Evans (1966a)
Stictiella evansi Gillaspy	Moths and butterflies	Lep.	+PC(†?)	Gillaspy *et al.* (1962)
Stictiella formosa (Cress.)	Moths and butterflies	Lep.	+PC(†?)	Gillaspy *et al.* (1962)
Stictiella serrata (Handl.)	Moths and butterflies	Lep.	+PC(†?)	Gillaspy *et al.* (1962)
Stizus (= Gorytes) fertoni Handl.	*Phaneroptera nana*	Ort.	+I	Ferton (1908, 1910)
	Athysanus limbatus	Hem.	+	
Stizus pulcherrimus F. Smith	*Chorthippus dubius*	Ort.	+I	Tsuneki (1956b)
	Tettigoniidae	Ort.	+I	
Tachysphex bengalensis Iwata	*Oedaleus infernalis*	Ort.	+I	Tsuneki (1969a)
Tachysphex fluctuatus	*Manta religiosa*	Dic.	+PC	Cros (1936)
Tachysphex helveticus Kohl	*Galliptamus ?italicus*	Ort.	+	Krombein (1972)
Tachysphex julliani Kohl	*Manta religiosa*	Dic.	+	Ferton (1901)
Tachysphex mantiraptor Fert.	*Manta religiosa*	Dic.	+	Ferton (1911)
Tachysphex mediterraneus Kohl	*Oecanthus pellucens*	Ort.	+I	Ferton (1908)
Tachysphex pisonoides Spinola	*Phymata carinata*	Hem.	+C	Janvier (1928)
	Nabis punctipennis	Hem.	+C	

Tachysphex rufitarsis Spinola	Locusts	Ort.	+	Janvier (1928)
Tachysphex terminatus Smith	Grasshoppers	Ort.	+I	Rau and Rau (1918)
Tachytes crassus Patton	*Orchelimum* spp.	Ort.	+I	Evans and Kurzewski (1966)
Tachytes europaea	Acrididae	Ort.	+	Ferton (1923)
Tachytes mandibularis Patton	Grasshoppers	Ort.	+I	Strandtmann (1945)
Tachytes mergus (Fox)	*Tridactylus apicalis*	Ort.	+I	Kurczewski (1966)
Tachytes minutes Rohwer	Pygmy molecrickets	Ort.	+I	Kurczewski (1966)
Trachypus mexicanus (Saussure)	Halictinae	Hym.	+	Evans (1964a)
	Anthophorinae	Hym.	+	
Trigonopsis abdominale Perty	Cockroach	Dic.	+I	Williams (1928a)
Trigonopsis cameroni Kohl	*Riata fulgida*	Dic.	+	Eberhard (1974)
	Charisoneura translucida	Dic.	+	
Trigonopsis moraballi Richards	Cockroaches	Dic.	+I	Richards (1937)
Trypoxylon albospilosum Fox	Spiders	Ara.	+P	Peckham and Peckham (1898)
Trypoxylon clavicerum Lepeletier	Epeiridae	Ara.	+I	Hamm and Richards (1930)
Trypoxylon elongatum Ashm.	Attid spiders	Ara.	+I	Williams (1919c)
Trypoxylon figulus L.	Spiders	Ara.	+P(I)	Malyshev (1911), see Malyshev (1966); Degroot (1971)
Trypoxylon politus	Spiders	Ara.	+	Newman (1965)
Trypoxylon rubrocinctus Packard	Epeiridae	Ara.	+PC(I)	Peckham and Peckham (1898)
Trypoxylon texense Saussure	Tetragnathidae	Ara.	+TI	Hartman (1905); Kurczewski (1963)
	Araneidae	Ara.	+TI	
Trypoxylon xanthandrum Richards	Seconculidae	Ara.	+	Coville and Griswold (1983)
Xylocelia americana	Aphids	Hem.	–	Peckham and Peckham (1898)
Xylocelia metathoracica (Mickel)	Aphids	Hem.	–	Rau and Rau (1918)
Xylocelia occidentalis (Fox)	Aphids	Hem.	–	Powell (1963)
Superfamily Vespoidea				
Subfamily Masarinae				
Euparagia scutellaris Cresson	Curculionidae	Col.	+I	Williams (1927)
Subfamily Eumeninae				
Ancistrocerus fulvipes Sauss.	*Characoma nilotica*	Lep.	–?	Rau and Rau (1918)
Ancistrocerus tardinotus Bequart	Caterpillars	Lep.	+P(I)	Taylor (1922)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Discoelius zonalis Panz.	*Corcyra cephalonica*	Lep.	+PI	T. Piek and R. L. Veenendaal (personal observations)
Eumenes arbustorum Panzer	*Spilothyrus alceae*	Lep.	+	Chrétien (1896); Deleurance (1945)
	Lycaena cyllarus	Lep.	+	
	Metroptria monogramma	Lep.	+	
	Agrotera trabealis	Lep.	+	
	Plusia gamma	Lep.	+	
	Pterophorus monadactylus	Lep.	+	
Eumenes conica	Caterpillars	Lep.	+C(I)	Hingston (1928)
Eumenes curvata Sauss	Caterpillars	Lep.	+I	Williams (1919c)
Eumenes pomiformis Fabr.	*Heliothis dipsaceus*	Lep.	+	Fabre (1879–1910); Chrétien (1896); Ferton (1902);
	Lydia adustata	Lep.	+	Deleurance (1945)
	Pionea extimalis	Lep.	+	
	Plutella cruciferarum	Lep.	+I	
	Eupithecia oxycedrata	Lep.	+I	
	Cidaria unifasciata	Lep.	+I	
	Pyrausta sanguinalis	Lep.	+I	
Eumenes tinctor	*Plusia* sp.	Lep.	+I	Roubard (1916)
	Pieris sp.	Lep.	+I	
Euodynerus foraminatus	Caterpillars	Lep.	+I	Steiner (1983a)
Manobia quadridens Linn.	*Epipaschia* sp.	Lep.	+I	Rau and Rau (1918)
Odynerus ambiguus Spin.	Caterpillars	Lep.	+I	Janvier (1930)
Odynerus anormis	Lepidoptera	Lep.	+I	Peckham and Peckham (1905)
Odynerus antilope (Panzer)	*Tetralopha* sp.	Lep.	+T	Mauvezin (1886); Medler and Fye (1956); Cooper (1953)
	Mineola indigelella	Lep.	+T	
	Salebria subcaesiella	Lep.	+T	
	Nephopteryx	Lep.	+T	
	Archips ferridana	Lep.	+T	

Odynerus arvensis Sauss.	Caterpillars	Lep.	+I	Hartman (1905)
Odynerus capra	Caterpillars	Lep.	+I	Peckham and Peckham (1898)
Odynerus colocolo Sauss.	Caterpillars	Lep.	+I	Janvier (1930)
Odynerus conformis	Caterpillars	Lep.	+I	Peckham and Peckham (1898)
Odynerus dilectus Sauss.	*Hypera postica*	Col.	+	Bohart *et al.* (1982)
Odynerus dorsalis Fabre	Caterpillars	Lep.	+I	Hartman (1905)
Odynerus consobrinus Duf.	Tenthreid larvae	Hym.	+C	Bernard (1934)
Odynerus egregius Schaeffer	Noctuid larvae	Lep.	+I	Bernard (1934)
Odynerus fastidiossimus	Caterpillars	Lep.	+I	Ferton (1901)
Odynerus geminus Cress.	*Loxostege ?semilalis*	Lep.	+PI	Rau and Rau (1918)
Odynerus herrichi Sauss.	Tortricidae spp.	Lep.	+	Spooner (1934)
Odynerus humeralis	Caterpillars	Lep.	+I	Janvier (1928, 1930)
Odynerus labiatus Hal.	Caterpillars	Lep.	+PC	Janvier (1930)
Odynerus laevipes Shuck.	*Hypera* spp.	Col.	+I	Bristowe (1948)
Odynerus molinae Sauss.	Small caterpillars	Lep.	+C	Janvier (1930)
	Large caterpillars	Lep.	+I	Janvier (1930)
Odynerus nidulator Sauss.	*Lina populi*	Col.	+P	Fabre (1879–1910)
Odynerus parietum Linn.	Caterpillars	Lep.	+I	Ferton (1896)
Odynerus quadrifasciatus	Microlepidoptera	Lep.	+	Adlerz, see Nielsen (1932b)
Odynerus reniformis Latr.	*Phytotomus variabilis*	Col.	+PI	Fabre (1879–1910)
Odynerus scabriusculus Spin.	Caterpillars	Lep.	+I	Janvier (1930)
Odynerus suecicus	*Phyllodectus* spp.	Col.	+	Nielsen (1932b)
Odynerus tropicalis Sauss.	Caterpillars	Lep.	+I	Roubaud (1916)
Odynerus tuberculiventris Spin.	Caterpillars	Lep.	+PC	Janvier (1930)
Orancistrocerus drewseni Sauss.	Pyralidae	Lep.	+	Iwata (1976)
Pterochilus chevrierarus	Caterpillars	Lep.	+I	Ferton (1909)
Raphiglossa zethoides	Curculionidae	Col.	+I	Ferton (1923)
Rhynchium anceps Gribido	*Phycitina* spp.	Lep.	+C(I)	Roubaud (1916)
Rhynchium oculatum Scop.	Caterpillars	Lep.	+	Bonelli *et al.* (1980)

(continued)

Table I (*continued*)
Summary of Some of the Aspects of Host Paralysis by Solitary Wasps[a]

Wasp species	Host	Taxon[b]	Paralytic effect	Reference
Rhynchium medium Maindron	Caterpillars	Lep.	+I	Maindron (1882)
Synagris calida L.	Hesperiidae	Lep.	+	Roubaud (1916)
Synagris sicheliana Sauss.	Hesperiidae	Lep.	+C(I)	Roubaud (1916)
	Sphingidae	Lep.	+C(I)	
Zethus dicomba Spin.	Caterpillars	Lep.	+C	Janvier (1930)
Zethus cyanopterus Sauss.	Caterpillars	Lep.	–	Williams (1919c)

[a]The names of wasps and hosts are usually cited as given by the authors noted in the references. Legend: +, paralysis; –, no paralysis; P, permanent; T, transient; C, complete; I, incomplete; D, delayed; (†) dead.

[b]Key to host taxa: Ara., Arachnida; Col., Coleoptera; Dic., Dictyoptera; Dip., Diptera; Emb., Embioptera; Hem., Hemiptera; Hym., Hymenoptera; Lep., Lepidoptera; Neu., Neuroptera; Ort., Orthoptera.

not affected by the sting of a solitary wasp. The most doubtful observations and records have been omitted from Table I but some of the information may be derived from faulty observation, or interpretation, by the author involved. An example might be the works of the Peckhams (1898) who studied 45 species of solitary wasps from the genera *Ammophila, Sphex, Rhopaleus, Crabro, Salius, Aporus, Bembix, Oxybelus, Trypoxylon, Astata, Diodonthus, Cerceris, Philanthus, Pompilus, Agenia, Tachytes, Lyroda, Prionyx, Chlorion* and *Pelopaeus*. The Peckhams described about a third of these wasps as killing their prey instead of paralysing them. They often did not distinguish between dead and completely paralysed prey. In a few cases (e.g. in prey of *Cerceris nigrescens* and *Pompilus quinquenotatus*) they doubted that complete paralysis was possible. The Peckhams described bees stung by *Philanthus punctatus* as being killed immediately. Also, Fabre (1879–1910) recognised that the bees were killed by *P. triangulum*, an observation which was not confirmed by the work of Rathmayer (1962a,b).

The Peckhams described a wasp which paralysed its prey as a 'beginner' in the art of stinging and not a 'master'. They also believed that it was not important whether the prey was killed or paralysed. Olberg (1959) has criticised the Peckhams' conclusion, arguing that it is very difficult to distinguish between death and complete paralysis. The simplest explanation of the presence of a well-developed and specialized sting apparatus is that the paralysis and resulting conservation of prey is not a coincidence and is not without significance for the wasp larva (Olberg, 1959).

The following example may illustrate the way in which data from the literature have been interpreted in composing Table I. Tsuneki (1969b), discussing the condition of pentatomid hosts (Hemiptera) which have been attacked by the sphecid wasp *Astata boops*, found that almost all the bugs removed from wasps after they had attacked them were completely immobile:

> this immobility is not a result of deep paralysis of the prey but of their death. Were the immobility a result of deep paralysis then the prey would be capable of recovery. The immobile prey does not have this ability and, after being taken from the wasp and placed in a small bottle, they become putrefied within a few days. Those few bugs which are not completely immobile may respond to stimuli by sluggish movements of the antennae or legs.

Tsuneki's conclusion that the bugs were immobile because they were dead and not because they were paralysed is obviously erroneous. His description mentions a few bugs that were alive and obviously partially paralysed. Putrefied bugs are obviously dead but their death may be a result of respiratory failure caused by long-term paralysis. Records of dead prey do not necessarily show that these prey were directly stung to death. The interpretation of this report, included in Table I, is that the bugs are paralysed,

sometimes incompletely. This conclusion has also been reached by Evans (1957) for bugs stung by *Astata occidentalis*.

While some individual data in Table I may not be completely reliable, a survey allows the general conclusion that the stings of many solitary Hymenoptera, belonging to families within both the Terebrantia and the Aculeata, paralyse their hosts.

1. Venoms of Terebrant Wasps

The terebrant wasps include three important superfamilies: the Ichneumonoidea, the Cynipoidea and the Chalcidoidea.

a. Ichneumonoidea. This superfamily is one of the largest within the order of Hymenoptera. Of the four families that belong to this group, two are important: the Ichneumonidae and the Braconidae.

All species of the Ichneumonidae are internal or external parasites of larvae of Holometabola (i.e. insects that hatch from the egg into a form, for example a caterpillar, which is quite different in appearance from the adult, in this example a butterfly). They show a marked preference for lepidopteran hosts. Only some species completely paralyse their hosts, the majority do so only incompletely or temporarily. A transient spastic paralysis has been seen in larvae of the greater wax moth, stung by *Coccygomius* (= *Pimpla*) *turionella*. This effect might be a result of a direct induction of contraction of muscle fibres, without neuromuscular transmission being affected. This interpretation is supported by the observation that spastic paralysis also occurs when *C. turionella* stings a wax moth larva which has first been treated with a presynaptic neuromuscular blocking agent from the venom of the braconid wasp *Microbracon hebetor*, (see Section II,B,2), to induce a flaccid paralysis (R. L. Veenendaal, personal communication).

The ichneumon wasp, *Nemeritis canescens*, has been observed to induce a paralysis of short duration in larvae of *Ephestia kuehniella* and *Galleria mellonella* (Piek and Simon Thomas, 1969). Preparations from the venom organs of *N. canescens* were only active when taken from wasps aged 2 or 3 days (Fig. 1). Richards and Thomson (1932), who reported that larvae stung by *N. canescens* were not paralysed, missed observing this very short-lasting paralysis, possibly by studying wasps of the wrong age.

The Braconidae are also parasites of larvae of Holometabola with a marked preference for lepidopteran hosts. In general the endophagous species parasitize free-living hosts and these are not, or only very temporarily, paralysed. Short-lasting paralysis has been described in hosts of many Braconidae: *Apanteles glomeratus* (~30 min) (Piek and Simon Thomas, 1969); *Monoctonus paulensis* (5–8 min) (Calvert and van den Bosch, 1972);

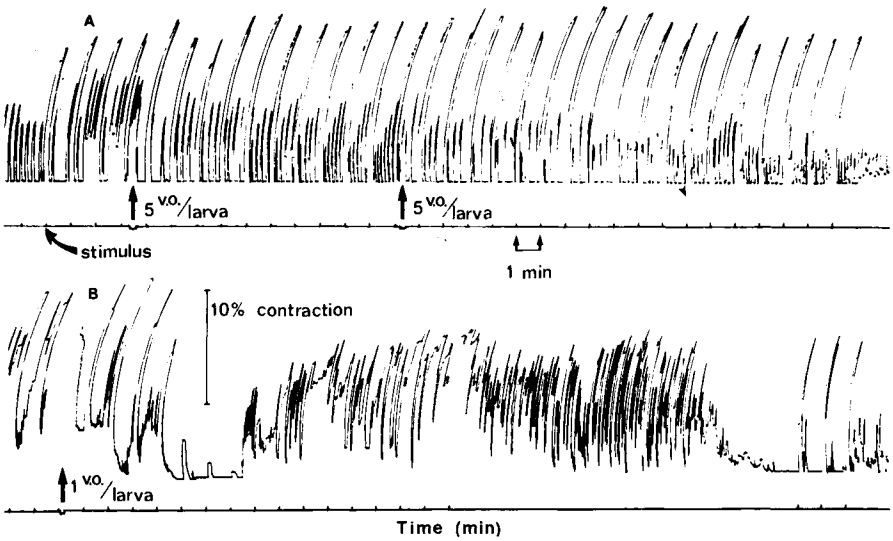


Fig. 1 Isotonic contraction of a *Galleria mellonella* larva, stimulated with pulse trains of 10 sec, pulses of 30V, 100 msec pulse duration and 50 Hz via two copper wires contacting the ends of the larva. Spontaneous contractions are visible between evoked contractions. At times marked by arrows an extract of whole venom organs (v.o.) of *Nemeritis canescens* was injected into the haemolymph. (a) Injection of a relatively large amount of venom solution (five venom organs per larva) made from wasps aged 1.0–1.5 days, followed by injection of a comparable venom solution from wasps aged 5.5–6.5 days. Both venom solutions did not affect the evoked spontaneous contractions. (b) Injection of venom solution (one venom organ per larva) from wasps 2.0–3.0 days old. Note the transient and incomplete paralysis 4–8 min after the injection, followed by a hyperactivity of the larva. Vertical bar represents a contraction of 10% of the relaxed body length.

Rhogas testaceus (3–10 min) (Amad, 1943); *Bassus acrobasidis* (1 min) (Nickels *et al.*, 1950). In contrast, the exophagous species parasitize hosts which have a cryptic life style. The host is usually stung and this often results in a complete and permanent or long-term paralysis (Table I). It is interesting that the onset of paralysis may take some time in the exophagous species. For example, *Iphiaulax kimballi* stings the caterpillar of *Diatraea grandiosella* to a complete paralysis, but with a delay of 10 to 20 min (Kirkland, 1982), and *Cedria paradoxa* attacks caterpillars of *Hapalia machaeralis* and induces a paralysis which is only complete after 24 hr (Beeson and Chatterjee, 1935). The beetle larva *Dendroctonus pseudotsugae* is completely paralysed several hours to two days after being stung by *Cedria brunneri* (Ryan and Rudensky, 1962). This phenomenon has also been observed in the genus *Microbracon*. According to Salt (1931), all *Microbracon* species are exophagous on cryptic hosts. Hosts are generally completely and permanently paralysed, although

appropriate dosages of the venom of a number of *Microbracon* species (*M. hebetor, M. gelechiae, M. kirkpatricki*) cause a reversible paralysis.

The stings of *Microbracon hebetor* and *M. gelechiae* cause a complete and permanent paralysis of their prey within 15 min. Injections of a dilute venom solution results in a paralysis which has a very slow onset and is partly reversible (*M. gelechiae*) or completely reversible (*M. hebetor*) (Fig. 2, see also Figs. 7, 44, and 45 for the effect of two toxins from the venom of *M. hebetor*). It appears that a wasp sting contains so much activity as to be an enormous overdose and that the host has no chance of recovery. This phenomenon could be biologically significant in that it allows the wasp to paralyse host species which have a much lower sensitivity to the venom than the normal host. There is certainly variation in host sensitivities; for example, larvae of *Ephestia figulilella* recover rapidly after they have been stung by *M. hebetor* (Donohoe, see Clausen, 1940) and larvae *Ostrinia nubilalis* are not paralysed at all by the venom of *M. hebetor* (Beard, 1952). Of course, the above wasps will fail to paralyse these hosts, but they might be more successful in hosts with a moderate sensitivity to the venom.

b. Cynipoidea. This superfamily includes ~1600 species of small insects (Imms, 1960). The subfamily Figitinae generally consists of parasites of Diptera, but *Figitis anthomyiarum* transiently paralyses larvae of the mealmoth *Ephestia kuehniella* (Doutt, 1963). A transient paralysis of larvae of flies has been described by Sweetman (1958).

c. Chalcidoidea. The superfamily Chalcidoidea includes many families, most of which are entomophagous, with the eggs, larvae or pupae of holometabolous insects being used as hosts (Clausen, 1940). Within this superfamily larvae and sometimes adults of representatives of nearly all insect orders are accepted as hosts (Table I). In most cases paralysis occurs, often incompletely and/or transiently. However, lack of paralysis is mostly not reported. Thus the relative number of wasps which do not paralyse their larval prey may be much larger than is suggested by Table I. This superfamily also includes many families which are entomophagous on eggs or pupae (Clausen, 1940). In a discussion on paralysing venoms, egg parasites could be excluded. However, it would be interesting to study effect of 'venom' secretions of these egg parasites. One could imagine that also parasites of insect pupae do not necessarily paralyse their hosts. However, *Pleurotropis passei* stings and immediately paralyses beetle pupae (Taylor, 1937).

Hase (1924) observed that *Lariophagus distinguendus* stings its prey at random and not specifically in the direction of ganglia. Wilbert (1964) observed the behaviour of *Aphelinus semiflavus* as it stung into the leg of the aphid *Macrosiphon solani*. After the onset of paralysis the sting was

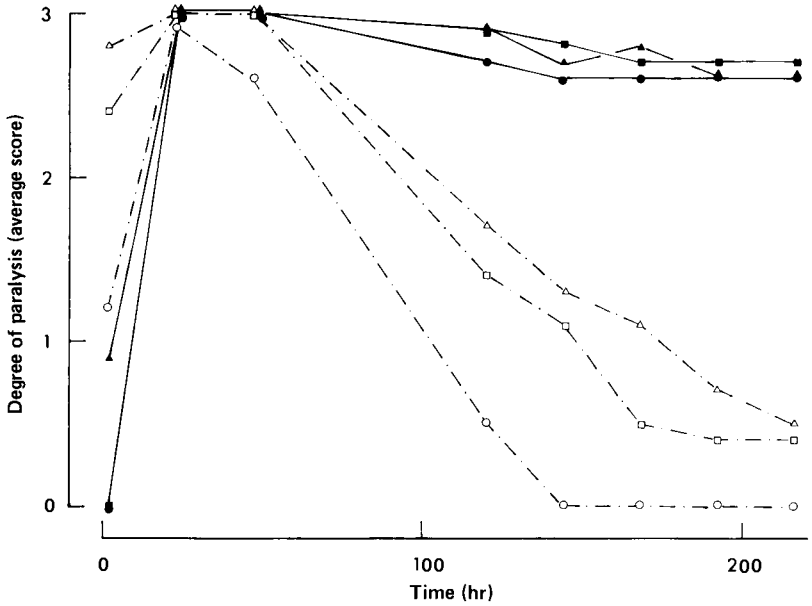


Fig. 2 Time courses of paralysis and recovery of larvae of *Galleria mellonella* (at 20°C) injected with the venom of *Microbracon gelechiae* (——) or *M. hebetor* (-•-). Note the slow onset of the paralysis. Each point is the averaged score (using the system of Beard, 1952) of 10 larvae tested. A score of 3 means 10/10 completely paralysed larvae; larvae unable to turn when laid on their back are scored 2; larvae showing uncoordinated movements are scored 1; and unaffected larvae are scored 0. Venom concentration is expressed in equivalents of the number of venom organs per larva: for *M. hebetor*, 40×10^{-5} (Δ), 20×10^{-5} (□) and 8×10^{-5} (○); for *M. gelechiae*, 10×10^{-3} (▲), 5×10^{-3} (■) and 2×10^{-3} (●). The relationship between rate of onset of paralysis and rate of recovery is different for *M. hebetor* venom and *M. gelechiae* venom. From Piek *et al.* (1974). See also Fig. 44 for comparable effect of two toxins from the venom of *M. hebetor*.

inserted further into the host before oviposition took place. The available data on the stinging behaviour of the Chalcidoidea supports the conclusion that these wasps do not sting in, or adjacent to, the central nervous system. Although this might be generalized to the whole section of Terebrantia, some terebrant wasps seem to be able to locate internal organs precisely (see Chapter 4, Section II,A).

2. Chemistry of Microbracon Venoms

a. The Venom of Microbracon hebetor. The chemical work performed to elucidate the nature of *Microbracon hebetor* venom has been published by a few authors. The first of them, Beard, wrote in 1952 his brochure *The*

Toxicology of Habrobracon Venom: A Study of a Natural Insecticide (*Habrobracon* = *Microbracon*). Beard foresaw already that the chemical work would be laborious since the quantities of the venom available are very small. In spite of the difficulties Beard succeeded in laying a thorough base for the following work by others. He supposed the paralysing principle in the *Microbracon* venom to be a protein or proteinlike material, for the venom showed a marked heat lability and was inactivated at a temperature of 65°C. The venom of *M. brevicornis* was also completely inactivated at temperatures above 50°C (Lee, 1971). The active principle in the venom of *M. hebetor* was not dialysable and was precipitated by ammonium sulphate. After adsorption on calcium phosphate gel the toxin could be eluted with an alkaline buffer. As a conclusion of his experiments Beard expected that the toxic principle in the venom should have a high molecular weight and in view of the enormous potency of the substance he assumed that it might be an enzyme.

Tamashiro (1971) studied two *Microbracon* species, *M. hebetor* and a species which was called 'Indian Bracon' because it was sent from India, probably identical to *M. brevicornis*. In order to show that the paralysing substances in the venoms were proteins, he incubated 'Indian Bracon' venom solution with pancreatin, a mixture of proteolytic and other hydrolytic enzymes. He concluded from the experiments that the venom was proteinaceous in character.

b. Stability. During the first physiological and chemical experiments in our laboratory, the great lability of *Microbracon* venom solutions was noticed. Beard (1952) did not compare different preparations quantitively and had not given any information about the stability of his venom preparations. Drenth (1974a,b), applied the biological standardization developed by Beard, using *Galleria mellonella* larvae. A paralysing unit, called the *Galleria* unit (G.U.) was defined as the dose injected per 100 mg (larva of *Galleria mellonella*) causing a 50% score (see Fig. 2 for definition) after 2 hr (i.e. an average score of 1.5), according to Beard (1952). Drenth (1974b) used for his experiments an extract of the venom glands from the female *Microbracon hebetor* wasps and also the so-called trilene preparation (for a description see Chapter 3). He found that venom solutions kept at 20 or 30°C were much more labile than the same solutions kept in melting ice or frozen at −20°C. Venom gland extract even remained stable when stored for 3 years at −20°C. After freeze-drying of solutions of venom in concentrations lower than 1 mg ml^{-1}, loss of activity of 70 to 80% occurred. This is probably not due to the freeze-drying, but to the freezing of the sample. We found that freezing of purified toxin solutions followed by thawing caused a considerable loss in toxin activity (Visser *et al.*, 1976). *Microbracon* venom and toxin solutions can be protected by addition of sucrose against damage by freezing. First

we used 150 mg sucrose ml^{-1} solution but later we found that 10 mg ml^{-1} sucrose had the same cryoprotecting effect. However, the loss in activity was still 10–20% following freeze-drying of purified toxins (Visser *et al.*, 1983).

Microbracon venom and toxin solutions are very labile. The highest stability of the biological activity was found for solutions kept at 0°C. A solution of venom gland extract at the concentration of 100 μg ml^{-1} showed a half-life for biological activity of 7 days (Drenth, 1974b). Purified toxin solutions showed a somewhat longer half-life of 18 days (Visser *et al.*, 1983). Therefore, the best way to preserve solutions containing *Microbracon hebetor* venom or toxins is to keep them in melting ice or in a cold room or in a refrigerator with a temperature not higher than 4°C.

Another difficulty when experimenting with *Microbracon* venom is the loss of activity caused by contact of venom solutions with filter and dialysing materials consisting of cellulose or cellulose derivatives. We had bad results with Visking tubing, Millipore filters and Lsg ultrafilters. The only usable type of ultrafilters were Diaflo membranes manufactured by Amicon. Contact with Diaflo membranes did not result in loss of activity, but ultrafiltration through these membranes, irrespective of the type used, normally caused a loss of up to 50%.

Another source of instability is the pH of the solution in which the *Microbracon hebetor* venom is dissolved. For crude venom preparation, Drenth (1974b) found an optimum for the stability at pH in the range from 8 to 9 at 30°C. Much lower and higher pH values were disastrous for the activity of the venom and the purified toxins, demonstrated for the A- and B-toxins in Fig. 3 (Visser *et al.*, 1983). Both toxins show an optimum in stability in the pH range 10–11. Lee (1971) found that the venom of another species, *M. brevicornis*, was inactivated below pH 5 and above pH 9.

In search of a stabilizing substance, Drenth (1974b) tried to stabilize *Microbracon hebetor* venom solutions by addition of other proteins and also non-protein compounds. At a relatively high concentration of 1 mg ml^{-1} most of the proteins used had a moderate stabilizing effect on the crude venom, but purified toxins were only slightly stabilized by bovine serum albumin. Addition of insect haemolymph (e.g. from *Galleria mellonella*, a protein-rich substance) has also a slight stabilizing effect on crude venom solutions.

The search for a nonprotein stabilizer did not result in a satisfying resolution of the problem. Compounds such as zinc sulphate or mercuric chloride had some stabilizing effect on crude venom solutions, but on purified toxins zinc sulphate had no stabilizing effect (Drenth, 1974b; Visser *et al.*, 1983). Numerous other compounds were examined without any result (Drenth, 1974b). Due to the nature of the *Microbracon* toxins, which are very labile proteins, the chance that a useful stabilizer will be found is very small.

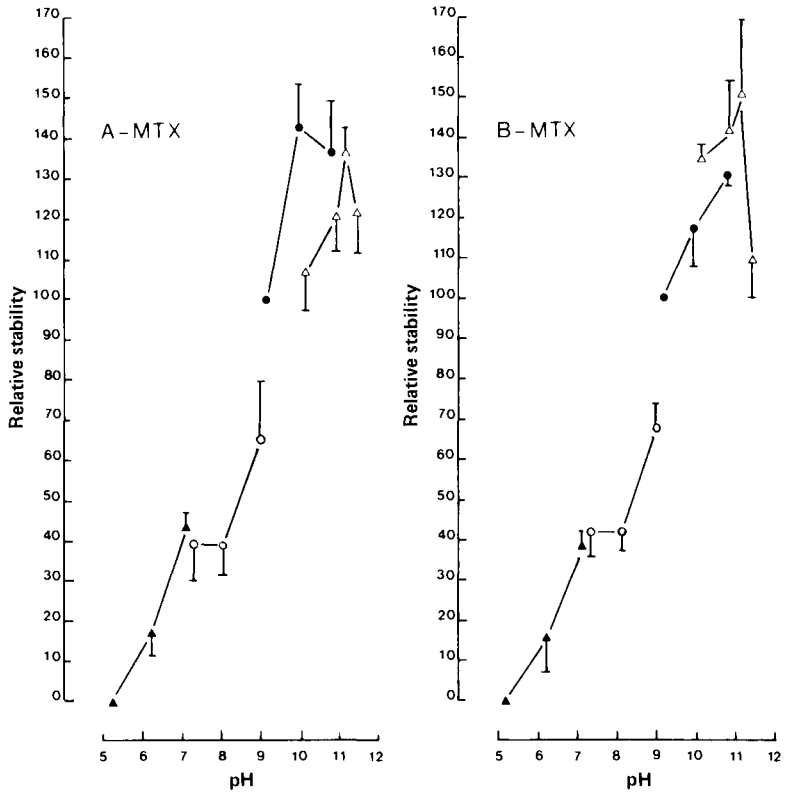


Fig. 3 Effect of pH on the stability of A-MTX and B-MTX. Activities are shown as percentages of the activities in controls (ammonium carbonate, pH 9.2) after storage at 0°C 24 hr. Buffers used were: Na_2 HPO_4/NaOH, pH 10.0–11.5, Δ; ammonium carbonate-ammonia, pH 10–11, ●; Tris-HCl, pH 7–9, ○; citric acid-Na_2HPO_4, pH 5–7, ▲. Each buffer was present at a final concentration of 0.05 mol litre^{-1}. The pH values shown were those measured at 22°C. Results are means ± SEM (n = 4). From Visser *et al.* (1983).

c. Purification. Based on the evidence from his experiments, Beard (1952) concluded that the paralysing principle of *Microbracon hebetor* venom was a protein or proteinlike substance. We decided to purify the toxin or toxins in order to get more convincing information about the biochemical and the physicochemical character of these compound(s) and also to obtain toxin preparations useful for neurobiological work which had been set up to elucidate the pharmacological action of the venom.

The dominating problem in the purification of the *M. hebetor* venom is the great instability of the active principle. A favourable circumstance is the enormous potency of the toxic substance. Even at low levels of activity, the

resulting activity is detectable with the sensitive biological testing method on *Galleria mellonella* larvae.

Most of the purification has been done using extract of whole female wasps as starting material (Visser *et al.*, 1976; Spanjer *et al.*, 1977; Visser *et al.*, 1983). The wasps, a nonflying mutation (Petters *et al.*, 1978) originally obtained from the University of North Carolina (United States of America), were bred on larvae of *Galleria mellonella* (L.), *Ephestia kuehniella* Zell. and *Corcyra cephalonica* (Staint.) as hosts. Adult female wasps were stored at −20°C. Extracts were obtained as follows: using ice-cooling 10,000 to 20,000 wasps (about 10–20 g) were homogenized (Sorvall Omnimixer, 16,000 revolutions min^{-1}) in 70 ml of 0.05 mol $litre^{-1}$ ammonium chloride-ammonia buffer, pH 9.2. After centrifugation at 20,000 revolutions min^{-1} (48,000*g*) for 45 min, a fatty layer was removed by pouring out the supernatant through nylon gauze with 30 μm mesh width. A freeze-dried extract with a weight of ~20% of the total wasps and a protein content of ~45% was obtained.

Other starting materials, such as extract of isolated venom glands and trichloroethylene preparations, have also been used. These preparations are described in Chapter 3. The trichloroethylene preparation, however, is not as useful since the biological activity in this preparation is very labile. Venom gland extract is a good starting material; however, the production is very laborious. Since purified active components isolated from extracts of whole female wasps could originate from parts of the wasp other than the venom apparatus, some work has been done with venom gland extracts to confirm the origin of the isolated substances.

d. Ion Exchange with DEAE-Sephadex. The next step in the purification was an anion exchange procedure with DEAE-Sephadex A-50 gel (Pharmacia) on a column (Visser *et al.*, 1976) or batchwise (Spanjer *et al.*, 1977). The columns used had diameters of ~2.5 cm and volumes of ~300 ml. The ion exchange chromatography started in 0.05 mol $litre^{-1}$ ammonium chloride-ammonia buffer pH 9.2 with venom extract solutions of ~2 g $(100\ ml)^{-1}$. The elution was continued with a linear concentration gradient of sodium chloride up to 0.3 mol $litre^{-1}$ (Fig. 4). The combined fractions containing the *Galleria mellonella* larvae-paralysing substances were concentrated to a volume of ~20 ml by ultrafiltration over a Diaflo UM-10 membrane.

In the batchwise method preswollen and settled ion exchange gel was combined with the venom extract solution. After stirring and centrifuging, the precipitated gel with the biological activity adsorbed on it was washed with the buffer and cetrifuged again. The supernatants were discarded. The compounds we were interested in were released from the gel by adding, with

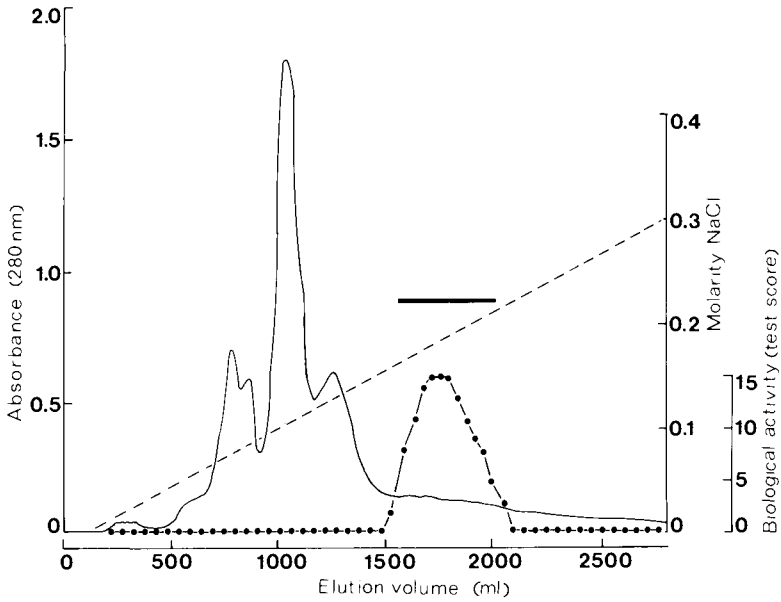


Fig. 4 Ion exchange chromatography on DEAE-Sephadex A-50. Fractionation of 2173 mg extract of female *Microbracon hebetor* wasps using a continuous NaCl gradient (---). Absorbance at 280 nm (——); biological activity in the fractions after 50-fold dilution with water, ●. The fractions indicated by a bar were pooled and concentrated by ultrafiltration. From Visser *et al.* (1976).

stirring, buffer containing sodium chloride so that a final chloride concentration of 0.35 mol litre^{-1} was obtained. After centrifugation and washing, the supernatants were concentrated to a volume of ~20 ml by ultrafiltration over a Diaflo UM-10 membrane. The ultrafiltration is time- and also activity-consuming; this can be avoided by using a large column in the next gel chromatography step. The batchwise method described hereafter is relatively rapid and gives good results in the recovery of the biological activity.

e. Chromatography on Sephadex G-100. In the first purification experiments columns with a diameter of 4 cm and gel volumes of ~700 ml were used. An example of the separation is shown in Fig. 5. When using, instead of the DEAE-Sephadex column separation the batchwise DEAE-Sephadex fractionation method without the concentration by ultrafiltration, a much larger Sephadex column with a diameter of 10 cm and a length of 100 cm was used (gel volume ~8 litres). The active part of the eluate was concentrated to a volume of ~20 ml by ultrafiltration over a Diaflo UM-10

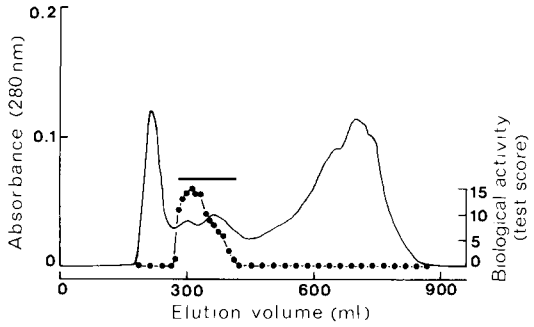


Fig. 5 Gel chromatography on Sephadex G-100. Gel chromatography of the biologically active material after DEAE-Sephadex fractionation: absorbance at 280 nm, ——; biological activity of fractions diluted 100-fold with 0.05 mol litre^{-1} ammonium carbonate, pH 9.2, ●. The fractions indicated by a bar were pooled and concentrated by ultrafiltration. From Visser *et al.* (1976).

membrane and freeze-dried after addition of 150 mg ml^{-1} sucrose for cryoprotection. This amount of sucrose seems fairly high; lower amounts of sucrose (e.g. 10 mg ml^{-1}) may also have the same effect (Visser *et al.*, 1983). Table II shows that after the first steps in the purification the recovery of the biological activity is very low. In an attempt to get better results we used QAE-Sephadex A-50 instead of the DEAE-Sephadex anion exchange chromatography. The next section shows the unexpected results.

f. QAE-Sephadex Ion-Exchange Chromatography. Using columns with 2- to 2.5-cm diameter and volumes of 50 to 250 ml, 0.3 mol litre^{-1} ammonium carbonate pH 9.2 as buffer and an elution gradient up to 1 mol litre^{-1} ammonium carbonate, a separation of two different activities could be accomplished (Fig. 6). The molecular weights of the two activities were estimated by analytical gel chromatography to be 42,000 and 57,000, respectively. The preparation containing the activity with the lower molecular weight was eluted first from the QAE-Sephadex column and was called the A-preparation; the second active preparation was called the B-preparation. When the activities of the various toxin preparations were determined by the standardization method described by Drenth (1974b), the slopes of the log dose-effect lines of the A- and B-preparations showed a statistically significant difference. These differences are shown in Fig. 7. The paralysing components in the A- and B-preparations of *Microbracon hebetor* venom are now called *Microbracon* toxin A and B, abbreviated A-MTX and B-MTX. During this separation of the A and B components using the QAE-Sephadex column, again, considerable loss in toxin activity occurred, as Table II shows. Due

Table II

Purification of A- and B- *Microbracon hebetor* Toxins[a,b]

Purification step	Total protein (mg)	Total biological activity (10^5 Units)	Recovery biological activity (%)	Specific activity (Units/ μg protein)	Purification (−fold)
Extract whole insects	1460	956	(100)	65.5	(1)
DEAE-Sephadex	813	692	72.4 ± 6.5[c]	85.1	1.3 ± 0.1[c]
Sephadex G-100	146	249	26.0 ± 3.2	170.3	2.6 ± 0.4
QAE-Sephadex					
A-MTX	13.5	33.5	3.5 ± 0.6	248.9	3.8 ± 0.8
B-MTX	18.9	105.2	11.0 ± 3.7	556.8	8.5 ± 1.4
A- + B-MTX	32.4	138.7	14.5		
Polyacrylamide gel electrophoresis					
Ultrogel AcA 44					
A-MTX	0.490	5.74	0.6 ± 0.2	1172	17.9 ± 4.9
B-MTX	0.886	16.25	1.7 ± 0.4	1834	28.0 ± 5.1
A- + B-MTX	1.376	21.99	2.3		

[a]From Visser *et al.* (1983).

[b]Data represent values obtained from an average of 10 different preparations each using 20 g female *M. hebetor* wasps as starting material.

[c]Mean ± SEM.

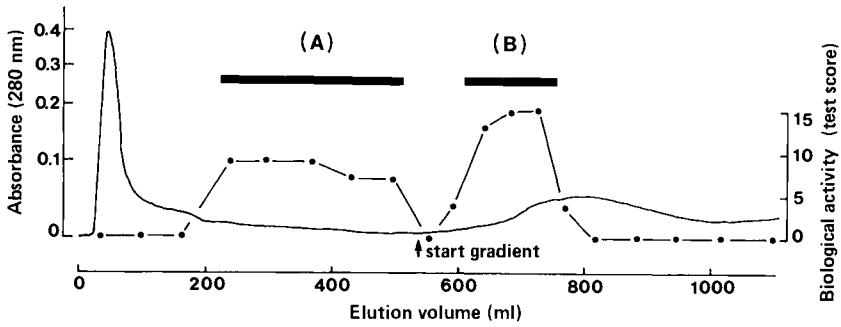


Fig. 6 Ion exchange chromatography on QAE–Sephadex A-50. Fractionation of 86 mg partly purified *Microbracon hebetor* homogenate using a continuous gradient of 0.3 mol litre^{-1} up to 1 mol litre^{-1} ammonium carbonate; ——, absorbance at 280 nm; ●, biological activity in the fractions after 10-fold dilution with distilled water. Fractions indicated by the bars were pooled and concentrated by ultrafiltration. From Spanjer *et al.* (1977).

to these losses, the specific activity of the various preparations does not show much progression.

g. Column Electrophoresis on Polyacrylamide Gel. The first purification steps were based on differences in molecular dimensions and in electrical charge. Further purification can be obtained by making use of the difference in mobility of molecules in an electric field. For preparative purposes electrophoresis on polyacrylamide gel has been developed, using a column apparatus type Uniphor, LKB (Visser *et al.*, 1983). Elution beneath the gel column was performed with 0.05 mol litre^{-1} Tris/0.38 mol litre^{-1} glycine buffer pH 8.3. Quantities of about 10 to 20 mg of partly purified A-MTX and B-MTX were subjected to the electrophoresis, the elution profiles of which are shown in Fig. 8. The solutions containing the respective toxin preparations were concentrated by ultrafiltration and at last separated from small molecules originating from the buffer and the electrophoresis column material by gel chromatography on Ultrogel AcA 44 (column 1.5 × 75 cm in 0.05 mol litre^{-1} ammonium carbonate, pH 9.2). After a final concentration by ultrafiltration, the pure toxin solutions could be stored at ~0°C with inevitable further loss of activity or could be freeze-dried after addition of 10 mg ml^{-1} sucrose and then stored dry at −20°C without further loss of activity.

The whole purification procedure has been summarized in Table II. The enormous loss in biological activity is reflected in the total recovery of only

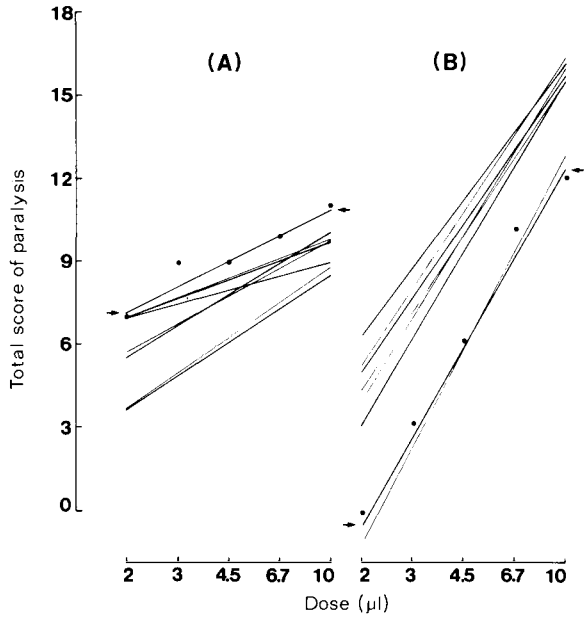


Fig. 7 Log dose-effect curves of A-MTX and B-MTX preparations isolated from eight different preparations of female *Microbracon hebetor* homogenates. The activities were tested by injecting into groups of six wax moth larvae of 150–200 mg, 2, 3, 4.5, 6.7 and 10 µl of venom preparation. Measured scores (according to Beard, 1952) are indicated by dots for two of the lines with arrows. Calculated linear regression lines. From Spanjer *et al.* (1977).

2.3% and the low purification factors of about 18 for A-MTX and 28 for B-MTX. Despite the low production, almost pure toxins have been obtained. This was demonstrated by analytical disc electrophoresis according to the standard method (Maurer, 1971) and is shown by Fig. 9. The localization of the protein bands in the pure preparations coincided with the localization of the toxin activities recovered of slices in parallel gels.

h. Properties of Microbracon Toxins. Beard (1952) and Tamashiro (1971) were right in their assumption that the paralysing principles in *Microbracon hebetor* venom should be proteinaceous. The toxins have all the characteristics of proteins (Visser *et al.*, 1976). They showed high molecular weights, estimated using analytical gel chromatography. For A-MTX and B-MTX the molecular weights were 43,700 and 56,700, respectively (Visser *et al.*, 1983). This is in agreement with the values of 42,000 and 57,000 found by the same method for the crude toxin preparations (Spanjer *et al.*, 1977).

The toxins are inactivated by the proteolytic enzymes chymotrypsin A,

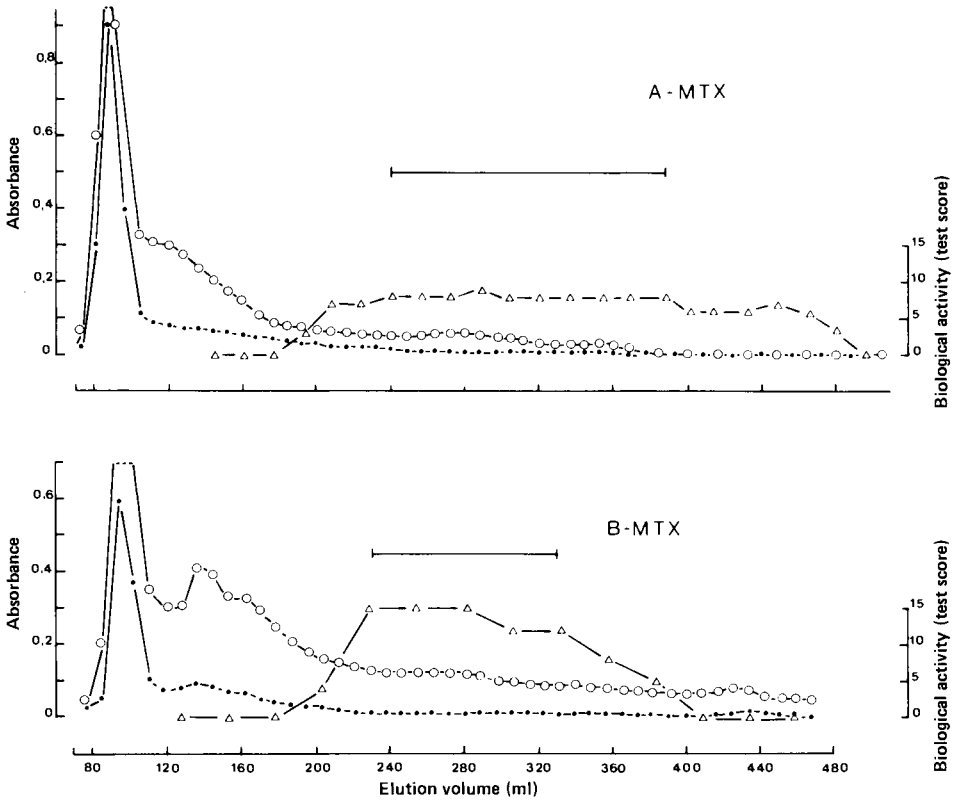


Fig. 8 Elution profiles obtained when preparations of A-MTX and B-MTX were submitted to column electrophoresis on polyacrylamide gel. Elution rate 12 ml hr^{-1}. Fractions of 4.5 ml: ○, absorbance at 230 nm; ●, absorbance at 280 nm; Δ, biological activity in 20-fold diluted fractions. The fractions indicated by a horizontal bar were pooled. From Visser *et al.* (1983).

trypsin, subtilisin A and pronase P; A-MTX appears to be more resistant to the enzymes than B-MTX. The amino acid compositions of both toxins show a great similarity, as is demonstrated in Table III.

Isoelectric points could be determined by isoelectric focusing. The values were 6.85 for A-MTX and 6.62 for B-MTX. Ultraviolet absorption and fluorescence emission spectra of both toxins were identical and typical for proteins. There was a maximum ultraviolet absorption at 278 nm and a minimum at 250 nm. Excitation at 278 nm caused maximum fluorescence at 345 nm. Typical protein inactivators, such as urea, sodium dodecylsulphate, dithiothreitol and β-mercaptoethanol, inactivated the toxins. B-MTX was more susceptible for the action of dithiothreitol than A-MTX. *N*-

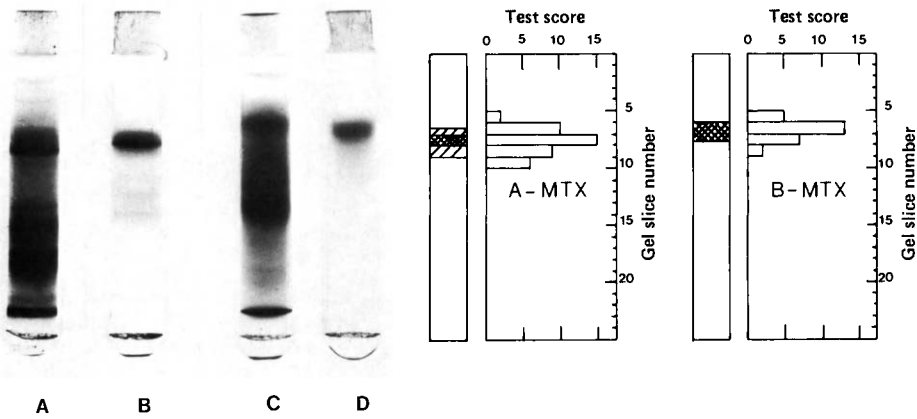


Fig. 9 Analytical polyacrylamide gel electrophoresis of *Microbracon hebetor* toxins. Left: staining for proteins (a) crude preparation of A-MTX, (b) purified A-MTX, (c) crude preparation of B-MTX, (d) purified B-MTX. Amounts of 182, 25, 308 and 23 μg of proteins, respectively, were applied to the gels. Staining with Coomassie Brilliant Blue R-250. Right: Histograms show distribution of biological activities eluted from gel slices after electrophoresis of purified toxins; 24 μg of protein (30,500 paralysing units) of A-MTX and 22 μg of protein (35,500 paralysing units) of B-MTX were applied to the gels. The peaks of activities coincide with the protein bands in the parallel gels. The anode is on the lower end of the gels. From Visser *et al.* (1983).

Bromosuccinimide also inactivated both toxins, indicating that the tryptophan residue plays a role in the active part of the molecules. The toxin activities were not affected by phenylmethanesulfonyl fluoride, indicating that the serine residues in the molecules are not essential for the biological activities. Also, *p*-chloromercuribenzoate was without effect on the activities, indicating that free SH-groups are not essential. Heavy metal ions are not necessary for the paralysis, for EDTA had no effect on the activity of the toxins.

i. Other Microbracon Venoms. In addition to *Microbracon hebetor*, two other species, *M. brevicornis* and *M. gelechiae*, have been studied regarding the properties of their venoms. The venom of *M. brevicornis* was studied by Lee (1971). It appeared that this venom lost its biological activity completely at temperatures above 50°C and was inactivated below pH 5 and above pH 9. Gel chromatography experiments were not successful for molecular weight determination, but using sucrose density gradient sedimentation an estimated molecular weight of ~100,000 was supposed. The proteinaceous character of the active component in *M. brevicornis* venom was evident. The venom of *M. gelechiae* appeared to be labile, like the venom of *M. brevicornis* and

Table III
Relative Amino Acid Compositions of A-MTX and B-MTX[a]

Amino acid	Molar ratios[b]	
	A-MTX	B-MTX
½ Cys	0.42±0.07	0.37±0.02
Asp	1.69±0.02	1.62±0.04
Thr	0.66±0.06	0.72±0.02
Ser	0.68±0.09	0.68±0.01
Glu	1.48±0.07	1.50±0.03
Pro	0.76±0.04	0.66±0.02
Gly	1.14±0.05	1.17±0.05
Ala	1.00	1.00
Val	0.72±0.04	0.79±0.01
Met[c]	0.24±0.03	0.32±0.01
Ile	0.55±0.03	0.61±0.01
Leu	0.90±0.03	0.99±0.04
Tyr	0.36±0.04	0.40±0.04
Phe	0.54±0.03	0.54±0.03
Lys	0.86±0.03	1.03±0.03
His	0.32±0.01	0.40±0.01
Arg	0.46±0.01	0.46±0.02

[a]Data represent the averages ±SEM of duplicate determinations of two preparations, of both toxins. Cys, as cysteic acid after oxidation. Tryptophan was present but was not determined. From Visser *et al.* (1983).
[b]Relative to Ala.
[c]As methionine sulfone after oxidation.

M. hebetor. A preparation of 100 venom organs in 1 ml distilled water or saline lost half of its activity in ~1 week during storage at −20°C (Piek *et al.*, 1974). In order to get an estimation of the molecular weight of the active principle, an extract was prepared of 500 mg of female wasps (corresponding to ~500 wasps). Homogenization was performed in 50 ml 0.05 mol litre^{-1} ammonium carbonate, pH 9.2, at 0°C as already described for *M. hebetor* wasps. After centrifugation and freeze-drying a preparation of ~100 mg weight was obtained. Using analytical gel chromatography, a molecular weight of ~60,000 could be determined (Piek *et al.*, 1974). The gel chromatography fractions containing biological activity were freeze-dried and had a weight of ~5 mg (only 1% of the original 500 mg of wasps), but during this first purification nearly 80% of the original biological activity in *Galleria mellonella* larvae was lost. The high molecular weight of ~60,000

and the high lability of the active principle of the *M. gelechiae* venom make it plausible that it is a protein like the *M. hebetor* toxins.

3. Venoms of Solitary Aculeate Wasps

The Aculeata can be divided into two groups: the solitary aculeate wasps on the one hand, and the ants (Formicidae), bees (social and solitary Apidae) and the social wasps (Vespinae) on the other hand. Members of the first group frequently produce paralysing venoms, used by the wasp to partly or completely immobilize (and not to kill) a prey (insect or spider), which in this condition is used by the larva of the wasp as food. Solitary aculeate wasps belong either to the superfamily Bethyloidea, Scolioidea, Pompiloidea or Sphecoidea, or to the Vespoidea. The latter superfamily is regarded by Imms (1960) as a single family, the Vespidae and contains the solitary Massarinae and Eumeninae as well as the social Vespinae (Chapter 6). In contrast to the terebrant wasps, which prey predominantly on Holometabola (see Section II,B,1), the Aculeata also prey on Hemimetabola* as well as on spiders. The depth of paralysis resulting from a sting is used to categorize paralytic states. Hosts which are still able to move part of their integumental muscles, either spontaneously or in response to a given stimulus, are considered to be incompletely paralysed. The time for which a host remains paralysed is used as a division between permanent paralysis and transient paralysis. The classification of a venom as causing either a permanent of a transient paralysis may be rather arbitrary, since in many cases it depends on the time for which the paralysed animal was observed.

a. Transient Paralysis. Examples of short-lasting paralysis are: *Tiphia popilliavora* (Scolioidea) (Complete paralysis reversible in 30 min) (Clausen *et al.*, 1927), *Pompilus cingulates* (Pompilidae) (45 min) (Ferton, 1910), *P. vagans* and *P. republicanus* (Ferton, 1897), *Anospilus barbilabris, A. orbitalis, Dicyrtomellus luctuosus* and *Agenioideus ursurarius* (Pompiloidea) (10–60 min) (Gros, 1982a,b) and *Agenioideus coronatus* (Pompilidae) (5 min) (Gros, 1982b), as well as *Larra analis* (Sphecidae) (5–10 min) (Smith, 1935), *L. anathema* (5–6 min) (Malyshev, 1941, see Malyshev, 1966) and *L. amplipennus* (68 sec) (Nambu, 1970, see Iwata, 1976). A number of wasps that sting the prey into a transient paralysis of very short duration remove the legs of the prey. This, for example, has been described for different species of *Pompilus* and *Pseudagenia* (Ferton, 1897, 1911). In other examples in which the wasp transiently paralyses the prey, the latter, after having recovered, seems to show a different (or adapted) behaviour. This has been described for larrine

*Insects in which the larva resembles the adult form.

wasps (Chapter 4, Second II,D) and for Ampulicinae (Section III,B,1).

A number of members of the Pompilidae have been shown to inflict a paralysis from which the host shows complete recovery at times between several hours and a few days after being stung; examples include *Anoplius apiculatus, A. cleora, A. imbellis, A. krombeini, A. marginatus, A. semirufus, A. ventralis* and *A. virginiensis* as well as *Aporinellus sexmaculatus, Agenoideus apicalis, A. nebecula* and *A. cinctellus* (Krombein, 1952, 1953; Wasbauer, 1957; Powell, 1958; Gros, 1982b,c). Certain Sphecidae (e.g. *Cercerus raui* and *Clorion auripes*) (Rau, 1928) produce a paralysis of similar duration.

Transient paralysis lasting a few weeks has been described in the hosts of other Pompilidae (e.g. *Anoplius americanus, A. amethystinus, Aporus fascinatus, Cryptocheilus affinis* and *Pompiloides tropicus*) (Peckham and Peckham, 1898; Rau and Rau, 1918; Richards and Hamm, 1939; Evans and Yoshimoto, 1962). Paralysis lasting a number of months has been described in the hosts of certain Pompilidae: *Anoplius biguttatus* (Peckham and Peckham, 1898), *A. viaticus* (see Fig. 12), *Priocnemis hyalinatus* (recovery started after 23 days, being complete within 25 days) (Ferton, 1897), *Pepsis mildei* (in which the host spiders show partial recovery in 5 months) (Williams, 1956), *Pepsis marginata* (Petrunkevitch, 1926) and also in caterpillars collected by the sphecid wasp *Sphex aberti* (Hicks, 1932a).

b. Permanent Paralysis. This has been described for a large number of prey of solitary wasps (Table I). The prey commonly die after a relatively short time, but this is not always the case. Ribi and Ribi (1979) found that immature female bush-crickets of the genus *Conocephalus* (Orthoptera, Tettigoniidae) paralysed by *Sphex cognatus* lived in the laboratory at 10°C for 3 to 4 weeks. Riley (1892) described how the cicada *Tibicen pruinosa* after being stung by *Sphecius speciosus* remained in a state of suspended animation, which under favourable conditions lasted for a year, and potentially longer. However, Evans (1966a, p. 107) found that *T. pruinosa*, although they appear fresh, have nevertheless been dead for a long time. He believes that Riley's statement is not true.

c. Delayed Paralysis. A delay between the time of stinging and the onset of paralysis has been observed in the hosts of a number of different wasp species. Rau and Rau (1918) have described a delay of 5 min before a grasshopper attacked by *Prionyx thomae* (Sphecidae) showed signs of paralysis. Wheeler (1928) described how queen ants, stung by *Aphilanthops frigidus* (Sphecidae), continued to move their palpi, legs and antennae, either spontaneously or when touched. These movements continued for several hours, or even for a few days, after the ants had been captured. All movement

then ceased, although the insects retained a fresh appearance, showing limb flexibility, with no indications of the tissues drying up.

In some cases it is uncertain whether or not delayed effects result in death. Such observations have been described by Evans (1966a) for leafhoppers caught by *Gorytes canuculatus*. They may show movements of the legs for several hours, but soon they become immobile and appear dead. In no case were any movement of body parts noted after 1 day, but the leafhoppers remain in fresh condition for several days. The same author described how he caught a *Glenostictia scitula* and her prey, a small bee of the genus *Perdita*, which had not yet succumbed to paralysis and flew away when released. Prey taken from these wasps at the nest entrances often showed movements of appendages, but prey taken from nests a few hours after they were captured were invariably dead and often quite stiff (Evans, 1966a).

d. Bethyloidea. This superfamily consists of families Dryinidae, Bethylidae, Chrysididae, Sclerogibbidae, Embolemidae and Cleptidae; the latter two are highly unknown. Members of the family Dryinidae parasitise hemipteran nymphs or adults. It appears that dryniid venoms produce a temporary paralysis of their hosts (Newman, 1965). Members of the closely related Bethylidae attack the larvae of Lepidoptera or Coleoptera. These larvae may be paralysed incompletely and transiently, or completely and permanently (Table I). Some bethyliid species may attack humans. Oda *et al.* (1981) reported that a man was injured (eruptions on the abdomen) by multiple stings of a *Sclerodermus* sp. parasitizing larvae of longicorn beetles, which were under the bark of Japanese cedar used as firewood to heat a bath. *Cephalonica gallicola* also caused sting dermatitis (Yamasaki, 1982). Most members of the Family Chrysididae live as inquilines on bees or wasps. Their larvae usually prey on those of the host, but Chapman (1869, see Imms, 1960) has observed larvae of *Chrysis ignita* feeding upon a caterpillar stored by a wasp, *Odynerus* sp., which is the host of *C. ignita*. *Chrysis shanghaiensis* is the only member of the family known to directly attack host larvae (Du Buysson, 1898). It is a parasite of the caterpillars of *Monema flavescens*, which, according to Piel (1933) and Parker (1936), are incompletely paralysed by the wasp.

e. Scolioidea. This superfamily is subdivided into five families, two of which are of relative importance with regard to our knowledge of their venoms: the Scoliidae and the Tiphiidae. For another family, the Mutillidae, it is known only that the sting of some species can be extremely painful. Baer (1901) wrote that according to Tschudi the meaning of the Indian name *peru* was 'wasp that makes men cry'.

Members of both the Scoliidae and the Tiphiidae usually prey on larvae

of Coleoptera (Table I), which are paralysed and parasitized at the place where they are found by the wasp. An exception is *Diamma bicolor*, which paralyses mole crickets incompletely and transiently (Hardy, 1911). According to Owen (1969), the venom of the latter wasp contains more than one $\mu g\ ml^{-1}$ serotonin.

Other Scolioidea sting beetle larvae, before oviposition. The paralysis caused by the sting varies from incomplete to complete and from transient to permanent (Table I). A very large scoliid wasp, *Megascolia flavifrons*, stings larvae of the rhinoceros beetle, *Oryctes nasicornis* (Passerini, 1840). The venom reservoir contains histamine and a bradykinin-like substance, and no cholinergic or serotonergic activities (Piek *et al.*, 1983a). The pharmacological and immunological properties of the kinin from *M. flavifrons* were compared with those of bradykinin and a number of bradykinin analogues, including *vespakinins* (see Chapter 6, Section III). Piek *et al.* (1984c) postulated that the venom contains a peptide, *megascoliakinin*, with a bradykinin-like sequence of amino acids. The histamine-like activity, which could be fully antagonized by mepyramine, has also been found in the venoms of *Scolia hirta* and of *Campsomeris sexmaculata* and *C. villosa* (Table 4) (Piek, 1984). The venom of *C. sexmaculata* contains an unknown smooth muscle contracting factor (Piek, 1985b). The venom causes contraction of the rat colon which is not antagonized by mepyramine (Figs. 10, 11). This action is comparable with that of angiotensin; however, differences in time course of the contraction and relaxation of the rat colon indicate that the factor is probably not identical to angiotensin I or II (Fig. 11). Scolioidea stings may produce effects ranging from a complete and permanent paralysis (*Campsomeris* and *Megascolia*) to a very temporary immobility (*Methoca* and *Tiphia*) (Table I). Clausen *et al.* (1927) described that prey of *Tiphia popilliavora* have been restored to activity from a complete paralysis within 30 min.

f. Pompiloidea. This superfamily contains only one family, the Pompilidae (= Psammocharidae). The Pompilidae and the families and subfamilies (Sphecidae, Eumeninae) described in later sections differ from the Scolioidea in that they transport their prey to a nest.

The Pompilidae, without known exception, prey on spiders, most of which are temporarily paralysed (Table I). When observations are not continued for long periods, then permanent paralysis may be recorded in error, since examples of long recovery periods are known. The Peckhams (1898) described the slow recovery of spiders paralysed by *Anoplius* (= *Pompilus*) *biguttatus*, which took 2 months. Figure 12 summarizes observations of spiders (*Trochosa terricola*) paralysed by *Anoplius viaticus*, which show an average recovery period of more than a month. A number of paralysed spiders died during

Table IV

Smooth Muscle Agonists Present in Solitary Wasp Venoms (Hymenoptera, Aculeata)[a,b]

Taxa	ACh[c]	Hist[c]	Hist[d]	5HT[c]	bradyk[c]	Other[c]	References[e]
Scolioidea							
Diamma bicolor[f]	nd	nd	—[f]	1300[f]	nd	nd	1
Megascolia flavifrons[g]	—	500	300[h]	—	80	—	2
Scolia hirta[g]	—	2000	nd	—	—	nd	4
Campsomeris sexmaculata	—	100	nd	—	t	g	4
Campsomeris villosa[g]	—	15	nd	—	—	—	4
Pompiloidea							
Anoplius infuscatus[g]	—	—	nd	—	—	—	4
Anoplius samariensis[g]	—	40	nd	—	—	—	4
Anoplius viaticus[g]	—	—	nd	—	—	—	4
Batozonellus larcerticida[i]	—	15	nd	—	—	—	4
Batozonellus annulatus[g]	—	—	115[j]	—	—	m	5
Episyron rufipes[i]	400	—	nd	—	—	—	4
Hemipepsis ichneumonea[g]	—	—	115[j]	—	—	m	5
Sphecoidea							
Bembix rostrata[g]	—	120	540	—	—	—	3
Cercerus arenaria[i]	—	25	23	—	—	—	3
Mellinus arvensis[i]	—	125	173	—	—	—	3
Philanthus triangulum[g]	400[k]	—	—	—	—	—	3
Sceliphron spirifex[g]	—	400	732[j]	—	—	—	3
Sceliphron laetum[g]	—[k]	—	2471[j]	—	—	—	5
Palmodes occitanicus[g]	—	3000	1800	—	—	—	3

Eumenidae							
Eumenes arcuatus[g]	—	—	1381	—	—	—	5
Eumenes latreillii[g]	—	—	9034	—	—	—	5
Rhynchium aruliferum[g]	—	—	2611	—	—	m	5
Rhynchium mirabile[g]	—	—	1042	—	—	m	5
Abispa splendida[g]	—	—	5733	—	—	—	5

[a]ACh, Hist, 5HT and Bradyk are acetylcholine-, histamine-, 5-hydroxytryptamine- and bradykinin-like activities, respectively. Values are in ng per venom reservoir, or per venom apparatus, or per μl, which is roughly comparable.

[b]Key to symbols: —, values not significantly different from zero; t, trace; g, slow contractions of rat colon, different from angiotensin I, II (see Figs. 10 and 11); m, adrenaline/noradrenaline; nd, not determined.

[c]Bioassay on vertebrate smooth muscle.

[d]Radioenzymatic assay.

[e]Key to references: 1, Owen (1969); 2, Piek *et al.* (1983a); 3, Piek *et al.* (1983b); 4, Piek (1984); 5, Nakajima *et al.* (1983).

[f]Fluorescent assay (venom collected by electrical stimulation).

[g]Extracts of venom reservoirs plus venom glands.

[h]300 ± 25 ng/venom reservoir (n = 4).

[i]Extracts of venom reservoirs plus glands and sting apparatus.

[j]High performance liquid chromatography.

[k]Also detected using mass fragmentography: 424 ± 12 ng/venom reservoir (n = 8).

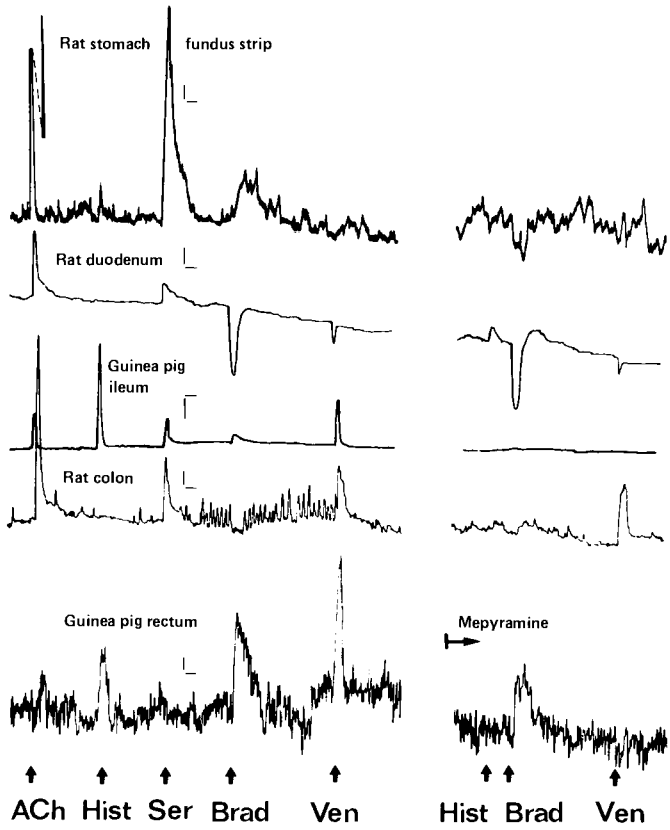


Fig. 10 Effect of a number of agonists: acetylcholine (ACh), histamine (Hist), serotonin (Ser) and bradykinin (Brad), and of the venom from *Campsomeris sexmaculata* (Ven), on contractions and relaxations of five different mammalian smooth muscle preparations, and the effect of the histamine antagonist mepyramine (100 mg ml^{-1}). Cascade technique (Ferreira and Vane, 1967) and superfusion with a Krebs solution to which is added in succession: 5 ng ACh, 10 ng Hist, 5 ng Ser, 10 ng Brad, 0.05 venom reservoir (Ven), and in the presence of mepyramine: 10 ng Hist, 10 ng Brad, 0.05 Ven. Note the contraction of the colon by the venom, without a contraction of the serotonin- and acetylcholine-sensitive fundus. The contraction of the colon is not caused by one of the above agonists. See also Fig. 11.

the observation period. If death of the host, which was first observed 10 days after the onset of paralysis, occurs in nature, the wasp's larva would have started to consume the host's tissue several days before this time. A similar slow recovery has been described for spiders paralysed by *A. relativus* (McQueen, 1979).

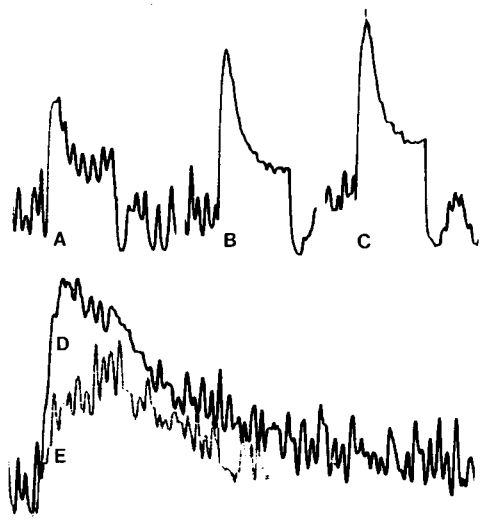


Fig. 11 Effect on the isolated colon of the rat of the venom from *Campsomeris sexmaculata* and angiotensin I and II. (a) 0.5 venom reservoir ml^{-1}, (b) 1.5 venom reservoir ml^{-1}, (c) 2.5 venom reservoir ml^{-1}, (d) 10 mg ml^{-1} angiotensin II, (e) 30 mg ml^{-1} angiotensin I. Note that the contractions by the venom and the relaxations are much faster than those by the angiotensins.

In other cases spiders may revive soon after being paralysed. An example is the spider *Cheiracanthium rufulum* stung by *Homonothus iwatai*. *Pompilus cinctellus* (Ferton, 1905) and *P. cingulatus* (Ferton, 1910) paralyse spiders only incompletely. In these cases the spiders were enclosed in a cell of the wasp's nest before they revived and therefore were unable to escape from the wasp larva.

Other pompilids whose venom produces only an incomplete paralysis amputate the legs of their spider prey in order to immobilize them. *Agenia subcorticalis* (Hartman, 1905), *A. variegata* (Maneval, 1939), *Pseudagenia mellipes* (Rau, 1928), *Ageniela* spp., *Phanagenia bombycina* and *Auplopus* spp. (Evans and Yoshimoto, 1962) have been described to exhibit this behaviour.

In the venoms of two pompilid wasps (*Anoplius samariensis* and *Batozonellus lacerticida*) histamine-like activity has been found, and in the venom of *Episyron rufipes* a large amount of acetylcholine has been found (Table 4) (Piek, 1984).

g. Sphecoidea. This superfamily is treated as a single family by Imms (1960). The Sphecidae attack a very wide range of host species, including

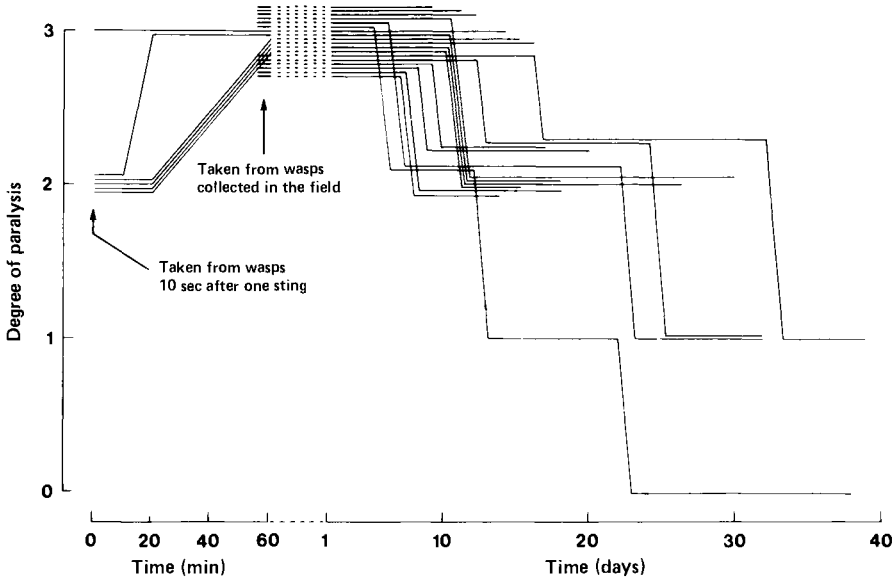


Fig. 12 Time course of paralysis and recovery of spiders (*Trochosa terricola*) stung by the wasp *Anoplius viaticus*. The degree of paralysis is scored as follows: completely paralysed spiders are scored 3, spiders unable to turn over when lying on their back but showing movements, either spontaneously or in response to tactile stimulation are scored 2, spiders showing only uncoordinated movements are scored 1, and unaffected spiders are scored 0. Twelve spiders were taken from wasps collected in the field. These spiders were completely paralysed and were assumed to have been stung 1 hr before the first observation. Six spiders were stung in the laboratory and were isolated within 10 sec after the wasp had inflicted a single sting. The degree of paralysis was determined 1, 10, 20 and 60 minutes after the sting and subsequently every day. This leads to the conclusion that, once the spiders are stung once, complete paralysis appears in between 1 and 60 min. It appears that some of the spiders are capable of recovering in ~40 days. Many spiders died and decayed before that time (indicated by the end of the lines). From Piek (1978).

Lepidoptera, Diptera, Hymenoptera, Hemiptera, Orthoptera, Dictyoptera and Arachnida (Table I). The effect of a sphecid sting ranges from complete and permanent paralysis to incomplete and temporary paralysis.

Grandi (1926) reported that a sting of *Ammophila campestris* produced local paralysis in larvae of the sawfly wasp of the genus *Salandriino*. This local effect could be the result of the sting being directed into a part of the ganglia instead of all major nerve centres being stung. Incomplete paralysis has been observed by Newcomer (1938) in caterpillars stung by *Podalonia luctuosa*. For a detailed description of the different stinging patterns see Chapter 4.

In general sphecid venoms are less potent than those of many other solitary wasps. This was demonstrated by Ferton (1902) in his observations of *Sphex subfuscatus italicus* stinging crickets. The first cricket to be stung was completely paralysed; the second was only partly paralysed. Ferton (1910) also showed that small *Halictus* bees attacked by *Cerceris emarginata* were completely paralysed, while bigger bees were incompletely paralysed. He described also that the small flies collected the first day by *Monedula chilensis* (= *Zyrzyx chilensis*?) (see Evans, 1966a) appeared dead, but the larger flies later on were imperfectly paralysed, although incapable of coordinating their movements.

Not all sphecid wasps seem to sting their prey. Hunters of aphids, such as *Passaloecus* and *Xylocelia* species, seem to be able to paralyse (or kill?) them without stinging, probably by strong bites with the mandibles just behind the head and in the thoracic region (Peckham and Peckham, 1898; Powell, 1963; Lomholt, 1973; Corbet and Blackhouse, 1975). A comparable situation exists for braconid wasps (Terebrantia) ovipositing aphids without paralysis and in a single case known with reduction of the venom glands (see Chapter 2). Other sphecine wasps, for example some of the sand wasps or Nyssoninae, may sting their prey to death. Although the majority of the Nyssoninae paralyse their prey in such a way that it remains alive and fresh for several days, some of those that exhibit progressive provisioning often bring in killed prey (Evans, 1966a). This has been reported for flies collected by *Bembix brullei* (Janvier, 1928), leafhoppers in the cells of *Alysson melleus*, flies found in the nests of *Steniola species* (Evans, 1966a) and of *Rubrica surinamensis* (Evans *et al.*, 1974) and moths and flies caught by *Stictiella* species (Gillaspy *et al.*, 1962).

Venoms of sphecid wasps contain agonists for vertebrate smooth muscle, which can also be considered to be active in central nervous systems of insects. The venom of *Philanthus triangulum* contains ~400 ng acetylcholine per venom reservoir. The venoms of five other sphecid wasp species (*Bembix rostrata, Cerceris arenaria, Mellinus arvensis, Sceliphron spirifex* and *Palmodes occitanicus*) did not contain acetylcholine, but a histamine-like activity (Table 4) (Piek *et al.*, 1983b). The venom of the mud-dauber wasp (*Sceliphron caementarium*) has been analysed by O'Connor and Rosenbrook (1963) and Rosenbrook and O'Connor (1964a,b). Twenty-two constituents which account for 96 ± 8% of the pure dried venom were revealed by various chromatographic techniques. Seventeen constituents were not polypeptide in nature. Among them were three free amino acids (histidine, methionine and pipecolic acid) and a lecithin-like compound. Acetylcholine and serotonin appear to be absent, similar to what was found in the venom of *S. spirifex*. Histamine could be present but at an amount of less than 0.01% of the dried

venom, (i.e. <20 ng). Piek *et al.* (1983b) have found in *S. spirifex* venom several hundred nanograms of histamine (Table 4).

The chemistry of *Philanthus triangulum* venom is described in detail in Section II,B,4.

h. Vespoidea. This superfamily is regarded by Imms (1960) as a single family, the Vespidae with subfamilies Massarinae, Eumeninae and Vespinae. Here we shall follow others and subdivide the superfamily into the families Masaridae, Eumenidae and Vespidae. The wings can be folded longitudinally and have a characteristic venation. However, in the Masaridae these characters are not always present. Moreover, Masaridae seem to be phytophagous. The solitary Eumenidae form one of the largest subfamilies of Hymenoptera. The social Vespidae are treated in Chapter 6.

The Eumenidae make vase-shaped nests of sand, mud, clay or leaves, or they may use cavities as nest sites. They normally prey on lepidopteran larvae (with few exceptions). Their prey is usually incompletely or temporarily paralysed (Table I). Janvier (1930) has studied a number of *Odynerus* species and concluded that all species sting caterpillars in the first three segments. These segments were paralysed immediately and permanently (Janvier, 1930, p. 340). Steiner (1983a) found that *Euodynerus foraminatus* stings in the direction of the three thoracic ganglia and in the suboesophageal ganglion (see also Chapter 4). Thus partial mobility of caterpillars (incomplete paralysis) (Table I) could be due to a complete paralysis of a limited number of segments, whereas the rest of the body may remain unaffected. Within the genus *Zethus* both solitary and social wasp type of feeding of the larvae and handling of the prey have been described. *Zethus dicomba*, for example, stings seven segments, and after the last sting the caterpillar remains in a complete paralysis (Janvier, 1930; p. 345). However, *Z. cyanopterus* feeds its larvae with freshly killed moth caterpillars. This wasp does not appear to sting, but chews off the head and most of the thorax to deliver to the offspring (Williams, 1919c). For the presence of active amines in the venom of euminid wasps see Table I of Chapter 6, and Nakajima *et al.* (1983).

4. Chemistry of Philanthus Venom

The chemical work on the venom of *Philanthus triangulum* (Piek and Spanjer, 1978; Spanjer *et al.*, 1982a,b) has been restricted for a long time by lack of material. The female wasps cannot be bred successfully in the laboratory. The large number of wasps needed for chemical work on the venom were collected one by one in the field. At first this was only possible in the south-west of France, later on also in Egypt, Belgium and the

Netherlands. The wasps were kept in liquid nitrogen. This field work was performed by Dr R. T. Simon Thomas and Mr R. L. Veenendaal both from our Laboratory. Due to natural circumstances the quantities of wasps were not always large enough for rapid progress on the chemical work, the purification of the paralysing components in particular. Progress has also been delayed by the fact that paralysis in honey-bees is caused by a combination of components, each of which has a much lower effect when administered alone than in combination or even has no effect at all. The active principles in *Philanthus* venom causing the paralysis of honey-bees were not peptides, as was demonstrated when extracts of venom reservoirs were incubated with the enzymes carboxypeptidase A and leucine aminopeptidase. The paralysing principle of the venom was not affected by these enzymes.

a. Purification. Venom-containing wasp extracts were prepared by two different methods (Spanjer *et al.*, 1982a). A fine extract was obtained using the isolated venom reservoirs of some hundreds of wasps. This is excellent for a small-scale preparation but it is very laborious. The venom reservoirs, collected in distilled water of 0°C, were homogenized in a Potter–Elvehjem homogenizer and after centrifugation at high speed the supernatant was freeze-dried. This resulted in a yellow powder, weighing 16–26 mg per 100 venom reservoirs.

Another, larger-scale extract was obtained by starting with the wasp abdomens. Usually 2500 wasps were processed for one extract. The wasps were kept frozen in liquid nitrogen, but when carefully shaken the abdomens break off and will float on the nitrogen. Thus the abdomens can easily be separated from the rest of the wasps' body parts. They were homogenized in ice-cold methanol in a Sorvall Omnimixer at 16,000 revolutions min^{-1}. After centrifugation and dilution of the supernatant with distilled water for better processing conditions during the following freeze-drying, the resulting brown powder had a weight of ~8 g per 2500 abdomens.

For the first purification step of the crude extracts, gel chromatography on Sephadex G-200 or G-100 in distilled water at 3°C was used. The low molecular weight components appeared as one peak in the 280-nm absorption elution diagram and contained all the substances causing paralysis in honey-bees. When venom reservoir extracts were used, the total biological activity expressed in bee units (B.U.) (see Chapter 3) (Piek *et al.*, 1971) was improved by a factor of ~2.6 after the gel chromatography. This phenomenon, for which there is no good explanation, has not been confirmed for crude abdomen extracts, since such extracts contain too many nonspecific toxic impurities to test them on bees. They are lethal to the bees.

The active fractions obtained after the first gel chromatography separation

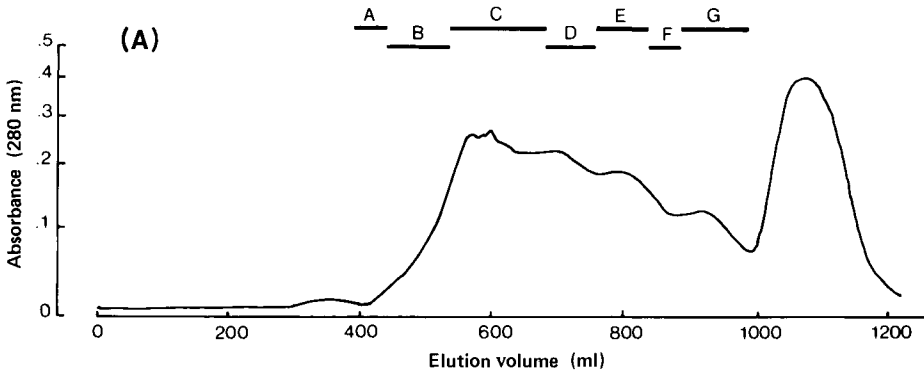
were further purified on Sephadex G-25 gel columns in 0.2 mol litre^{-1} ammonium acetate made acid to pH 4.75 by acetic acid. Figure 13(a) shows the separation during the second step in the purification of abdomen extract, and Fig. 13(b) shows the thin-layer chromatogram of the different groups of fractions combined as designated in Fig. 13(a).

Paralysis in honey-bees was found after injection of the B preparation (Fig. 13) but only for a part of the original activity. This preparation provisionally was called *Philanthus* Venom B, abbreviated PVB. The total original activity was recovered when PVB was combined with the groups of fractions A and C (Fig. 13), similarly called PVA and PVC. These two preparations alone had no visible paralysing effect in honey-bees, but PVC was active in the central nervous system of the cockroach *Periplaneta americana* (Piek *et al.*, 1980b, 1982a) and seems to contain the acetylcholine which Piek *et al.* (1983b) found in the venom reservoir of *Philanthus triangulum*.

Using ion exchange chromatography on SE- or SP-Sephadex C-25, the combination of the preparations PVA, PVB and PVC could be separated into a number of different fractions. The column chromatography was started in 0.4 mol litre^{-1} ammonium acetate pH 4.75, followed by a linear gradient up to 0.7 mol litre^{-1} ammonium acetate. Figure 14(a) shows the ultraviolet absorption–elution diagram of the separation. After repeated freeze-drying to remove the volatile ammonium acetate, five preparations called α, β, γ, δ, and ϵ were obtained. These were still unhomogeneous, as Figure 14(b) shows. The ϵ preparation did not show any venom activity, either in bees or in isolated insect nerve–muscle preparations. The γ and δ preparations caused paralysis when injected into honey-bees but at a low level of activity compared to that originally present in the combination of the PVA, PVB and PVC preparations. Approximately the whole original activity was recovered when the γ and δ preparations together were combined with the β preparation, which itself did not cause paralysis in honey-bees. The β preparation enhances the paralysis in honey-bees caused by the separate δ and γ preparations. This is shown for the δ and β preparations in Fig. 15. The calculated dose–effect lines are very flat, but the regression is significant.

The γ preparation showed a completely different dose–effect curve (Fig. 16) for the paralysis in honey-bees. When low doses were injected there was no visible effect, but when a certain threshold value was reached, a sharp increase in the effect was seen. Combination of the γ preparation with the ineffective (by itself) β preparation produced longer paralysis in the bees. This potentiating effect of the β preparation on the paralysing effect of both the γ preparation and the δ preparation is dose-dependent (Figs. 15 and 16).

The α preparation blocked the transmission in the sixth abdominal ganglion of the cockroach *Periplaneta americana* (Piek *et al.*, 1980b). This observation



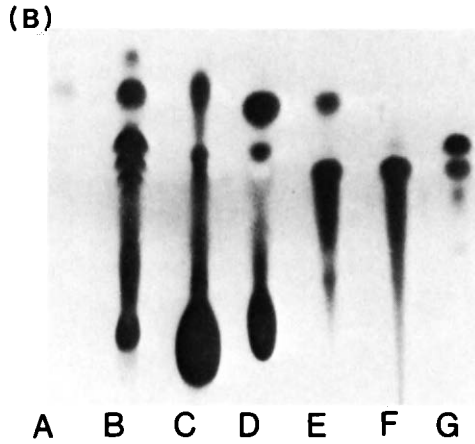
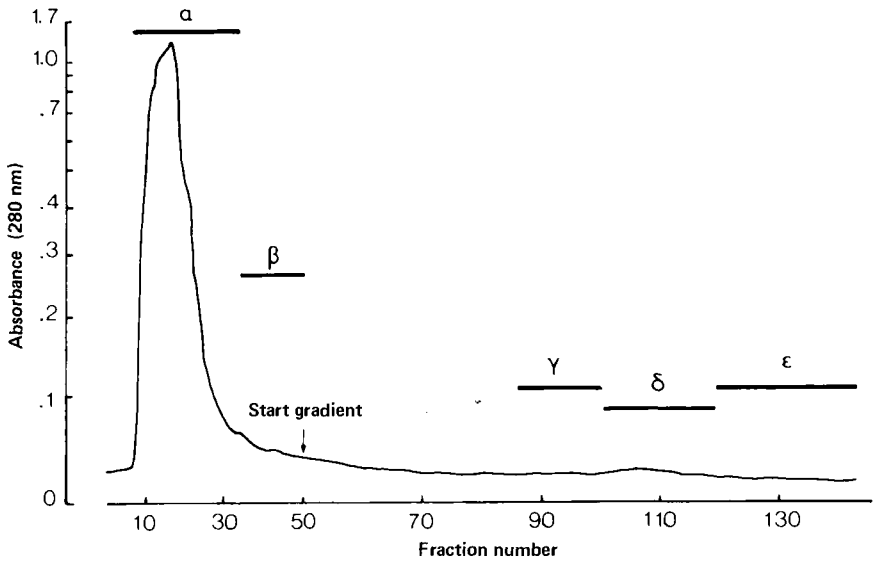


Fig. 13 Chromatography of the venom of *Philanthus triangulum*. (A) Gel chromatography on Sephadex G-25 of the low molecular weight fractions (Sephadex G-100) of a freeze-dried methanol extract of 2500 female *P. triangulum* abdomens. Eluting solvent 0.2 mol litre^{-1} ammonium acetate, pH 4.75. Part of the original activity (after Sephadex G-100) was recovered only in fractions indicated with B (further specified as PVB). The total original activity could be recovered by combining PVB with PVA and PVC. (B) Thin-layer chromatography on 0.1-mm cellulose layer of pooled Sephadex G-25 fractions shown at (a). From each group 0.1% (equivalent to ~2.5 wasps) was used. Development by butanol-1/acetic acid/water, 4:1:2 (v/v). Detection by spraying with ninhydrin solution. From Spanjer *et al.* (1982a).



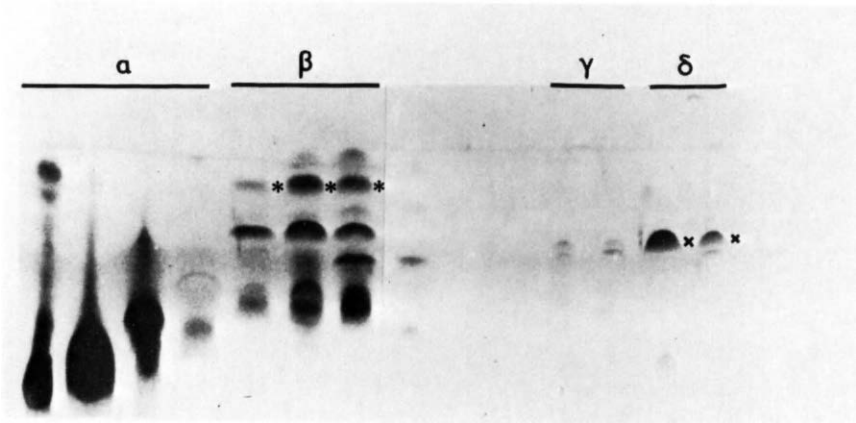


Fig. 14 Chromatography of the venom of *Philanthus triangulum*. (a) Ion exchange chromatography on SP-Sephadex-C-25. Starting solvent 0.4 mol litre^{-1} ammonium acetate, pH 4.75. Gradient up to 0.7 mol litre^{-1}. The starting material was the combination of PVA, PVB and PVC. Groups of fractions α to ε are indicated by bars. The γ and δ fractions have different paralysing activities in honeybees. These activities are potentiated by the β fractions (see Figs. 15 and 16). The α fractions are active in the sixth abdominal ganglion of the cockroach, probably due to the presence of acetylcholine. The ε fraction had no biological activity. (b) Thin-layer chromatography on 0.1-mm cellulose layer of SP-Sephadex groups of fractions shown in (a). From each group of fractions 0.4% (equivalent to about 10 wasps) was used. For details of development and detection, see Fig. 13. The β-PTX spot is indicated by *, the δ-PTX spot by ×. From Spanjer *et al.* (1982a).

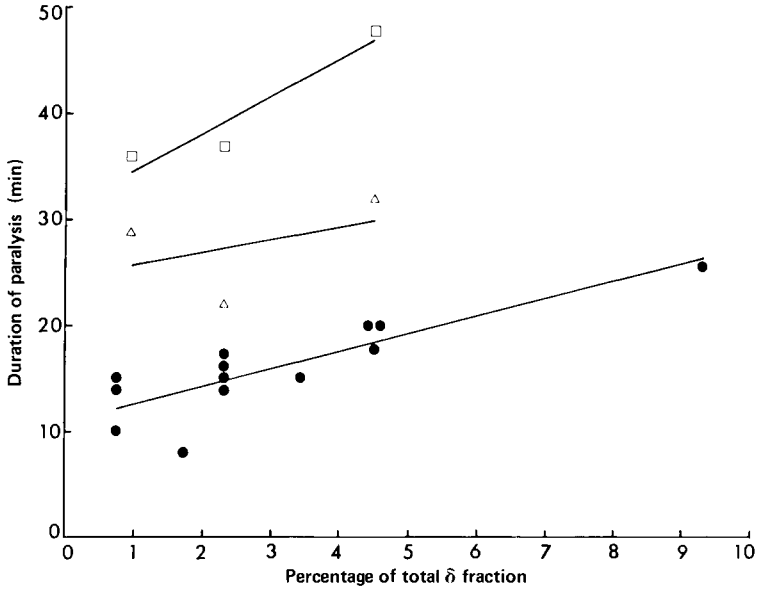


Fig. 15 Effect in honey-bees (*Apis mellifera*) workers of partly purified philanthotoxins. The duration of the paralysis (50% turnover moment) is plotted against the dose of δ-PTX, expressed as the percentage of the total fraction, injected into the thorax of 10 honey-bee workers: δ-PTX alone, ● ; each dose combined with 2% of the β-PTX fraction, Δ; or 4% β-PTX, □. One percent of each of the toxins is roughly equivalent to 5–10 wasps. Calculated linear regression lines. From Piek and Spanjer (1978).

can be explained now since this fraction contains a cholinergic factor that acts like acetylcholine on the guinea pig ileum. The presence in this fraction of acetylcholine was confirmed by a radioimmunoassay (W. Spanjer, N. de Haan, P. Mantel, and W. Th. Labruyère, personal communication). Although the venom of *Philanthus triangulum* contains a high concentration of acetylcholine (Piek *et al.*, 1983b), the acetylcholine present in the α fraction was much more than could be explained as originating from the content of the venom reservoirs alone. About 75% of the cholinergic activity probably originates from other body parts, since the starting material for the preparation of the above fractions was a homogenate of wasp abdomens.

The active principles in the β and δ preparation could be localized by analytical and preparative thin-layer chromatography on cellulose (Fig. 14) and were called β- and δ-philanthotoxin (abbreviated β- and δ-PTX). The localization on the thin layer was not possible for the activity in the γ preparation. In this case, for unknown reasons there was no recovery of biological activity after preparative thin-layer chromatography experiments.

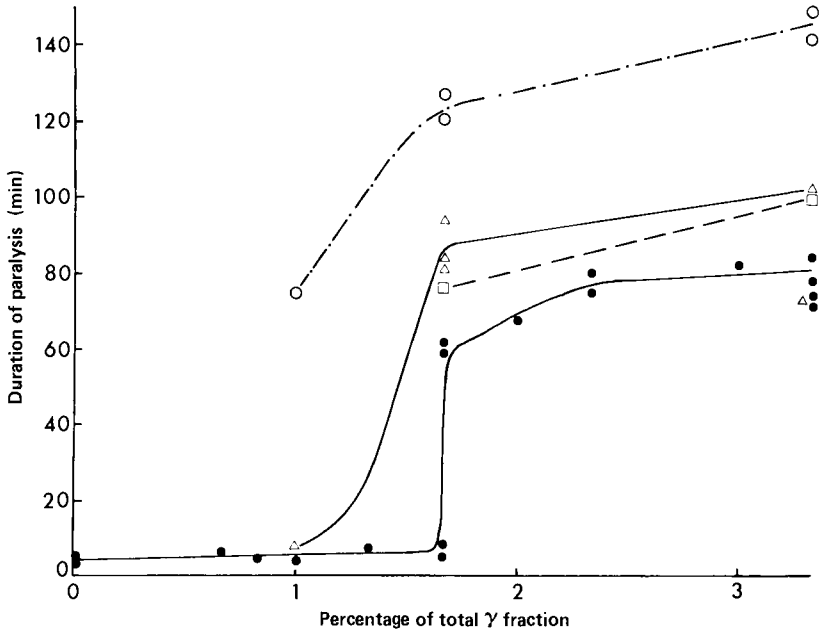


Fig. 16 Effect in honey-bee workers of the γ-PTX fraction alone, ● ; and combined with 1%, □; 2%, Δ; or 4%, O, of the β-PTX fraction. For explanation see Fig. 15 and text. From Piek and Spanjer (1978).

The pure toxins β-PTX and δ-PTX were isolated from the β and δ preparations by high pressure liquid chromatography (HPLC) on Lichrosorb RP 18 columns in 0.005 mol litre^{-1} hydrochloric acid, 2% methanol solution (Spanjer *et al.*, 1982b). An example for the purification of δ-PTX is given in Figs. 17 and 18. The total quantities of obtained pure toxins were very low. From a total of 21,000 wasps the production of β-PTX was 3.1 mg. This means that one female *Philanthus* wasp may have only about 150 ng of this component in its venom.

The average amount of δ-PTX per wasp is usually ~200 ng, but sometimes wasp venom contained a higher quantity of this component and a production of 2.2 mg δ-PTX from 2500 wasps was possible. This is ~0.9 μg per wasp, and in an exceptional case even 1.3 μg was noted. Using field desorption mass spectrometry, for β-PTX a molecular weight of 243 was determined, and using the method of exact mass determination by electron impact mass spectrometry, a molecular formula of $C_{13}H_{29}N_3O$ could be calculated for

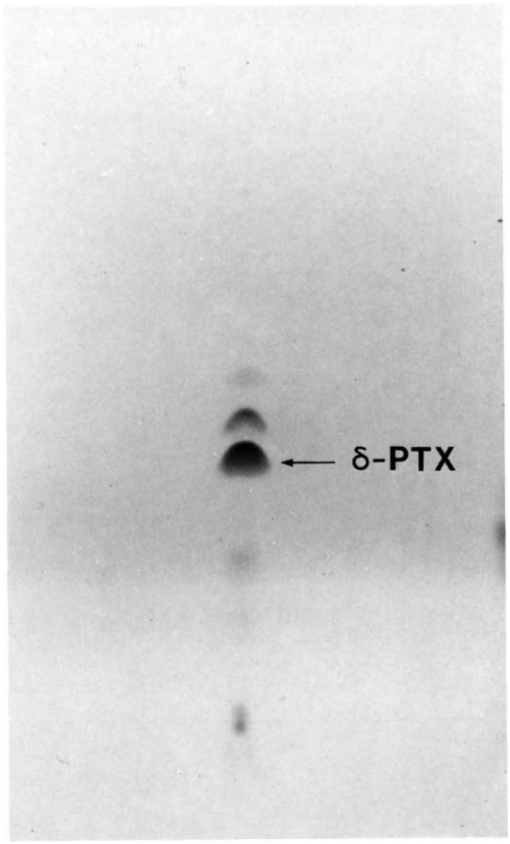


Fig. 17 Thin-layer chromatography of δ-PTX, before the HPLC purification. Conditions similar to that of Fig. 12. The amount of δ-PTX is ~3 μg.

β-PTX. Similarly determined, the molecular weight of δ-PTX is 435 (Spanjer *et al.*, 1982b) and the most probable molecular formula is $C_{23}H_{41}N_5O_3$ (N. M. M. Nibbering and R. H. Fokkens, personal communication). Nuclear magnetic resonance (NMR) spectra show some similarity in structure for β-PTX and δ-PTX. They have a polyamine character (C. Kruk, personal communication).

The molecular weights of the different active components in the *Philanthus* venom determined from these studies are in accordance with the observation from Sephadex G-10 gel chromatography experiments that the molecular weights of the active components were lower than 700 (Spanjer *et al.*, 1982a).

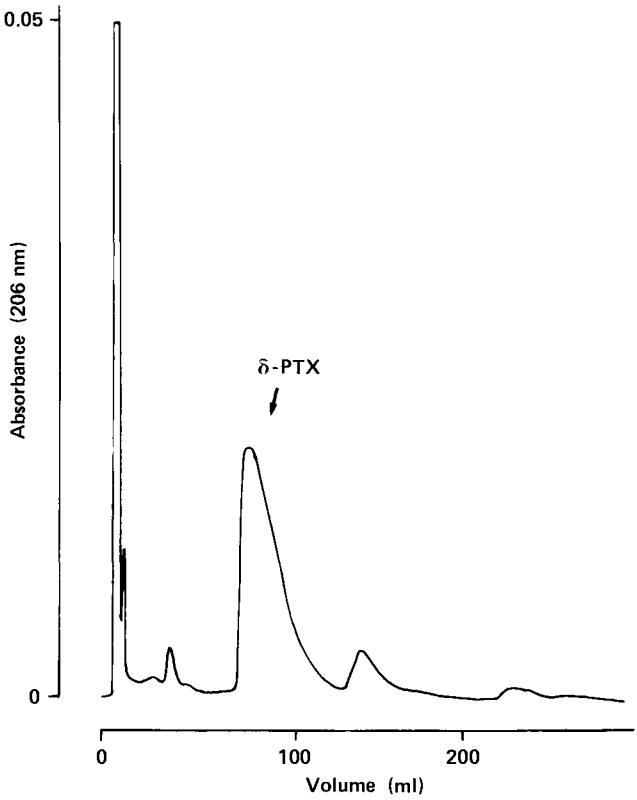


Fig. 18 Purification of δ-PTX by preparative HPLC on a Lichrosorb RP 18 column (0.9 × 25 cm). Eluting solvent 0.005 mol litre^{-1} hydrochloric acid at 10 ml min^{-1}. The amount of δ-PTX is ~70 μg. Ultraviolet absorbance was monitored by a Uvicord S (LKB).

III. PHARMACOLOGY OF SOLITARY WASP VENOMS

A. Effects on Metabolism and Endocrine Control

A consequence of the reduction in the host's mobility caused by the venom of solitary wasps is a fall in the rate of the host's metabolic processes. However, it is not easy to decide whether the effects of solitary wasp venoms on metabolic processes are direct or indirect. Nielsen (1935) compared the oxygen consumption of paralysed spiders *Epeira cornuta* stung by the pompilid wasp *Episyron rufipes* and of nonparalysed starving spiders. He also compared the oxygen consumption of paralysed caterpillars stung by

the sphecid wasp *Ammophila campestris* with that of nonparalysed starving caterpillars and concluded that the effects of paralysis and of starvation on the oxygen consumption of spiders and caterpillars were not significantly different.

Payne (1937) found that the respiration of larvae of *Ephestia kuehniella* stung by *Microbracon hebetor* was so low and so insensitive to temperature changes that she thought it to be comparable with the metabolic rates measured in diapausing insects. In fact Payne's measurement of the fall in larval respiration was ~50% while the respiration level in larvae in diapause condition is between 25 and 10% of normal levels. A comparable reduction in the rate of respiration has been demonstrated by Waller (1964, 1965) with larvae of *Galleria mellonella*, also stung by *M. hebetor*. Waller found a difference in the reduction of oxygen consumption of larvae paralysed by the wasps compared with larvae injected by an amount of venom organ extract just large enough to completely paralyse the larvae (i.e. equivalent to 0.005 venom organ). In the first group the oxygen consumption was reduced to less than 50%, in the second group the oxygen consumption was ~80% of the control (Fig. 19). This difference cannot be due to the different routes of venom administration, since Waller (1965) showed that the oxygen consumption of a just-paralysed larva could be further reduced by increasing the concentration of injected venom from 0.005 to 0.01 venom organ (V.O.) per larva. When the venom concentration was increased from 0.01 up to 0.1 V.O. $larva^{-1}$, there was no additional change in oxygen consumption. Waller interprets these results by suggesting that *M. hebetor* venom might have two effects: a first effect to stop the locomotor activity, this being accompanied by a 20% reduction in oxygen consumption, and a second direct effect on oxygen consumption, appearing at higher venom concentrations and resulting from a more direct inhibition of respiratory metabolism. However, a preliminary experiment in which the oxygen uptake of homogenates of whole larvae was measured showed no inhibition of respiration by *M. hebetor* venom (Waller, 1965).

The effect of *Microbracon hebetor* venom on *Galleria mellonella* has also been examined by Edwards and Sernka (1969), who injected 1- and 2-day-old pupae with an amount of venom equivalent to 0.5 venom organ. Contrary to the larvae, the pupae at this stage in development do not show muscular activity unless disturbed. Venom-treated pupae had respiratory rates similar to those of controls injected with an equal volume of 0.9% NaCl (Fig. 20). From these results it was concluded that the effect of the venom of *M. hebetor* on the metabolic rate is restricted to the decrease in metabolism resulting from paralysis. This conclusion does not agree with that of Waller (1965) but agrees with his preliminary experiment on oxygen uptake. Moreover, the above-described reduction in oxygen consumption can be explained by

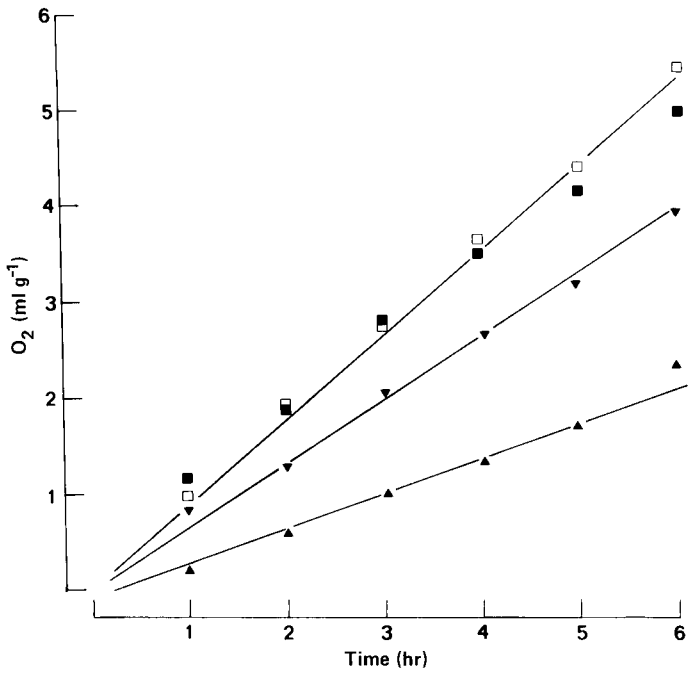


Fig. 19 The reduction in the oxygen consumption of larvae of *Galleria mellonella* caused by the venom of *Microbracon hebetor*. ■ and □, The oxygen consumption of unparalysed larvae; ▼, larvae paralysed by injection of a venom solution, just enough to completely paralyse them; ▲, larvae paralysed by a wasp sting. From Waller (1964).

assuming an inhibition of a subthreshold synaptic activity by doubling the venom concentration.

Using partly purified venom preparations from *Microbracon brevicornis*, Lee (1971) found a 50% inhibition of the aerobic oxydation-driven NAD^+ reduction by succinate when mixed with 0.5 mg of calf heart submitochondrial particles. This is reached with a 100,000 times higher venom concentration than that required to cause paralysis in a wax moth larva. A comparably high amount of venom could increase the oxygen consumption of a preparation of rat liver mitochondria, even in a oligomycin-inhibited preparation. Lee (1971) compared the effect of the venom with the effect of the uncoupler DNP. In Lee's experiments (see also Beard, 1978) the oxygen consumption in the ADP-activated state (state 3, according to Chance and Williams, 1956) exceeds the idling state (state 4) by a factor of 2.5. For intact mitochondria this ratio of oxygen consumption in state 3 over that in state 4 is normally 10 or higher. Lower values indicate damage of mitochondria

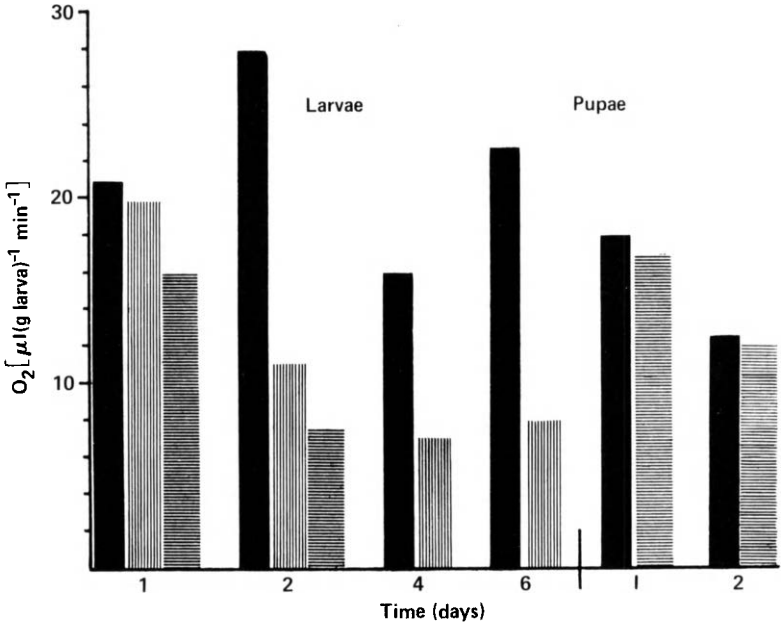


Fig. 20 The effect of *Microbracon hebetor* venom on the oxygen consumption of larval and pupal *Galleria mellonella*. Controls, ■; stung larvae, ▥; injected larvae and pupae, ▤. Each bar is based on five tests measured over a 2-hr period. From Edwards and Sernka (1969).

(Lehninger, 1975, page 518), which obviously was the case in Lee's experiments.

Despite the limited value of Lee's experiments (damaged mitochondria and an extremely high venom concentration), it could be valuable to compare the effect of dinitrophenol and *Microbracon* venom in a preparation of intact insect mitochondria. According to Mitchell's theory (see Mitchell, 1973, 1979) an uncoupling of the oxydative phosphorylation by a specific proton conductor such as DNP could explain the effect of the venom.

Structural deformities and inhibition of host development as well as synchronization of the development of the host with that of the parasite has been described many times for terebrant wasps. For a recent review, see Vinson and Iwantsch (1980). Structural deformities of alate ants, including complete suppression of the development of the wings, were described for hosts of the chalcid wasp *Orasima* in the beginning of this century (Wheeler, 1910). Changes in the development of the aphid *Aphids craccivora* by the braconid wasp *Aphidius platensis* are thought to be brought about by interference with the titre of juvenile hormone (see Vinson and Iwantsch,

1980). However, the question arises whether these effects may be due to the presence of the parasite or may have been caused by the wasp's venom. Guillot and Vinson (1972b) have investigated the way in which the action of the venom of *Cardiochiles nigriceps* (Braconidae) may be involved in the regulation of growth in its host, the tobacco budworm *Heliothis virescens*. They suggest that the venom gland material acts synergistically with the substances produced by the host to regulate growth. Holdaway and Evans (1930) showed that when *Alysia manducator* (Braconidae) stings the sheepfly maggot, *Lucilia sericata*, the wasp-induced pupation occurred without parasitization, and thus must be the result of the stinging action. The venom of the braconid wasp *Clinocentrus gracilipes* causes in caterpillars a transient paralysis, with a recovery time of ~10 min. However, the venom employed by *C. gracilipes* switches the host to a pharate pupal stage (cuticle separated from hypodermis) irrespective of its larval instar (Shaw, 1981). On the contrary, the venom of the wasp *Eulophus larvarum* (Chalcidoidea) prevents separation of the cuticle from the epidermis (apolysis) in larvae of *Orthosia stabilis* (Lepidoptera) after a depressed feeding period (Shaw, 1981). Although many physiological and biochemical changes which occur after parasitism may be due to the presence of the parasitizing wasp larva, the few examples above show that at least some of these phenomena (paralysis as well as changes in growth, feeding and development) may be caused by the venom.

Much work has to be done before it will be possible to say that solitary wasp venoms might affect endocrine systems of their hosts.

B. Neurotoxic Effects

Solitary wasp venoms often cause changes in the behaviour of the host. In most cases it has been possible to specify the action of the venom as an effect on the function of the nervous system. A simple way to study in insects some aspects of nervous system functions is to isolate parts of the body by ligatures. This technique is easy to apply to insect larvae, for example, caterpillars. Beard (1952) used this technique to demonstrate that in larvae of the greater wax moth *Galleria mellonella*, the venom of *Microbracon hebetor* is transported through the haemolymph. In his experiments venom was introduced on one side of the ligature; paralysis set in only on that side. If the ligature was cut and the flow of haemolymph restored, the previously unparalysed larval region then became paralysed. Piek (1966a) used ligatures to divide larvae of the moth *Philosamia cynthia* into three parts (A, B and C in Fig. 21). Careful electrical stimulation of the ventral part of region C resulted in a simultaneous contraction of all parts of the ligated caterpillar, indicating that the nervous system still conducted impulses through the length of the ligatured larva. Injection of a large dose of *M. hebetor* venom into

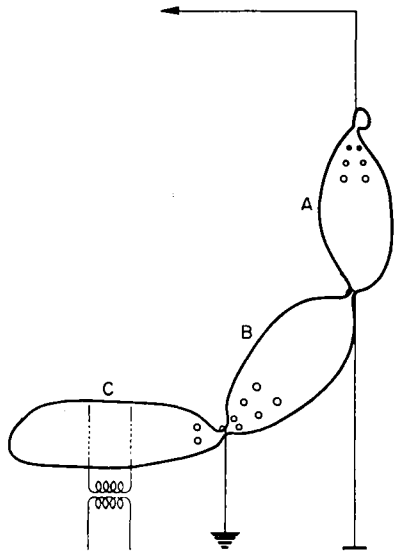


Fig. 21 Ligated larva of *Philosamia cynthia*. Injection of *Microbracon hebetor* venom into region (B) results in paralysis of this region, and not of the anterior or posterior regions. Electrical stimulation of the ventral nerve cord in region (A) results in simultaneous contractions of regions (A) and (C). Mechanical stimulation of region (B) also induces contractions in (A) and (C). From Piek (1966a).

region B rapidly paralysed this region, leaving the regions A and C unaffected. Penetration of the venom into regions A and C had obviously been prevented by the ligatures. Stimulation of region C resulted in contractions of the regions A and C only. Thus nervous conduction was not affected by the venom. Tactile stimuli given to the integument of region B resulted in contractions of regions A and C. The sensory cells in region B were therefore not affected by the venom.

Valuable as such experiments on intact, or nearly intact, animals may be, further information can only be obtained from dissected animals or isolated organ systems. In a dissected larva of *Galleria mellonella* paralysed with *Microbracon hebetor* venom, Beard (1952) was the first to record action potentials from stimulated as well as unstimulated nerves. Piek *et al.* (1971), studying isolated parts of the nerve cord of the locust *Schistocerca gregaria*, found that treatment with very high concentrations of *Philanthus triangulum* venom did not change the electrical activity. The insensitivity of the nervous system to these venoms is therefore likely to be due to a barrier preventing venom penetration into the nervous system. Another possibility might be that these venoms do not affect the nervous system at all. In the next we shall

see that the latter hypothesis is not true for the venom of *Philanthus triangulum*.

In his *Souvenirs Entomologiques* Fabre (1879–1910) presented the view that solitary Aculeata sting their victims in the central nervous system. In contrast to this, Ferton (1902–1920) thought that this seeming evidence was caused by the fact that aculeate wasps sting through the soft membranes between legs and body. When Rathmayer (1962a,b) found that the venom of *Philanthus triangulum* was very active on the locomotor system of a honeybee, even when the venom was introduced into the haemolymph, the conflict seemed to be even further from being solved. However, two lines of research have been developed to solve the problem. First, painstaking study of stinging behaviour of aculeate wasps suggests that they sting into the insect ganglia (Steiner, 1962, 1981a,b) (see Chapter 4), and second, pharmacological work described here suggests that venoms of the aculeate wasps are active in the insect central nervous system.

1. Effects on Behaviour

In a review on host regulation by insect parasites, Vinson and Iwantsch (1980) conclude that this regulation results in varying degrees of a pathological condition in the host, including changes in growth rate, food consumption, development, morphology, behaviour, respiration, etc. These changes may be brought about by the developing larva of the wasp, although according to Vinson and Iwantsch (1980) there is increasing evidence that many of these effects are caused by factors injected by the female parasitic wasp at oviposition. This has been well documented for a special change in the host's behaviour, called paralysis. Since paralysis in insects caused by a sting of solitary wasp varies from incomplete to complete and from transient to permanent, it might be possible that changes in behaviour other than paralysis are wrongly described as some sort of paralysis. For example, Iwata (1932) described that the pompilid wasp *Homonotus iwatai* stings the spider *Cheiracanthium rufulum* to a complete but transient paralysis. After recovery in about 30 min the most noticeable effect of the sting is the complete prevention of oviposition by the host. The spider remains sluggish. Crickets stored by *Sphex lobatus* (Sphecidae) recover almost completely in 10–15 min. Yet apparently a considerable lethargy follows, for they show no indication to escape from the burrow. (Hingston, 1925a, 1926). A number of sphecid wasps belonging to the subfamilies of Ampulicinae and Larrinae seem to sting their prey to a transient immobility, which is followed by a docile behaviour (*Dolichurus stantoni, Larra luzonensis, Notogonoidea williamsi* and *Cratolarra pitamawa*) (Williams, 1919c). It is probable that in Sphecidae this lethargy or deactivation is caused by the wasp's sting into the suboesophageal ganglion.

This has been described in detail by Steiner (1962) (see for details Chapter 4, Section II,D,1). Another example started with the description by Réaumur (1742) of a wasp which was most probably *Ampulex compressa*. Réaumur described the change in behaviour of the prey, a cockroach, as a loss of forces. This has been wrongly interpreted as a paralysis (Lepeletier, 1841) (see also Chapter 1, p. 6), despite the fact that changes in behaviour have also been described for victims of other species of *Ampulex*: 'The wasp (*A. sonnerati*) has taken away the cockroach ability to defend himself' (Sonnerat, 1776). Cockroaches stung by *A. compressa* were dazed with fear (Bingham, 1897). After a sting by *A. sibirica* (= *A. compressiventris*) (according to Williams, 1929), 'the cockroach became instantly quiet and submissive, and suffered itself to be led away . . .' (Sharp, 1901; referring to Perkins). According to Maxwell-Lefroy (1909), *A. compressa* stings a cockroach (*Periplaneta*) along the side of the prothorax, and in the direction of the suboesophageal ganglion (Figs. 22 and 23). The cockroach did not, however, 'appear much worse after the sting, and if the wasp after this so-called paralysis strays away in search of a hole, the cockroach manages to slip away slowly ...' (Maxwell-Lefroy, 1909). However, normally the cockroaches do not escape and are consumed by the wasp's larva. Hingston (1925a) did not understand how a cockroach (*Shelfordella tartara*) from the Bagdad Oasis, revived after being stung by *A. assimilis*, endured such a catastrophe as being eaten alive. The double sting action as described by Maxwell-Lefroy (1909) has been confirmed for *A. caniculata* from Missouri (Williams, 1929) and for *A. compressa* (Williams, 1942). Comparable phenomena have been described by Ferton (1895b) for the cockroach *Loboptera decipiens* stung by the ampulicinine wasp *Dolichurus haemorrhous*.

Recently, the sting and nesting behaviour of female *Ampulex compressa* in captivity has been redescribed and photographed (Piek *et al.*, 1984b). As soon as the cockroach is introduced into the wasp's cage, hunting starts. This finally results in an attack in which the wasp, using his mouthparts, firmly holds the cockroach along the thin edge of the dorsal thorax (Fig. 22, left). The wasp first stings into the prothorax and then stings a second time in the throat of the cockroach. The result is a transient flaccid paralysis (Fig. 22, right). After a period varying from 15 min to 2 hr and after having found a cavity to store the cockroach, the wasp returns to the partly revived cockroach and cuts the host's antennae to a third or a quarter from their base (Fig. 23, right top). A drop of haemolymph often appears at the cut end of the left antenna (Fig. 23, left and right top). The wasp often sucks haemolymph from the cut antennae (Fig. 23, left and right bottom). The cockroach is now guided by the wasp to a cavity. The cockroach follows passively (Fig. 24). Once the cockroach has been stored, the wasp deposits an egg (Fig. 25, top) and closes the cavity loosely with any material available (in this case filter paper, see Fig. 25, bottom).

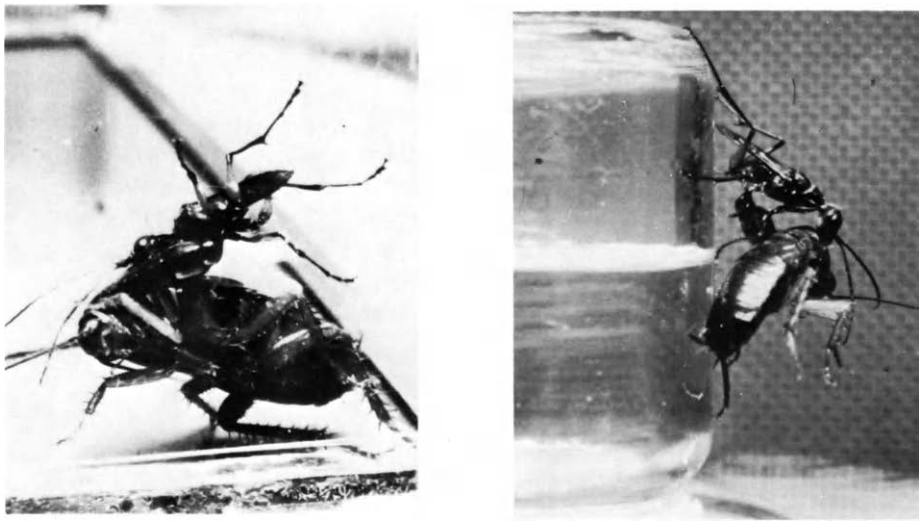


Fig. 22 Stinging action of *Ampulex compressa*. From Piek *et al.* (1984b).

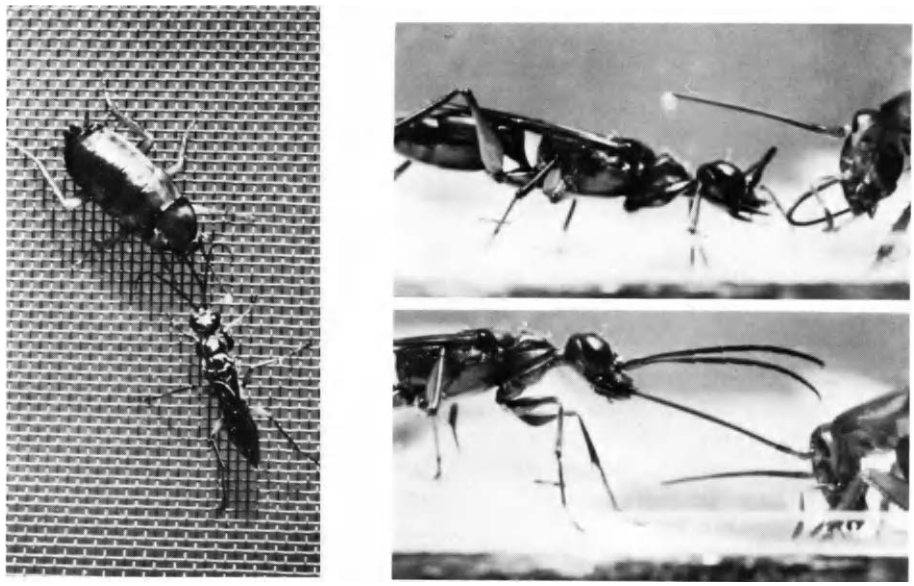


Fig. 23 Amputation of the antennae is followed by sucking haemolymph from the cut ends of the antennae. From Piek *et al.* (1984b).

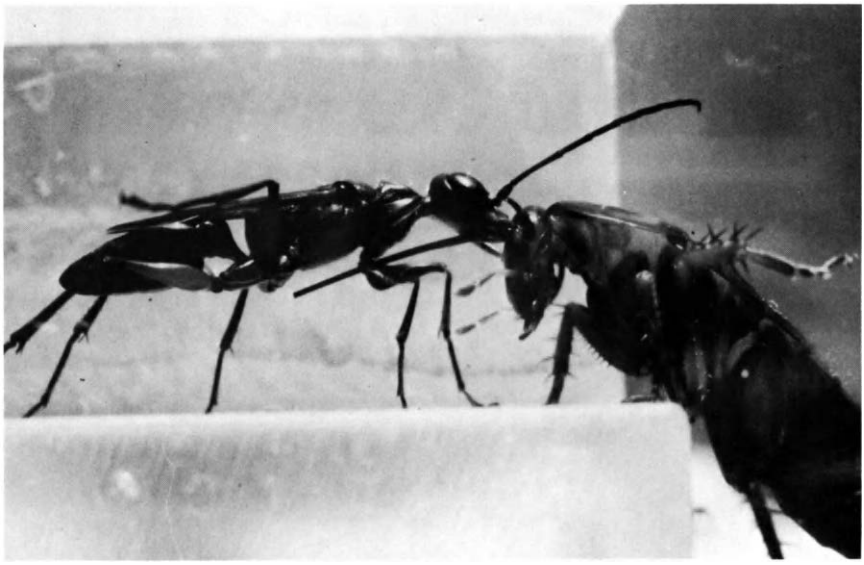


Fig. 24 *Ampulex compressa* guides the deactivated cockroach to climb in a cavity. From Piek *et al.* (1984b).

The submissive behaviour of the cockroach is obviously due to the wasp's stinging and has nothing to do with the amputation of the antennae.

The first sting is always directed to the base of the prothoracic legs. Once the cockroach is immobilized by this first sting, the wasp stings the host near the suboesophageal ganglion. Figure 26 shows the two different stinging positions in relation to the CNS of the cockroach. From these observations two questions arose (Piek *et al.*, 1984b): (1) Is the initial and transient paralysis followed by a second phase of (incomplete) paralysis, or is it followed by a change in behaviour without reduction in the cockroach's locomotor abilities, and (2) Are the two phases of relative immobility related to the two different sting actions and stinging sites?

In order to study the behaviour of cockroaches stung by the wasps, they were removed from the wasp's territory either after the sting was given in position I (see Fig. 26) or after a subsequent sting in position II. Paralysis recovery time was estimated by laying a paralysed cockroach on its back and measuring the period of time from the first or the second sting, respectively, until the moment the cockroach was able to turn over. The first sting (position I) results in a short-lasting paralysis, and that recovery is significantly ($p < .05$, Student's t test) delayed after a subsequent sting in the direction of the suboesophageal ganglion (position II). Nevertheless, even after two stings the paralysis is very short-lasting (a few minutes).

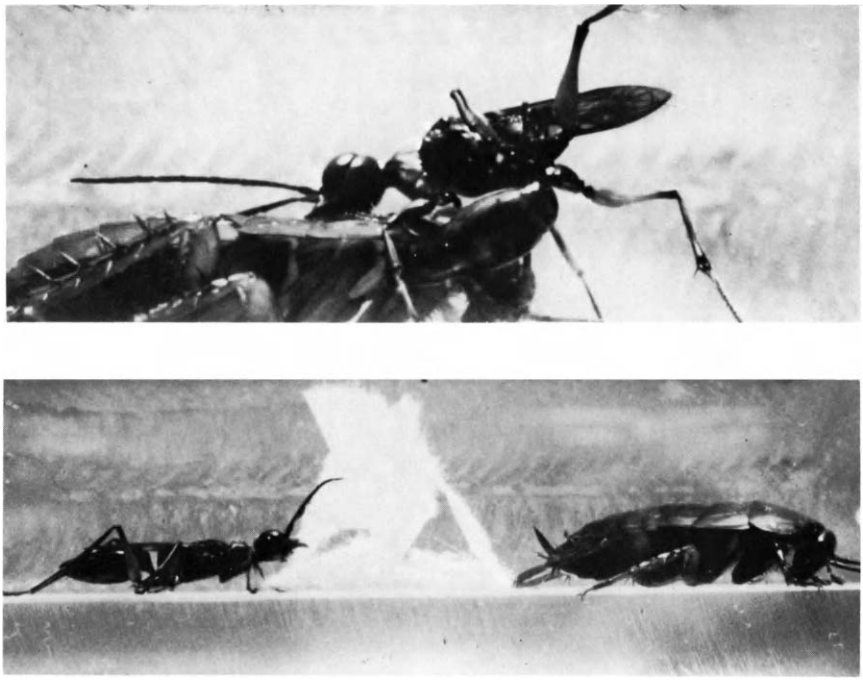


Fig. 25 Oviposition (top) and closing of the nest (bottom) with filter paper. The cockroach, recovered from the initial paralysis, is deactivated and does not try to escape. From Piek *et al.* (1984b).

After recovery from the initial paralysis the locomotor activity of cockroaches stung once or twice was compared with that of control animals. Locomotion was studied with just enough light to observe and stimulate the cockroaches by blowing air puffs onto the cerci and using a locomotion compensator coupled to an analysing computer system and x–y plotter. Three groups of adult male cockroaches were studied 1–3 days after they were stung: control animals, animals which had been stung once, in position I, and animals which had been stung twice, in positions I and II. Piek *et al.* (1984b) concluded from their experiments that the first sting resulted in a short-lasting and completely reversible paralysis, and the second sting (into the suboesophageal ganglion) caused an irreversible change in behaviour: undisturbed cockroaches seem to be lethargic, but if stimulated they are able to run with speeds that equal that of control animals. The observations by Sonnerat (1776), Bingham (1897), Williams (1929) and Maxwell-Lefroy (1909) as well as the observations described here indicate that after recovering from

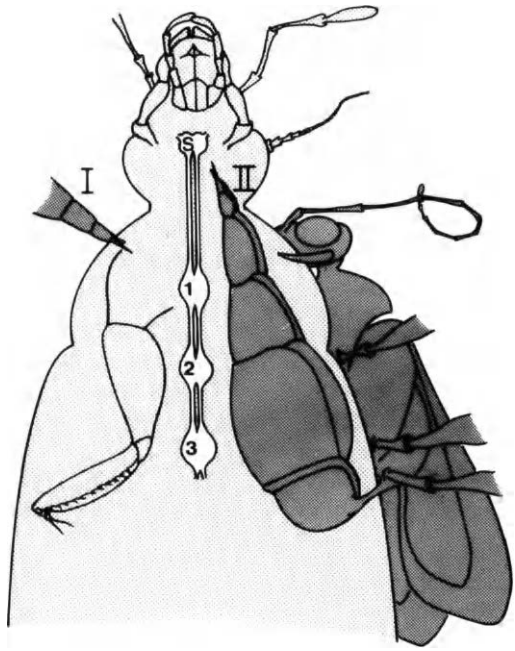


Fig. 26 Position of the two stinging sites: I, the approximate position of the first sting into the prothorax at the base of the prothorax leg. II, the position during the second sting in the direction of the suboesophageal ganglion(s). Numbers 1, 2, 3 indicate the pro-, meso- and metathoracic ganglia. For the sake of clarity both stinging sites are drawn at different sides of the cockroach, although the wasp always stings at one side. From Piek *et al* . (1984b).

the initial paralysis the cockroaches were not really paralysed, but lethargic. In a recent study (T. Piek and J. H. Visser, in preparation), it was found that the lethargic (deactivated) state increases during the first 30–60 min and then partly recovers to a steady state.

Although many members of solitary wasps produce paralysing venoms only a few of these venoms have been studied in detail. Therefore, the generalization that paralysis is the only action of solitary wasp venom is inadmissible. The above observations demonstrate that the stings of the aculeate wasp *Ampulex compressa* cause transient paralysis and an irreversible change in the behaviour of the cockroach, this change being certainly not caused by the parasitizing larva of the wasp. The observations also indicate that the suboesophageal ganglion might be an important centre for regulating behaviour. According to Kien (1983), the locust suboesophageal ganglion plays a unique role in mixing and distributing motor information to the rest of the body and in maintaining behaviour.

2. Effects on the Central Nervous System

The preceding chapter discusses evidence favouring the idea that a number of Aculeata sting into the central nervous system of their prey. The question now arises as to whether the venom of these wasps, when brought into the ganglia, could interfere with synaptic transmission within the ganglia. Rathmayer (1962a) found that honey-bee workers, 2 hr after being stung by *Philanthus triangulum* in the first thoracic ganglion show morphological changes close to the site of the sting. Morphological changes in the second thoracic ganglion were not seen until 24 hr after the sting. The changes observed involved degeneration in the neuropil and the glial cells. The neurons seemed to be unaffected. Hartzell (1935) has described lesions in the central nervous system of cicadas attacked by *Sphecius speciosus*. Richards and Cutkomp (1945; see also Beard, 1952) criticized Hartzell's interpretation of the venom as a neurotoxin, arguing that the observed histological changes could be a result of degeneration in the paralysed host. Although this seems a reasonable criticism of Hartzell's experiments, it cannot apply to Rathmayer's (1962a) observations on honey-bees paralysed for only 2 hr in which the ganglia which were not penetrated by the sting showed no degeneration.

In a first attempt to solve this problem for the venom of *Philanthus triangulum*, Piek *et al.* (1980a, 1982a) demonstrated that certain venom preparations from *P. triangulum*, at concentrations which were probably lower than those injected by the wasp, caused a reversible depolarization of giant neurons in the sixth abdominal ganglion of the cockroach *Periplaneta americana* and a subsequent reversible block of synaptic transmission. The rates of restoration of both phenomena were not equal (Fig. 27).

As described in Section II,B,4, the venom of *Philanthus triangulum* (PV) contains a number of low molecular weight toxins called philanthotoxins, abbreviated as β-, γ- and δ-PTX (Piek and Spanjer, 1978; Spanjer *et al.*, 1982a). In current-clamp experiments using the single-fibre oil-gap technique (Callec, 1972; Hue, 1976), Piek *et al.* (1984a) showed that neither PV nor β-PTX and δ-PTX affect significantly the excitability of the desheathed cockroach giant axons. However, when PV was applied topically to the desheathed abdominal ganglion a depolarization of the giant neurons was followed by a complete and slowly reversible block of excitatory transmission, in agreement with the effect described above, which was recorded with the mannitol gap technique (Piek *et al.*, 1982a). At a concentration of ~ 20 μg ml^{-1}, δ-PTX caused a block of synaptic transmission without any change in resting membrane potential, and β-PTX had no effect on either the resting membrane potential or the postsynaptic potential (Piek *et al.*, 1984a). At a concentration of 10 μg ml^{-1}, δ-PTX caused a partial block to a plateau

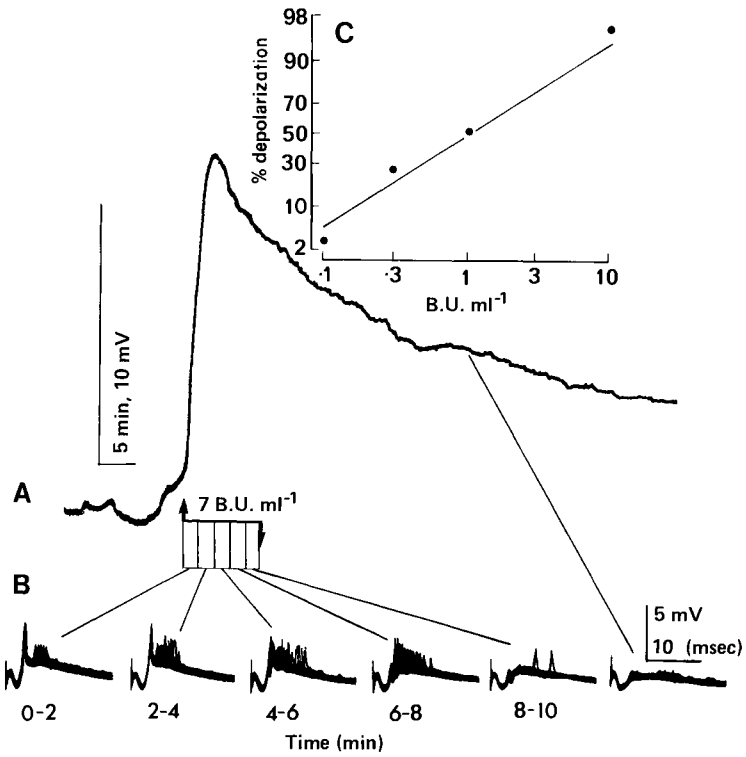


Fig. 27 Effect of the contents of venom reservoirs of *Philanthus triangulum* from Egypt at a concentration of 30 venom reservoirs (v.r.) per millilitre, that is, ~7 B.U. ml^{-1} (Bee unit, see Fig. 34) (saline flow 22.5 ml hr^{-1}), on the mannitol gap-measured DC potential (a) and on the AC-coupled synaptic events (b), recorded from the sixth abdominal ganglion of *Periplaneta americana*. The presynaptic nerves (number XI, according to Roeder *et al.*, 1960) were stimulated once every 2 sec. The lower records consist of 60 superimposed sweeps. The records start with a stimulus artefact followed by a small presynaptic potential and a postsynaptic potential, and a number of active membrane responses (spikes). (c) Dose–response curve of depolarization of the giant neurons in the sixth abdominal ganglion of *P. americana* caused by the venom preparation. The response is plotted as the percentage of maximal depolarization (33 mV = 100%) on a probability scale against log dose, B.U. ml^{-1}. From Piek *et al.* (1982b).

varying from 40 to 60% of the control value. The block was slowly reversible (Fig. 28). Acetylcholine potentials, which were iontophoretically evoked, were highly sensitive to both PV and δ-PTX, even more sensitive than excitatory postsynaptic potentials (EPSPs) (Fig. 29). This indicates that the effect of δ-PTX is on the postsynaptic side. It is now obvious that even if the wasp (*P. triangulum*) injects only 10% of the content of its venom reservoir (i.e. 10% of ~1 μl), into the bee's thoracic ganglion complex, the initial

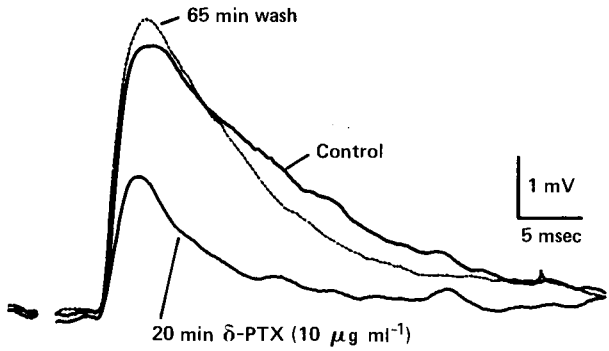


Fig. 28 Effect of δ-philanthotoxin on the amplitude of subthreshold excitatory postsynaptic potentials of the sixth abdominal ganglion of the cockroach, *Periplaneta americana*. At a concentration of 10 μg ml^{-1} the toxin causes a slowly reversible block of ~50%. From Piek *et al.* (1984a).

concentration may be ~1000 μg ml^{-1} δ-PTX, and the final concentration within the whole ganglion at least 100 μg ml^{-1}. Even when the venom was distributed equally throughout the haemolymph, the final dilution to about 10 μg ml^{-1} may be sufficient to maintain subthreshold transmission for a very long time, thus explaining the long-term paralysis of honey-bees stung by *P. triangulum*.

3. Effects on the Sensory System

Information on the effects of solitary wasp venoms on sensory receptor cells is very limited. Tactile stimuli applied to paralysed regions of ligated moth larvae (Fig. 21) still initiate contraction in the nonparalysed parts of the larvae. This indicates that the tactile sense cells are not affected by the venom of *Microbracon hebetor*. The rear legs of locusts injected with a venom solution of *Philanthus triangulum* at a concentration 50 times higher than needed for complete paralysis of skeletal muscles still show phasic activity of the chordotonal organ (Fig. 30). It appears that this organ involved in mechanoperception, as well as its afferent axons, is not affected by *P. triangulum* venom. The available evidence, albeit very limited, suggests that sensory receptors might not be sensitive to the venoms of these solitary wasps.

4. Effects on Neuromuscular Transmission

According to Beard (1952) electrical stimulation of muscles in larvae of *Ephestia kuehniella* paralysed by the venom of the terebrant wasp *Microbracon hebetor* results in contractions of the muscles. Isotonic and isometric

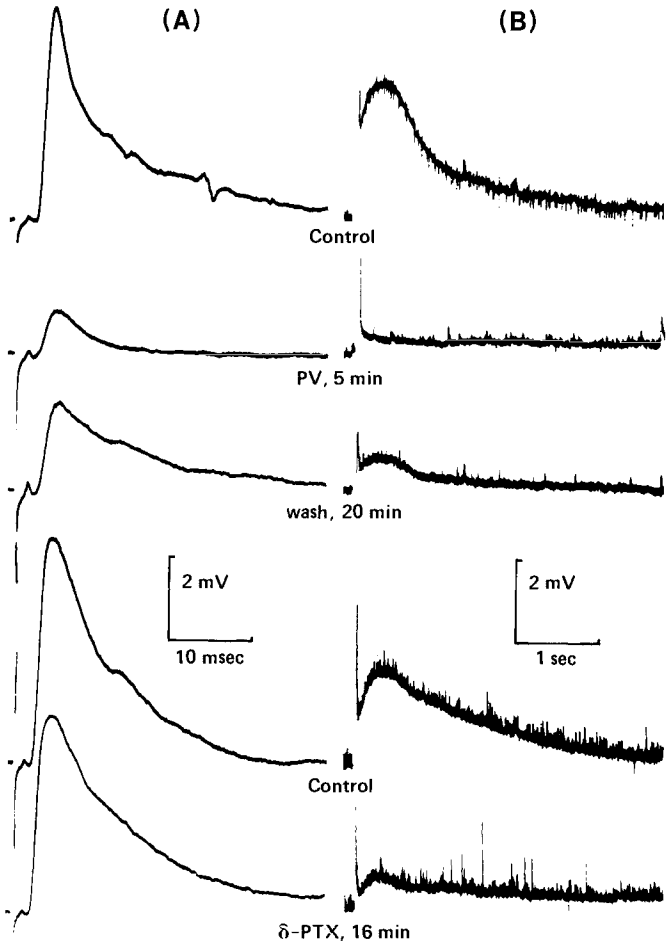


Fig. 29 Effect of the venom of *Philanthus triangulum* (PV) (two drops of 130 v.r. ml^{-1}) and δ-PTX (5 $\mu g\ ml^{-1}$) on iontophoretically applied acetylcholine (B) (300-nC pulses of 50 msec) compared with alternatingly evoked subthreshold excitatory postsynaptic potentials (EPSPs) (A). Note that the decrease in amplitude of acetylcholine potentials is more pronounced than that of the EPSPs both with PV and δ-PTX. From Piek *et al.* (1984a).

contractions have also been recorded from larvae of *Galleria mellonella* and *Philosamia cynthia* before and after these larvae were paralysed by *M. hebetor* venom. An increased stimulatory pulse width was needed to evoke a contraction in paralysed larvae (Fig. 31). This phenomenon has also been observed in larvae of the beetle *Oryctes nasicornis* stung by the aculeate wasp *Megascolia flavifrons* (Fig. 31). Both venoms increased the chronaxie of electrically stimulated muscles (see legend of Fig. 31 for explanation). This

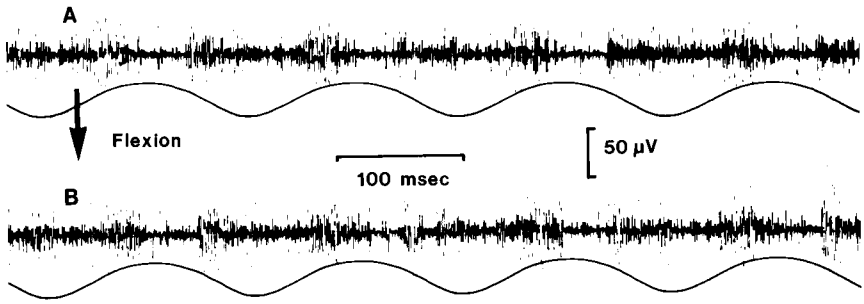


Fig. 30 The effect of *Philanthus triangulum* venom on the chordotonal organ and its efferent axons is *Schistocerca gregaria*. (A) Nerve action potentials (upper trace) and movements of the tibia (lower trace) before administration of the venom. (B) Thirty minutes after administration of the venom at a final concentration of 50 B.U. ml^{-1} (see Fig. 34). Despite the fact that this venom concentration is ~100 times higher than needed for a complete paralysis of the skeletal muscles, the frequency and distribution in time of the chordotonal afferents were not affected. Vertical scale bar, 150 angular degrees. From Piek *et al.* (1971).

indicates that before and after paralysis different kinds of excitable membranes were stimulated. It is likely that muscles were indirectly stimulated before paralysis was induced and that they were directly stimulated after paralysis.

It is clear that the venom of *Microbracon hebetor* does not affect the contractile processes of muscle fibres and that excitation–contraction coupling is also not affected. These experiments give no information on possible changes in the electrical responsiveness of muscle fibre membranes, that is, the active membrane response which follows a postsynaptic current. Piek and Engels (1969), Piek *et al.* (1971) and Walther and Rathmayer (1974) have approached this problem by stimulating muscle fibre membrane with long-lasting depolarising currents applied across the membrane. They studied the effects of the venom of *M. hebetor* on muscle fibres of the moths *Philosamia cynthia* and *Ephestia kuehniella* and worked with the venom of *Philanthus triangulum* acting on muscle fibres of the locust *Schistocerca gregaria*. Their measurement of active electrical responses to depolarising currents show that the membrane responses are not affected by the solitary wasp venom studied.

Beard's (1952) assumption that the venom of *Microbracon hebetor* probably affects neuromuscular transmission in skeletal muscles appears to be confirmed by these experiments. The same conclusion may be drawn for the site of action of the venom of *Philanthus triangulum*, though this venom may also have a central effect (see Section III,B,2).

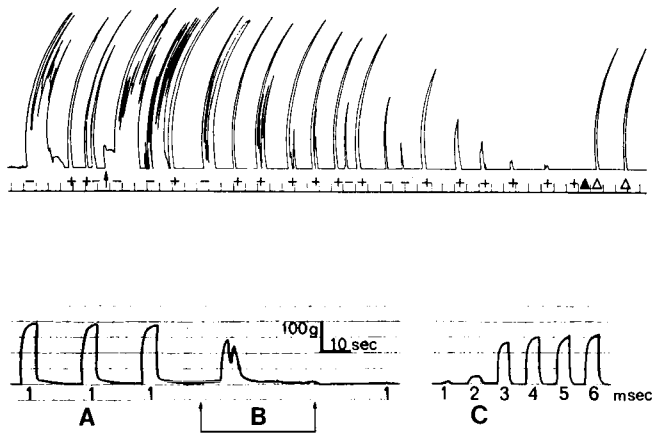


Fig. 31 Contractions of larvae of the moth *Philosamia cynthia* and on the beetle *Oryctes nasicornis*. The larvae were placed vertically between two wires. The free ends of the wire were stuck into the larvae to assure good electrical contact. Top: *P. cynthia* was stimulated (indicated with +) by pulse trains of 50 Hz, 25 V, 0.1 msec pulse width, during 10 sec. The contractions following this supramaximal stimuli as well as the spontaneous contractions (indicated with −) were ~10% of the body length. Arrow: injection of 20 µl venom of the braconid wasp *Microbracon hebetor*, equivalent to four venom glands (▲) 50V, 50 Hz, 0.1 msec, during 10 sec; (Δ) 25V, 50 Hz, 1 msec, during 10 sec. From Piek (1966a). Bottom: Effect of a sting by the scoliid wasp *Megascolia flavifrons* in the direction of the thoracic ganglion mass of *O. nasicornis*: (A) 25 V, 50 Hz, 1 msec pulse width applied for 5 sec; (B) stinging act; (C) increase in pulse width from 1 to 6 msec. From Piek *et al.* (1983a). In both records the venom caused an increase in the chronaxie. Chronaxie is the stimulus duration, expressed in milliseconds, required to produce a contraction when the strength of the stimulus is twice the threshold value measured at very large pulse widths.

a. Effects on Heart Muscle. Table I summarizes more than 600 reports, which nearly all show that prey of solitary wasps are paralysed and not killed, at least in an early stage of paralysis. Transient paralysis has been described in more than a quarter of the cases. Recovery from paralysis lasting as long as 1 or more months has been observed (Peckham and Peckham, 1898; Hicks, 1932a) (see also Fig. 12). It does not seem likely that deeply paralysed animals are able to survive and recover without some circulation of haemolymph, and, indeed, heartbeat has been observed in many paralysed insects.

In larvae of *Galleria mellonella* and *Ephestia kuehniella* paralysed by the venom of *Microbracon hebetor* the heartbeat appears to be perfectly normal, but gradually slows down after several days, presumably because of the general disability of the insect (Hase, 1924; Beard, 1952). Larvae of the beetle

Dendroctonus pseudotsugae stung by the braconid wasp *Coeloides brunneri* become paralysed in ~12 hr and survive in the paralysed state for ~2 weeks, maintaining a distinct heartbeat throughout this time (Ryan and Rudensky, 1962). However, Myers (1927) described that maggots of the genus *Calliphora* and *Lucilia* are completely paralysed for several seconds to 1 min after the withdrawal of the ovipositor of the braconid wasp *Alysia manducator*, and that before resuming activity the maggots are to all appearance dead, and there is no perceptible beating of the heart. This organ does not recommence beating until the violent struggles of the recovered maggot have been continued for a couple of minutes (1–14 min, in 40 observations). Williams (1928a) observed a slow heartbeat in a beetle larva paralysed by the scoliid wasp *Pterombus iheringi*. A fly, which was caught in the field by the sphecid wasp *Mellinus arvenis*, was completely paralysed, and since it possessed a hyaline thorax the heart could be seen beating (T. Piek, personal observation). Observations of paralysed workers of the honey-bee (Rathmayer 1962a,b) showed that the heart was still beating 37 hr after they were stung by the bee wolf, *Philanthus triangulum*. The frequency of the heartbeat was not constant; periodic bursts of beats often occurred. Rathmayer (1962a,b) has suggested that this might be caused by a block of the neurogenic pacemaker while the myogenic mechanism initiating contraction was not affected. However, it may be possible that the trauma of dissection needed to make the heart visible caused heartbeat variations. Sherman (1978) examined the heart of spiders (crab spider, *Misumena vatia*) that had been stung by a mud-dauber wasp, *Sceliphron cementarium*. The myocardial cell depolarizations in unparalysed as well as in paralysed spiders were 20–40 mV in amplitude and 300–500 msec in duration. Therefore, Sherman (1978) concluded that the normal electrical events in the heartbeat were not affected.

In contradiction to what is described above, in the 'tarantula' *Cyrtopholis portoricae*, paralysed by a number of stings from the wasp *Pepsis marginata*, the heart did not beat. Petrunkevitch (1926) described a tarantula which had been paralysed for 5 weeks, in which the heart did not beat but the spider's legs contracted when stimulated by blowing. In a similarly paralysed spider the injection of adrenaline restored the heartbeat for a couple of hours. Two months after paralysis was induced the tarantula had recovered sufficiently to be able to move when disturbed and to defaecate occasionally, but the heartbeat had not returned.

The idea that the enduring heart activity in insects paralysed by solitary wasp venoms is due to a myogenic pacemaker is based on the theory of heart control proposed by Krijgsman (1952). This theory suggests that the systole is initiated by a myogenic pacemaker while the heart rhythm is controlled by a cholinergic, neurogenic pacemaker. Miller and Usherwood (1971) found evidence for a control of the cockroach heart organised on three levels. The

basic rhythm of heart beat is myogenic. The timing of the contractions of the heart is influenced by inputs from the intrinsic cardiac ganglion cells, possibly through a feedback mechanism involving the contractions of the heart muscle. Finally, the activities of the heart muscle and the cardiac ganglion cells are influenced by inputs from the central nervous system.

Further research is obviously needed to determine whether or not the myogenic mechanisms of heart muscle are isolated from their neural controls by the venoms of solitary wasps.

b. Effects on Intestinal Muscle. Until the recent papers by Dunbar and co-workers (see further on insect visceral function) the idea was that intestinal muscles were not affected by solitary wasp venoms. Hase (1924) and Beard (1952) reported that the alimentary tract in larvae of *Galleria mellonella* and *Ephestia kuehniella* continued to function despite the induction of complete somatic paralysis by the venom of *Microbracon hebetor*. Gut movement continues and there is characteristically a discharge of accumulated faeces during 2 or 3 days after the onset of paralysis (Beard, 1952). Defaecation has also been observed in paralysed prey of other Terebrantia: for example, in larvae of *G. mellonella* paralysed by a sting of *M. gelechiae* (Fig. 32); in larvae of *Corcyra cephalonica* stung by *M. brevicornis* (Tamashiro, 1971) and in larvae of *Philosamia cynthia*, *Actias selene* and *Pieris brassicae* paralysed by the venom of *M. hebetor* (Piek and Simon Thomas, 1969). Faeces from paralysed insects are excreted as long strands, instead of normal pellets (Fig. 32). Tamashiro (1971) suggested that this results from the anal sphincter being paralysed. Defaecation has also been seen in the prey of Aculeata: for example, in beetles stung by the sphecid wasps *Cerceris tuberculata* (Fabre, 1855, 1879–1910); in beetle larvae stung by *Odynerus nidulator* (Fabre, 1879–1910); in the prey of *Cerceris rauii* (Rau, 1928); in the beetle *Strophosomus capitatus* paralysed by *Cerceris quadrifasciata* (T. Piek, personal observation); in caterpillars stung by *Ammophila sp.* (Malyshev, 1966; Rathmayer, 1966); in larval and adult locusts paralysed by *Sphex nigellus* (Piel, 1933); in a paralysed locust stung by *Sphex latreilli* and in mantids stung by *Sphex cyaniventrus* (Claude-Joseph, 1928); in a long-horned grasshopper stung by *Sphex occitanicus* (Fig. 32; Piek, 1978); in Orthoptera stung by *Stizus pulcherrimus* (Tsuneki, 1965b) and in noctuid moths completely paralysed by *Podalonia violaceipennis* (Krombein, 1936).

Beard (1960) has used two metal electrodes, one inside the foregut and the other in the haemolymph, to record the activity of the foregut of paralysed larvae of *Galleria mellonella*. He found the foregut to show spontaneous activity in a number of experiments. This activity was reversibly blocked by carbon dioxide. In other preparations the foregut did not show spontaneous activity, but activity could be induced by injection of 5-hydroxytryptamine

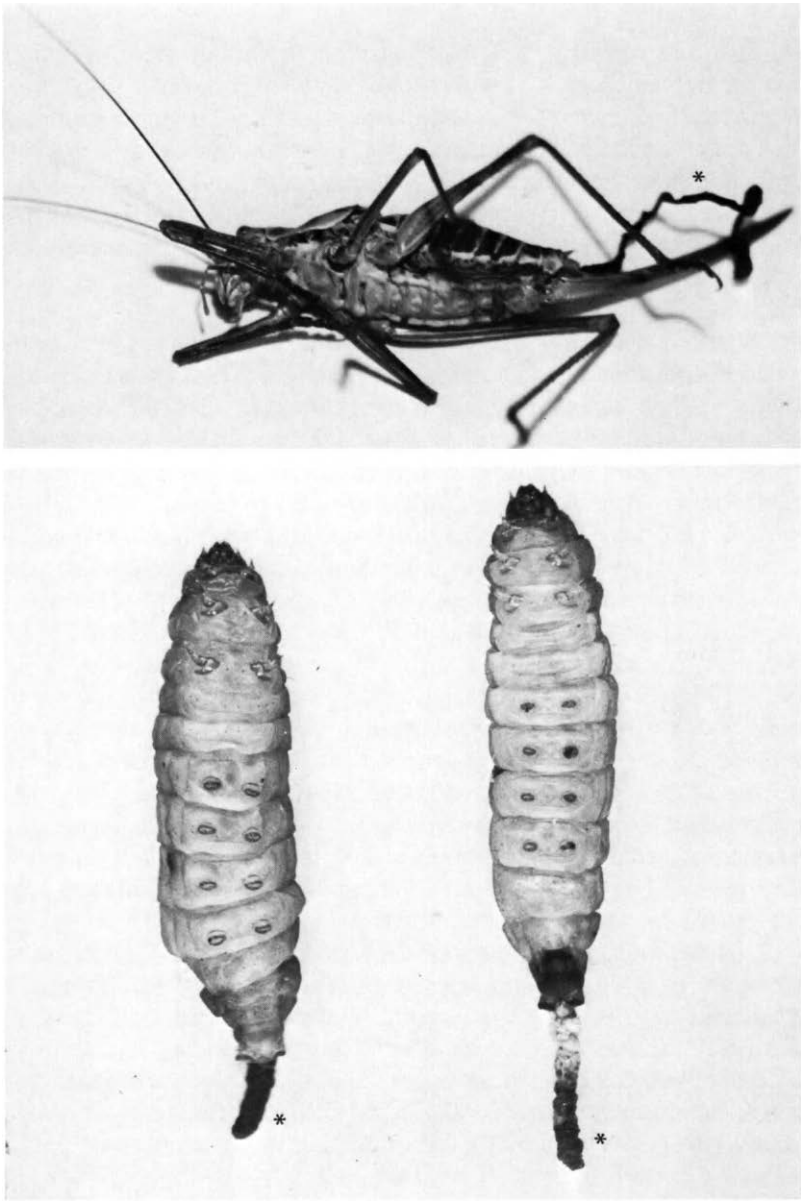


Fig. 32 A long-horned grasshopper paralysed by *Sphex occitanicus* (from Piek, 1978; photograph courtesy of R. L. Veenendaal) and larvae of *Galleria mellonella* paralysed by *Microbracon gelechiae* (from Piek *et al.*, 1974). Note the strands of faecal discharge, (*), which are probably caused by paralysis of the sphincter.

or adrenalin. We have used this technique to demonstrate spontaneous electrical activity in the foregut of larvae of *Philosamia cynthia* completely paralysed by the venom of *Microbracon hebetor*. The injection of venom from *Philanthus triangulum* into such a paralysed preparation did not decrease this spontaneous activity for more than a few minutes (Fig. 33), indicating that the contraction of the gut was also not blocked by the latter venom.

It is doubtful whether the activity of the alimentary tract described above could be considered normal (Beard, 1952). It is possible that, in paralysed insects, a myogenic control is exercised over the activity of visceral muscle fibres and that this determines gut activity when the regulation of the tissue activity by the nervous system has been blocked by the venom. Among the pieces of evidence supporting this idea are: (1) The demonstration of myogenic activity in the proctodeum of *Periplaneta americana* (Nagai and Brown, 1969) and (2) The block of the local contractions evoked by field stimulation in the gut of *Pieris brassicae* by the venom of *Microbracon hebetor*. The time taken for total paralysis to set in is dose-dependent. The slope of the line relating the dose of venom and the time needed to obtain complete paralysis of the stimulated part of the gut parallels the dose–time relationship for the paralysis of whole *Pieris brassicae* and *Philosamia cynthia* larvae (T. Piek and P. Mantel, personal observations). This suggests a similarity in the paralysing action of the venom on the innervation of the somatic and of the visceral muscles. It appears that the myogenic action of gut muscles of insects paralysed by the venom of *Microbracon hebetor*, *M. gelechiae* or *M. brevicornis* is not affected by these venoms. The neural control of visceral functions may be blocked by these venoms. More experimental evidence is needed to confirm this interpretation.

Recently we demonstrated that the venom of *Philanthus triangulum* inhibits, in the locust hindgut (proctodeum), nerve-evoked contractions to a dose-independent plateau level (Fig. 34). Here again, spontaneous contractions were unaffected by the venom. In addition to nerve-evoked contractions, the venom also inhibited responses to bath-applied glutamate, but the venom had no effect on responses to bath-applied proctolin (Fig. 35). Dunbar and Piek (1982) concluded that the venom-insensitive plateau contractions were the results of excitation by a nonglutamatergic transmission, possibly a proctolinergic transmission. Responses to iontophoretically applied L-glutamate were also blocked by the venom and by δ-philanthotoxin (Fig. 22 in Dunbar and Piek, 1983). For a description and definition of the different electrical phenomena evoked in a muscle fibre see the next section.

As is also described in detail in Section III,B,4,c, the venom of *Philanthus triangulum* and δ-PTX cause in skeletal muscles an activation-induced paralysis, and this phenomenon of activation dependency of the action of

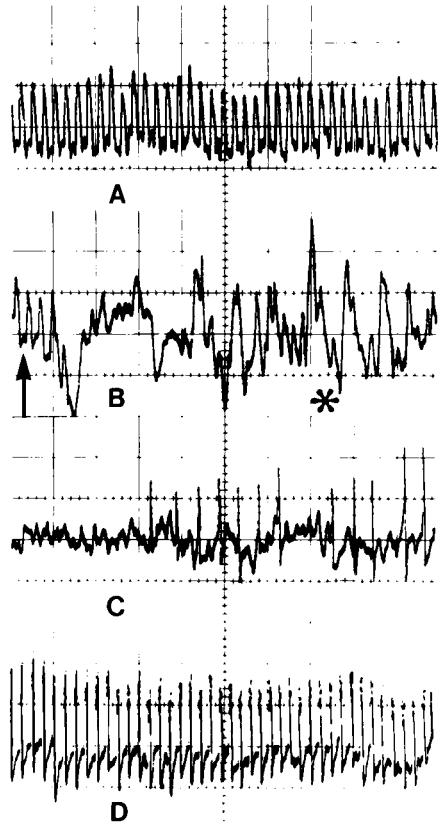


Fig. 33 Electrical activity of the foregut of a *Philosamia cynthia* larva in which the somatic muscles were completely paralysed by *Microbracon hebetor* venom. (A) Spontaneous electrical activity in the foregut. The record was made 30 min after the larva was injected with an amount of *M. hebetor* venom solution equivalent to five venom organs per gram of larva; (B) between the times marked by the arrow and the asterisk an amount of *Philanthus triangulum* venom solution equivalent to five venom organs per gram larva was injected into the larva; (C) between 3 and 4 min after the injection of *Philanthus triangulum* venom the electrical activity, which had been temporarily suppressed, recovered; (D) 7–8 min after injection of *P. triangulum* venom the electrical activity had returned to a normal level.

δ-PTX was also observed in the locust proctodeum (Fig. 36b). Kits *et al.* (1985) showed that δ-PTX blocks glutamatergic cation channels in the proctodeal muscle fibre membrane in a way comparable to what has been described earlier for skeletal muscle fibres of the locust (see Section III,B,4,c). The mean channel lifetime was slightly, but not significantly, reduced in the

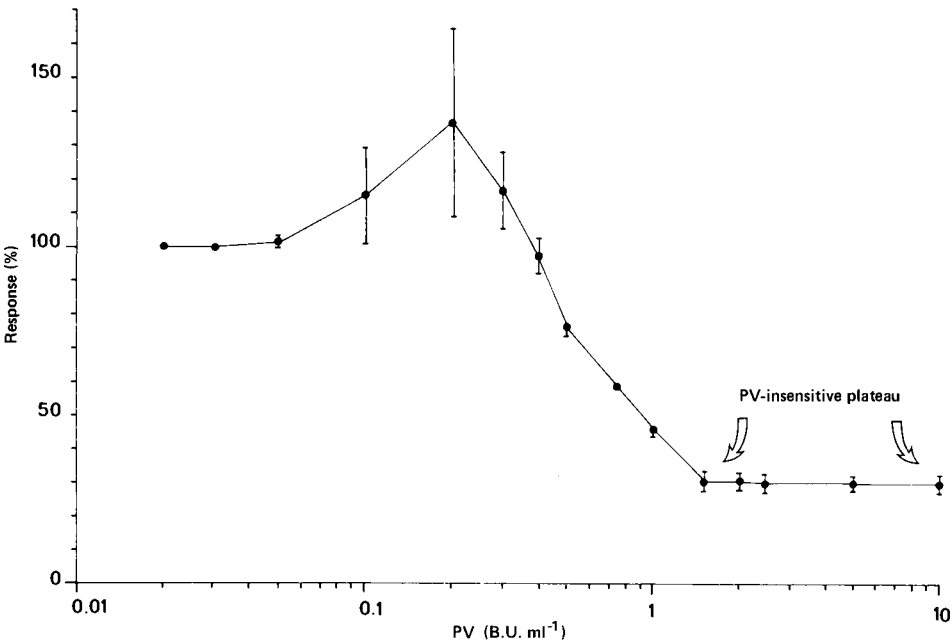


Fig. 34 Log dose-response relationship between concentration of venom of *Philanthus triangulum* (PV) and contraction height of the locust (*Locusta migratoria*) proctodeum. The response is expressed as a percentage of the control height, which was taken to be the average contraction height over a 10-min period immediately prior to addition of the venom to the bath. Each point represents the mean of four replicates ± SD. Note the dose-independent plateau at venom concentrations greater than 1.5 bee unit (B.U.) ml^{-1}. The increase in response at ~0.2 B.U. ml^{-1} is probably due to the presence of proctolin in the crude venom. With purified δ-philanthotoxin no increase has been recorded. From Dunbar and Piek (1982). 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 turn over within 1 hr when laid on their backs.

presence of δ-PTX. The mean closed times showed a pronounced increase with δ-PTX. The increase in mean closed time is mainly caused by an increase in the frequencies of long closed times, whereas the frequencies of short closed times is approximately the same. This has been explained by assuming that the excess in frequency of long closed times represents channel blocking by δ-PTX (Kits *et al.*, 1985).

c. Skeletal Muscle. Initial studies on the pharmacology of a few solitary wasp venoms have indicated that these venoms may block neuromuscular transmission in insects (Beard, 1952; Rathmayer, 1962a,b; Piek, 1966a). These and subsequent studies have shown that the venoms do not affect the excitability of the membranes of muscles and peripheral nerves. Therefore,

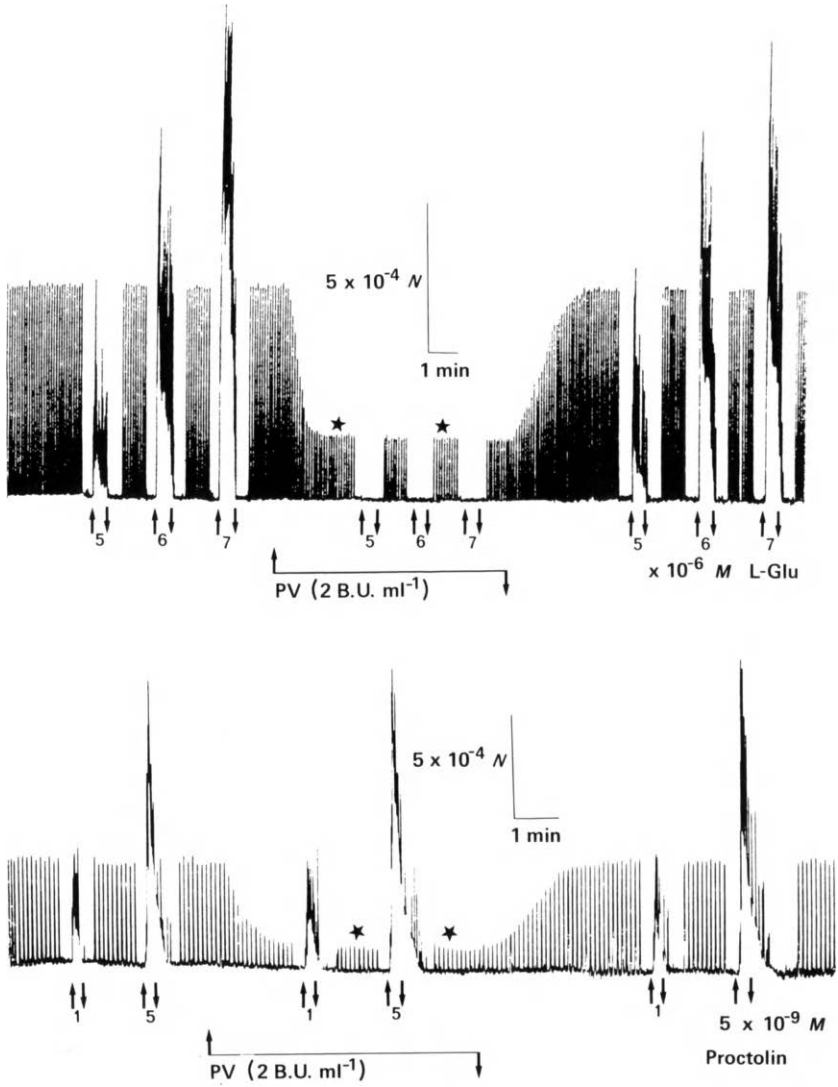


Fig. 35 Effect of the venom of *Philanthus triangulum* (PV) on nerve-evoked glutamate-evoked and proctolin-evoked contractions of the locust hindgut. Top: The effect of the venom (2 B.U. ml^{-1}) on neurally evoked and L-glutamate($5–7 \times 10^{-6}$ *M*)-evoked responses. Neural stimulation was stopped for 2 min prior to and 5 min after each concentration of L-glutamate. Note the total inhibition of L-glutamate-elicited responses by the venom and the plateau of venom-independent contractions (★). Downward-pointing arrows represent the removal of L-glutamate. Bottom: The effect of the venom (2 B.U. ml^{-1}) on neurally and proctolin-evoked contractions. Neural stimulation was stopped for 2 min prior to and 5 min after the addition of each concentration of proctolin. Note the lack of action of the venom on proctolin-evoked contractions and the venom-insensitive plateau (★). Downward-pointing arrows represent the removal of proctolin.

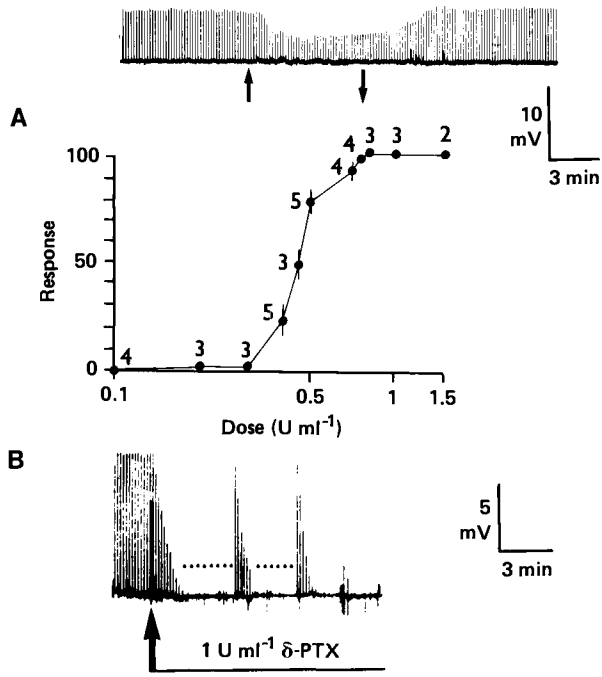


Fig. 36 The effect of δ-PTX on glutamate potentials recorded from the locust hindgut superior longitudinal muscle. (A) Dose–response curve illustrating the dose-dependent inhibition of glutamate potentials by δ-PTX. Response is plotted as the point of maximal inhibition by each concentration of δ-PTX, expressed as a percentage of a control value taken immediately before the addition of the toxin, ± SD. The figures by each point represent the number of replicates. Inset: An example of the inhibition of glutamate potentials by a concentration of δ-PTX (0.45 U ml^{-1}) (1 U is the amount of toxin extracted from 1 B.U. of venom). The upward-pointing arrow represents the addition of the toxin and the downward-pointing arrow represents washoff with normal saline. The preparation was stimulated using −7-nC pulses of glutamate at a rate of 0.1 Hz. (B) Activity-induced inhibition of glutamate potentials by δ-PTX (1.0 U ml^{-1}). Note that 1.0 U ml^{-1} δ-PTX totally inhibited all glutamate potentials, but that the response returned almost to control levels when iontophoresis was restarted after a period of no stimulation. The preparation was stimulated as in (A). The periods of no stimulation are indicated by the dotted lines. From Dunbar and Piek (1983).

the apparent peripheral action of the venoms must be on the neuromuscular junction.

It is beyond the limits of this review to do more than to introduce the general physiology and pharmacology of the insect neuromuscular junction. For a more detailed information see Usherwood and Cull-Candy (1975) and Piek (1985a).

Neuromuscular transmission in insects is mediated by at least two different transmitters: L-glutamate for excitation, and γ-aminobutyrate (GABA) for

inhibition. Brenner (1972) and Brenner and Rathmayer (1973) studied transmission in spiders. The little available information suggests that neuromuscular transmission in spiders is similar to that in insects.

Nerve impulses can evoke different types of depolarizing and hyperpolarizing postsynaptic potentials in insect skeletal muscles. Transmitter substance is released when a nerve impulse reaches a nerve terminal. It is generally assumed that binding of the transmitter to the postsynaptic receptor induces a transient change in ion permeability and a consequent local current in the postsynaptic membrane. This current passes the input impedence of the muscle membrane and therefore gives rise to a transient potential shift across the membrane (i.e. the postsynaptic potential, PSP). The PSP may either be excitatory (EPSP) or inhibitory (IPSP). In the muscle membrane an EPSP may evoke an electrically excited response. In records the active membrane response is superimposed on the EPSP. The EPSP and the active membrane response commonly cannot be distinguished with certainty. For this reason the combination of both types of potentials caused by nerve stimulation is referred to as 'neurally evoked potentials'.

Transmitter substances are generally believed to be released in discrete amounts, or quanta. At resting neuromuscular junctions quanta of transmitter substance are spontaneously released at low and random frequencies. This results in small but distinct changes in the postsynaptic potential. When these postsynaptic potentials result from the release of quanta of excitatory transmitter substance they are called miniature excitatory postsynaptic potentials (MEPSPs). In insects MEPSPs were first described by Usherwood (1961, 1963), in spiders by Brenner and Rathmayer (1973). Miniature inhibitory postsynaptic potentials (MIPSPs) also occur and have been described in insects by Piek and Mantel (1970a,b).

A commonly used method in the assessment of drug actions on neuromuscular transmission is to study various parameters of miniature potentials. Since miniature potentials occur at random intervals, changes in the mean MEPSP frequency provide a reliable indication for a presynaptic action of the drug, that is, on the motor nerve terminals. Less reliable, but still a source of information, are changes in the amplitude of the miniature potentials; such changes would possibly be an indication that a drug produces a postsynaptic effect. Since changes in miniature potential amplitude could also result from a change in the transmitter content of the quanta, a presynaptic effect cannot be excluded with certainty. A reliable indication of a presynaptic effect is a change in the ratio: EPSP amplitude over MEPSP amplitude, or IPSP amplitude over MIPSP amplitude. These ratios provide an indication of numbers of quanta involved in producing a postsynaptic potential. In multiterminally innervated muscle fibres the amplitude of the miniature potentials show considerable variation and in most cases the

amplitude distribution cannot be completely separated from electrical noise. Therefore, the evaluation of records of miniature potentials may be extremely difficult. Evaluation of amplitude distribution is ameliorated by plotting them reversibly and cumulatively on a double logarithmic scale (Huijbregts and Schreurs, 1975) (see also Figs. 40, 49, 52).

In order to avoid the generation of electrically evoked potentials, the muscle fibre membrane potential can be clamped to a fixed potential by a special circuit, the so-called voltage clamp. If this is done, the effects of venoms or their toxins on the postsynaptic current can be recorded without interference of electrically evoked potentials. A second advantage of voltage-clamping is that the current records are not attenuated by the relatively long time constant of the membrane, thus allowing one to study the effect of the venom on the time course of the synaptic current.

For the study of postsynaptic effects of venoms or toxins affecting the neuromuscular transmission of insects, the function of the nerve terminals can be replaced by a glutamate- or GABA-filled microelectrode from which the agonist is released with a current pulse. This iontophoretic application of agonists, that is, the presumed transmitter or transmitterlike components, results in a potential shift called, for example, glutamate potential, in the case that glutamate is the agonist.

Glutamate receptors are present not only in the junctional membrane, but also in the extrajunctional membrane of insect muscle fibres. To study the influence of toxins on the glutamate binding in the junctional and extrajunctional membrane three different methods can be used: (1) glutamate potentials can be evoked with bath-applied glutamate (see Fig. 43, left); in a comparable way glutamate contractions can be studied (see Fig. 37); (2) iontophoretically applied glutamate potentials or currents can be studied and (3) individual openings of ion channels can be recorded with the patch-clamp method. The study of contraction (nerve stimulus-evoked or high potassium- or glutamate-induced contractions), as well as the study of postsynaptic potentials (nerve-evoked, spontaneous miniature released or glutamate-induced), are all needed to complete the picture about the mode of action of solitary wasp venoms or their composing toxins.

Of all solitary wasp venoms for which we have some information (Table I) only two types of venoms have been studied in detail: (1) the venoms of the terebrant wasps *Microbracon hebetor* and *M. gelechiae*, and (2) the venom of the aculeate wasp *Philanthus triangulum*.

The venom of *Microbracon hebetor* causes a decrease in extracellularly recorded potentials from muscle fibres of the greater wax moth *Galleria mellonella* (Beard, 1952). Piek (1966a) has recorded a reversible decrease in the amplitude of the extracellular potentials from intersegmental muscles of larvae of the moth *Philosamia cynthia* after treating the preparation with *M.*

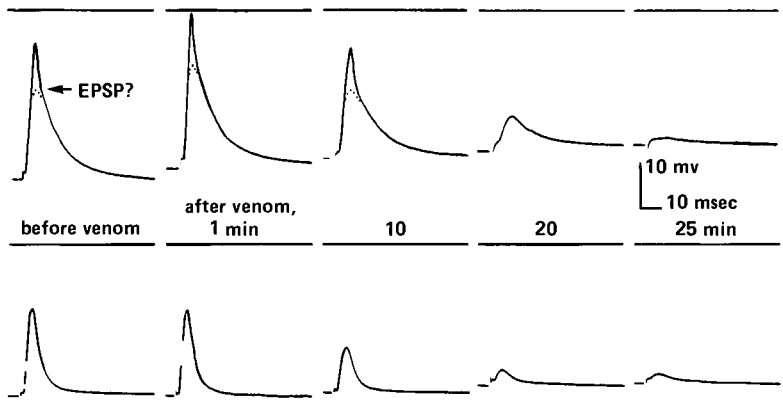


Fig. 37 Effect of *Microbracon gelechiae* venom on nerve-evoked potentials in flight muscle fibres of *Galleria mellonella*. Two different fibres were recorded from a preparation treated with 10 venom organs per millilitre. Note the small decrease in the resting potential (straight lines are zero potentials). In the top record the nerve-evoked potential may consist of an excitatory postsynaptic potential (EPSP, dotted line) plus an active membrane response.

hebetor venom. Neurally evoked potentials in flight muscle fibres of *G. mellonella* decrease in amplitude when the preparation is treated with venom of *M. gelechiae*. When the applied venom concentration is equivalent to 10 venom organs ml^{-1}, the excitatory postsynaptic potentials (EPSPs) are reduced and, about half an hour after venom application, they disappear (Fig. 37) (Piek *et al.*, 1974). The relatively slow suppression of the EPSPs correlates with the slow action of the venom in intact larvae of *G. mellonella* (see Figs. 2 and 39).

Walther and Rathmayer (1974) have used very high concentrations of *Microbracon hebetor* venom (see Table V) to produce a block of neuromuscular transmission in locust rear legs. The small residual EPSPs, recorded 2 hr after the application of venom, show a distinct facilitation at stimulation frequencies of 5 or 20 Hz (Fig. 38). Inhibitory postsynaptic potentials are not affected by *M. hebetor* venom (Piek and Mantel, 1970b; Walther and Rathmayer, 1974) and are also not affected by the venom of *M. gelechiae* (Piek *et al.*, 1974).

In moth muscle fibres treated with the venom of *Microbracon gelechiae*, there is a direct correlation between the rate at which the amplitude of EPSPs decreases and the reduction in contraction of the muscle fibres (Fig. 39). These results show that paralysis caused by solitary wasp venoms can be linked to the decrease in EPSP amplitude. This leaves the question whether the decrease in EPSP amplitude is the result of a presynaptic or of a postsynaptic effect

Table V

Relative Sensitivity of a Number of Insect Species for the Venom Preparations from Different *Microbracon* Species[a]

	M. kirkpatricki	*M. gelechiae*	*M. hebetor*[b]	*M. hebetor*[c]	*M. hebetor*[d] *A*	*M. hebetor*[d] *B*
Galleria mellonella larvae (Lepidoptera)	100	100	100	100	100	100
Apis mellifera workers (Hymenoptera)	2.2	690	5.6	3.9	1.5	5.9
Musca domestica adults (Diptera)	1.1	1.9	0.2	0.8	1.3	0.2
Locusta migratoria larvae (Orthoptera)	$<<1.0$	0.07	0.03	<0.01	0.04	0.01
Tenebrio molitor larvae (Coleoptera)	$<<1.0$	0.01	0.01	$<<0.01$	<0.04	<0.01

[a]The values are given as a percentage of the sensitivity of larvae of the greater wax moth, *Galleria mellonella*.

[b]Laboratory culture, University of Konstanz (see Walther and Rathmayer, 1974).

[c]Laboratory culture, University of Amsterdam (see Piek and Engels, 1969; Piek and Mantel, 1970b).

[d]Partly purified A and B toxins (see Spanjer *et al.*, 1977).

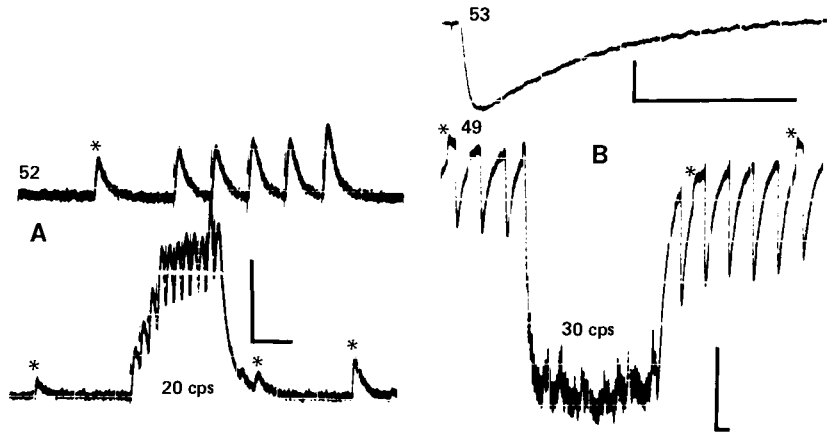


Fig. 38 Postsynaptic potentials in the extensor tibiae muscle in the metathoracic leg of *Locusta migratoria*, 2–3 hr after 5 μl saline containing an extract equivalent to half of the venom organ of *Microbracon hebetor* has been injected into the leg. The numbers at the beginning of the traces are the measured resting potentials in millivolts. (A) *Upper*: 2 hr after injection the amplitude of the EPSPs (5 Hz) has been reduced to that of the small spontaneous EPSPs (*). *Lower*: effect of stimulation of the main crural nerve (20 Hz). Note facilitation (upper trace) and summation (lower trace) of the evoked potentials. (B) Three hours after injection hyperpolarizing potentials (IPSPs) were recorded from the distal part of the extensor tibiae muscle (small bundles separated from the main part of the muscle). During stimulation of the nerve at 30 Hz the 'tetanus' was not smooth. This is probably caused by an interaction of highly suppressed EPSPs and unaffected IPSPs. Note the posttetanic facilitation of spontaneous IPSPs. Spontaneous EPSPs (*). Calibration, 1 mV and 200 msec. From Piek (1982a).

of the venom. This problem can be approached through an analysis of the spontaneous miniature potentials.

Applying the plotting technique described by Huijbrechts and Schreurs (1975), it was possible to study the effect of *Microbracon gelechiae* venom in spite of the unfavourable signal-to-noise ratio in records from fibres of flight muscles of *Galleria mellonella* (Fig. 40). The result clearly demonstrated that the venom markedly depresses the frequency of the miniature potentials and has very little effect on their amplitude (Piek *et al.*, 1974). Administration of 30 *Philosamia Units* (P.U.) (see Fig. 41) of *M. hebetor* venom/ml to a superficial fibre from an adult *Philosamia cynthia* skeletal muscle causes the frequency of the MEPSPs to decrease about a minute after venom application (Fig. 41). Between 20 and 25 minutes after venom application, the frequency of the MEPSPs decreases to less than one percent of the control frequency. The frequency of the MIPSPs is unchanged and remains unchanged when higher doses of the venom are tested. The MEPSP amplitude and the MIPSP

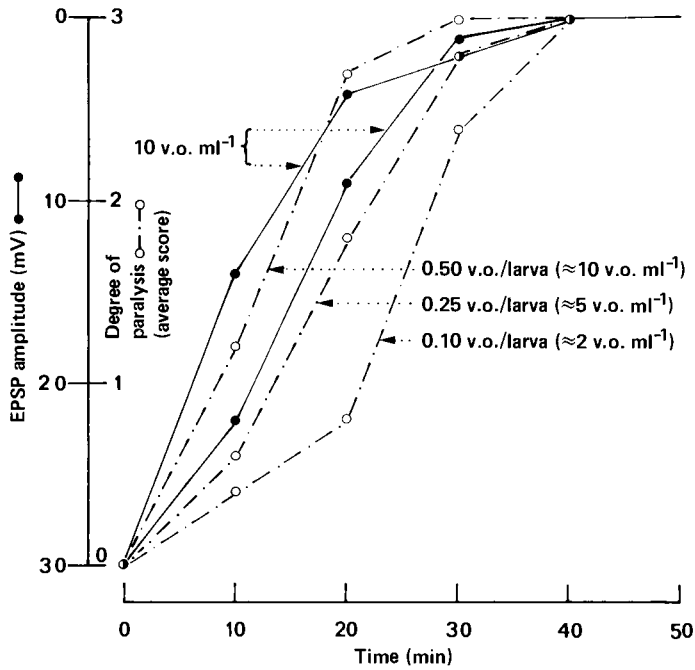


Fig. 39 Speed of paralysis in *Galleria mellonella* larvae (——) and rate of decay of the amplitude of excitatory postsynaptic potentials in flight muscle fibres (-·-) caused by *Microbracon gelechiae* venom. The concentrations are expressed in venom organs (v.o.) per larva and venom organs per millilitre respectively. For comparison the concentrations, noted as venom organs per larva, are converted by estimation into venom organs per millilitre (between brackets). The scoring system for the grade of paralysis has been adopted from Beard (1952) (see Fig. 2). After Piek *et al.* (1974).

amplitude remain unchanged. These results clearly show that *M. hebetor* venom only affects the frequency of the MEPSPs. This agrees with the observation that *M. hebetor* venom blocks the generation of the EPSPs and not that of the IPSPs.

It is generally assumed (for a review, see Piek, 1985a) that in insects excitatory and inhibitory transmission are not mediated by one and the same transmitter. Since the venoms of *Microbracon hebetor* and *M. gelechiae* specifically block excitation, these venoms might exclusively affect processes involving the excitatory transmitter, that is, synthesis, storage or release. This notion does not explain the species specificity of *M. hebetor* and *M. gelechiae* venoms and must be regarded as highly speculative.

If *Microbracon* venoms block the synthesis, the transport or storage of the excitatory transmitter substance, then the amounts stored in nerve terminals might be a function of the level of synaptic activity. If, however,

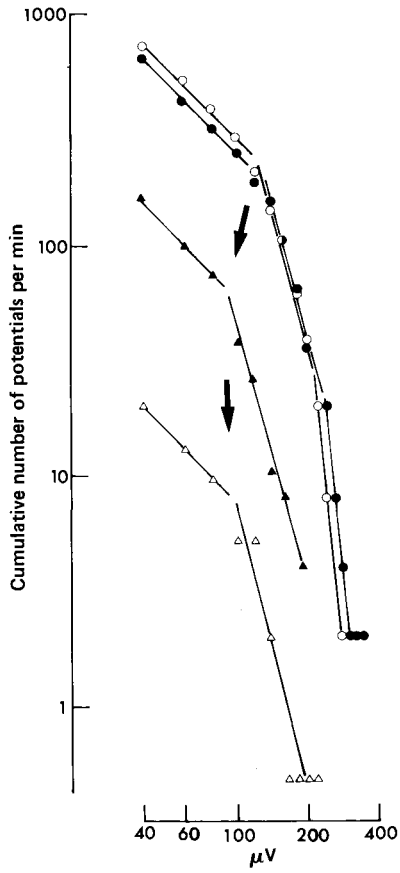


Fig. 40 The effect of the venom of *Microbracon gelechiae* (10 v.o. ml^{-1}) on the reverse cumulative amplitude distribution of excitatory miniature potentials in a skeletal muscle fibre of *Galleria mellonella*. Before venom, ● ; 30 min after, ○ ; 70 min after, ▲; and 85 min after, △. Note that the displacement is mainly vertical, which indicates that the main change is a reduction in frequency of the miniature potentials. After Piek *et al.* (1974).

these venoms block the release of transmitter substance, the speed of onset of paralysis then would not depend on the level of synaptic activity. In larvae of *Philosamia cynthia* injected with *M. hebetor* venom, paralysis is markedly delayed if they are immobilized by CO_2. Moreover, repetitive stimulation of the motor nerve supplying a venom-treated preparation speeded up the onset of paralysis (Piek and Engels, 1969). Nerve-muscle preparations of *P. cynthia* treated with *M. hebetor* venom were paralysed more rapidly when stimulated at 1 Hz than at 0.017 Hz. At the same time that stimulated muscles

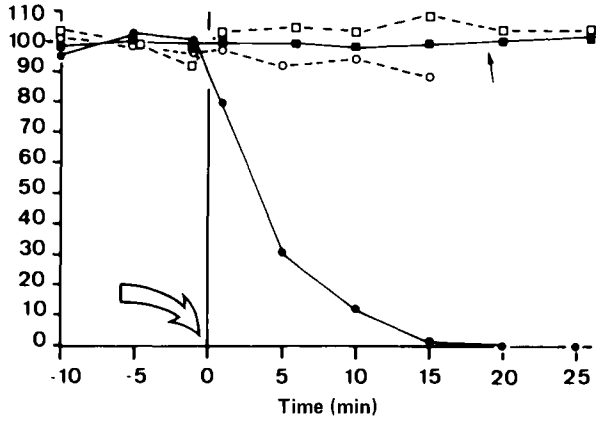


Fig. 41 Effect of *Microbracon hebetor* venom on the frequency and the median of amplitudes of miniature potentials (vertical axis, %) recorded from a muscle fibre of *Philosama cynthia*: ■, MIPSP frequency; □, MIPSP amplitude; ●, MEPSP frequency; ○, MEPSP amplitude, open arrow, addition of venom. From Piek *et al.* (1971). The venom concentration used was 30 *Philosamia* units (P.U.) per millilitre. The P.U. has been defined as the dose injected per gram that causes complete paralysis in 30 min (Piek and Engels, 1969). In subsequent work the *Galleria* unit (G.U.) was used, defined as the dose injected per 100 mg (larvae of *G. mellonella*) causing a 50% score after 2 hr, that is, an average score of 1.5, according to Beard (1952) (see Fig. 2). The ratio P.U./G.U. is ~30.

become completely paralysed, a nonstimulated muscle in the same preparation still showed spontaneous miniature potentials (Piek and Engels, 1969). The authors concluded that paralysis in insects may be caused by some sort of depletion. However, in initial studies Piek *et al.* (1974) as well as Rathmayer and Walther (1976) did not find any distinct changes in the number and distribution of presynaptic vesicles. In recent careful ultrastructural analysis of motor nerve terminals in locust skeletal muscle Walther and Reinecke (1983) concluded that the most likely target of *M. hebetor* venom is the release mechanism. This notion is mainly based on their observations that (1) the venom causes an increase in the number of presynaptic vesicles in both resting and stimulated nerve muscle preparations (Fig. 42) and (2) the proportion of vesicles in close contact with the presynaptic membrane found under paralysis was increased. According to Walther and Reinecke (1983), the venom of *M. hebetor* could interfere with an enzymatic process or could inactivate some kind of recognition site located in the presynaptic membrane, which could be required for an exocytotic release mechanism. As demonstrated via mitochondrial swelling the Ca^{2+} influx during electrical activity was not blocked under paralysis; on the contrary, the small and

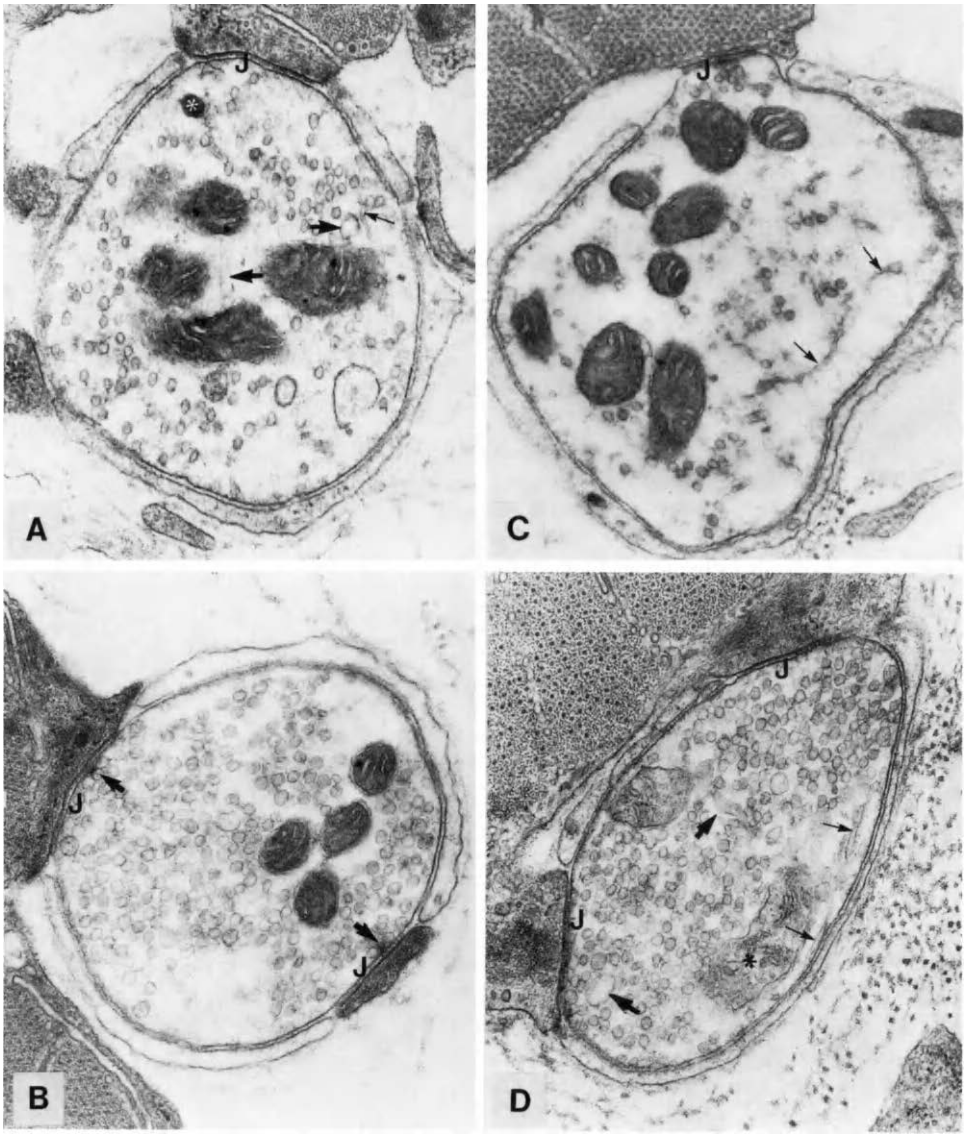


Fig. 42 Junctional regions of motor nerve terminals at the retractor unguis muscle. J, junction. (A) Unstimulated control preparation. The terminal contains synaptic vesicles, profiles of smooth endoplasmic reticulum (small arrow) and cisternae (large arrows). The occasionally occurring, large, electron-dense vesicles (white asterisk) were not included in the stereological investigations since both their average number and size remained unaffected with the different experimental conditions. (B) Unstimulated paralysed preparation. The numerical density and

significant swelling of mitochondria due to long-term stimulation was significantly enhanced. Walther and Reinecke (1983) concluded that Ca^{2+} influx *per se* may be not sufficient to induce exocytosis of vesicles. This is in contrast to the effect of black widow spider venom which, in terminals of mammalian motor neurons (Hamilton and Robinson, 1973) and of insect motor neurons (Cull-Candy *et al.*, 1973), changes the number and the distribution of synaptic vesicles in a way which correlates with observed changes in the spontaneous release of transmitter. The effects of *Microbracon* venoms parallel those of *botulinum* toxin in vertebrates (Thesleff, 1960). In both examples venom decreases the MEPSP frequency without causing depletion of presynaptic vesicles.

Partial purification of the venom of *Microbracon hebetor* resulted into separation of two active components of different molecular weight (see Section II,B,2). The two (A and B) components show different dose–response relations (Fig. 7) (Spanjer *et al.*, 1977), but their effects on the MEPSPs are identical: a decrease in frequency without a distinct effect on the amplitude distribution (Fig. 43).

Recently the purified toxins *Microbracon* toxin A, or A-MTX, and *Microbracon* toxin B, or B-MTX, have been characterized as being labile proteins with molecular weights of 43,700 and 56,700 (Visser *et al.*, 1983) (see Section II,B,2). The biological effects of the two purified toxins were identical to those found by Spanjer *et al.* (1977). Both toxins show very slow time courses of paralysis and recovery. Both time courses (Fig. 44) show sigmoid time–percentage effect curves, which become linear when plotted on a probability scale against log time (Litchfield, 1949). The values representing the degree of paralysis of *Galleria mellonella* larvae have been obtained using the score system according to Beard (1952): unaffected larvae were scored 0; larvae showing uncoordinated movements were scored 1; larvae unable to turn around but reacting to tactile stimulation were scored 2; completely paralysed larvae were scored 3. The activity of the venom solution is expressed in *Galleria Units*, defined by Drenth (1974b) as the amount of venom per 100-mg larva causing, after 2 hr, an average score of 1.5 (see also Fig. 41). In Fig. 44 the moment of maximal effect has been estimated by extrapolation of the calculated regression lines. At all doses the peak of paralysis lies at ~20 hr. The time courses of the onset of paralysis differ

the size of the synaptic vesicles has been increased after paralysis. Note the presynaptic dense projections (arrowheads) surrounded by vesicles. (C) Stimulated control preparation. Note the reduced number of synaptic vesicles (compare A). Long profiles of the smooth endoplasmic reticulum (small arrows) are present. (D) Stimulated paralysed preparations. The terminal has a similar overall appearance to those without stimulation. However, the mitochondria are enlarged and have a lighter matrix. One of them seems to be undergoing lysis (black asterisk). Some cisternae (large arrows) occur. ×37,400. From Walther and Reinecke (1983).

Fig. 43 (A) Effect of A-MTX on miniature excitatory postsynaptic potentials recorded from a small accessory flight muscle of *Pieris brassicae* (see Spanjer *et al.*, 1977). (B) Oscilloscope trace during control and after 20 min perfusion with toxin-containing saline. From Piek *et al.* (1982b).

for the two toxins; the slopes of the plots are less steep for A-MTX than for B-MTX. The plots of recovery do not show marked differences. As has been found earlier for the crude toxins, by Spanjer *et al.* (1977), the dose–response curves of A-MTX are much less steep than those of B-MTX (Fig. 45). *Piek et al.* (1982b) regarded this fact as an indication that the two toxins may have different pharmacokinetics.

Comparable time courses of paralysis and recovery have been described for *Corcyra cephalonica* larvae injected with the venom of *Microbracon brevicornis* (Tamashiro, 1971). He found a maximal paralysis at 11 hr at 27°C (and at 5 hr at 32°C).

A remarkable, and until now unexplained phenomenon, is that the curves for recovery shown in Fig. 44 run parallel. This indicates that of two groups of wax moth larvae, one group injected the day before with a relatively low dose and the other a week before with a relatively high dose, and both being already recovered to about the same average stage of 50% paralysis, further recovery takes place with distinctly different rates.

As already has been described for the crude venom, the purified toxins cause a decrease in amplitude of miniature excitatory postsynaptic potentials (Fig. 43), suggesting a presynaptic effect. The decrease in amplitude and the absence of any effect on the amplitude distribution has been investigated using the plotting method of Huijbregts and Schreurs (1975); see also Fig. 40 and Piek *et al.* (1982b).

Although the venom of *Microbracon hebetor* and A-MTX and B-MTX are very labile, production of the crude venom is easy, and it can be used in the crude form to block insect excitatory neuromuscular transmission selectively.

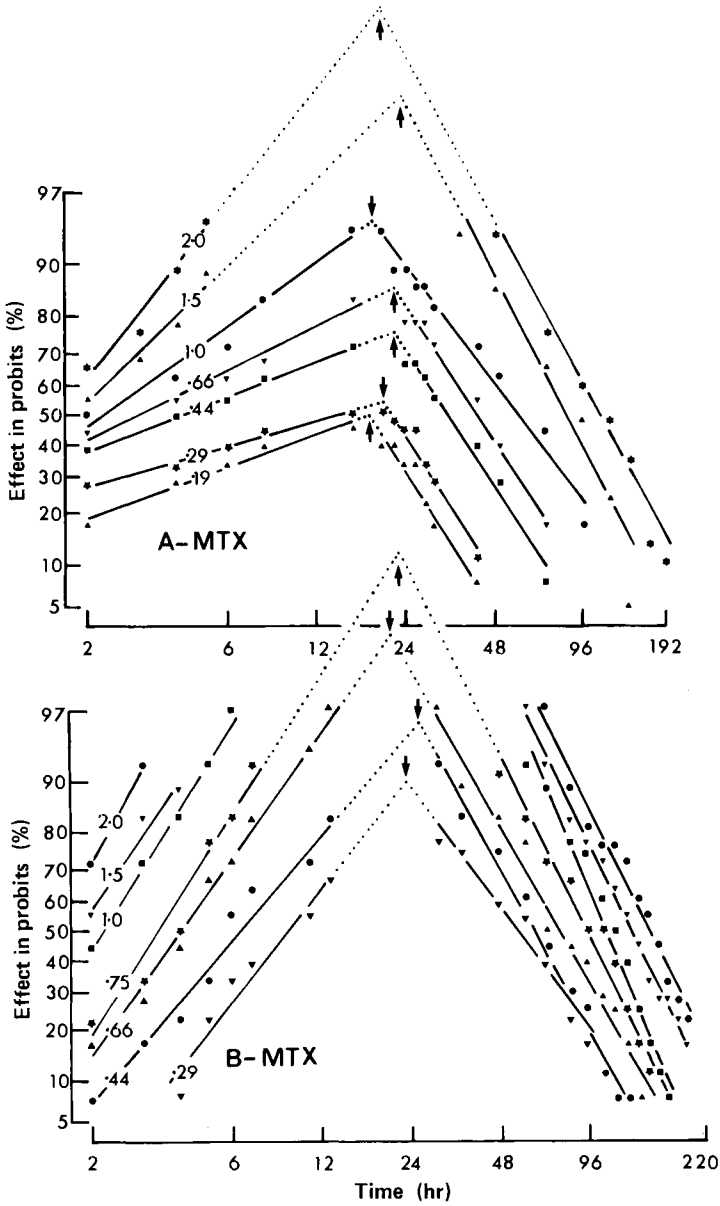


Fig. 44 Time–percentage effect plots of the paralysis of wax moth larvae (*Galleria mellonella*), injected with different doses of A-MTX and B-MTX. The degree of paralysis (see text) has been plotted on a probability scale against log time. The doses are indicated as the number of units per 100 mg of larva. Points of supposed maximal paralysis are indicated with arrows. From Piek *et al.* (1982b).

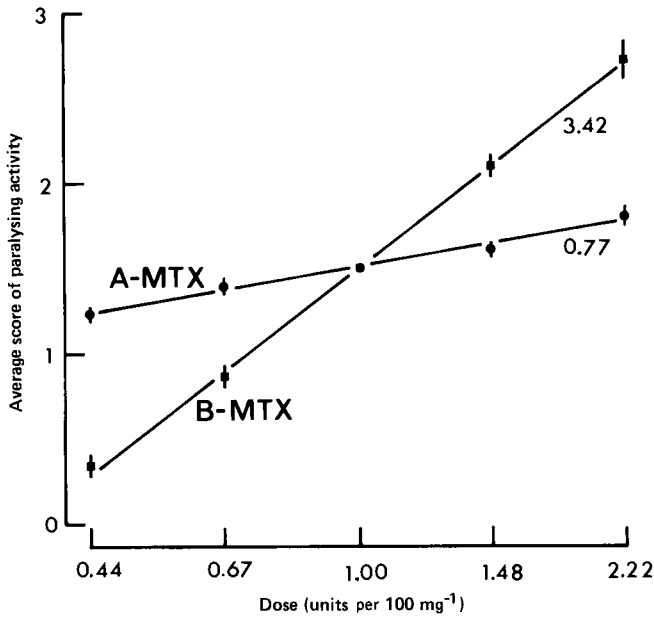


Fig. 45 Dose–effect curves of five A-MTX preparations and 16 B-MTX preparations in *Galleria mellonella*, plotted as the average score after 2 hr (see text) against log dose. The data are normalized to 1 U per 100 mg of larva. (1 U is the amount of toxin extracted from 1 G.U. of venom). The regression coefficients indicated are expressed as score units per decade in dose. Vertical bars are SEM values (n = 5 and 16, respectively). From Piek *et al.* (1982b). See also Fig. 7 from comparable results with partly purified toxins.

Figure 46 shows an example of blocking excitatory transmission in skeletal muscle of the locust without any effect on inhibition. The venom has also been used to selectively suppress the spontaneous and nerve-evoked excitatory release of transmitter so that the postsynaptic effect of another venom, in this case from the scorpion *Androctonus australis*, could be studied (Walther *et al.*, 1976). Also, spontaneous and stimulation-induced myogenic contractions of the locust extensor muscle are not affected by the venom and can be studied without interference of the normal contraction when the muscle is treated with *Microbracon* venom (Piek, 1981).

Mainly based on the experiments described in Figs. 43–45, it may be concluded that the venom of *Microbracon hebetor*, and possibly also the venom of *M. gelechiae*, blocks release of excitatory transmitter in the nerve terminals of skeletal muscle of insects, with the notion that some insect groups (Lepidoptera) are extremely sensitive to the venom and others (Orthoptera, Coleoptera) seem to be much less sensitive or almost insensitive to the venom. The original suggestions (Piek and Mantel, 1970b; Walther and Rathmayer,

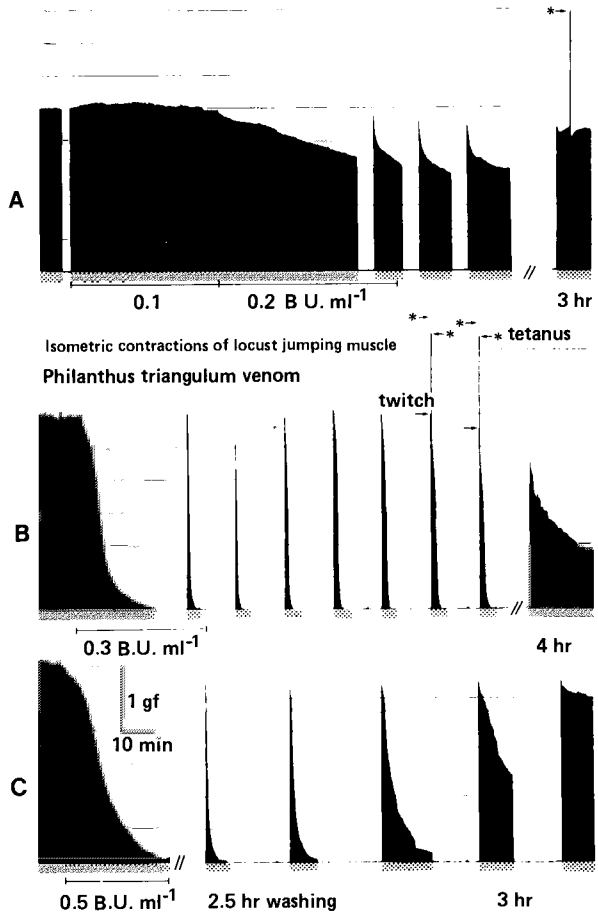


Fig. 46 Effects of different concentrations of the venom of *Philanthus triangulum* on the force of contraction of the musculus extensor tibiae of the rear leg of *Locusta migratoria*. Stimulation every 10 sec indirectly via nerve 5 (fast contraction), is indicated by dotted bars. (A) Effects of 0.1 and subsequently 0.2 B.U. venom ml^{-1} perfusion fluid. (B) Effect of 0.3 B.U. ml^{-1}. (C) Effect of 0.5 B.U. ml^{-1}. gf, Gram force. In the recovery period (washing) a single stimulus is sometimes followed by a short tetanic contraction (*). From Piek and Spanjer (1978).

1974) that this type of venom may specifically act on the glutamatergic transmission process has to be rejected. Still puzzling is the fact that in wax moth larvae the maximal paralysis is found after ~20 hr and that the rate of recovery (rate of elimination?) is dose-dependently decreased by the venom. The latter phenomenon indicates the presence of an additional effect of the venom on processes involved in elimination or inactivation of the toxins.

The venom of the digger wasp *Philanthus triangulum*, also called the bee wolf, causes paralysis in insects and spiders (Rathmayer, 1962a,b). For a long time the action of the venom was considered to be exclusively peripheral (Piek *et al.*, 1971), but Piek *et al.* (1980b, 1984a) have described a block of transmission in the cockroach central nervous system (CNS) (see Section III,B and Fig. 15). For the peripheral effects three different components have been described (Piek and Spanjer, 1978; Spanjer *et al.*, 1982a). The reversible paralysis of insects stung by *P. triangulum* or treated with the wasp venom is the final result of a complicated action of a number of active components on the neural control of muscle contraction, including effects on pre- and postsynaptic events at the neuromuscular junction.

Addition to the bath of an extensor tibiae muscle of the locust of the venom of *Philanthus triangulum* at concentrations from 0.2 B.U. ml^{-1} (see legend to Fig. 34 for the definition of B.U.) caused a decrease of contraction height of the twitch at stimulation frequency of 0.1 Hz (Fig. 46); depending on the individual sensitivity of the nerve–muscle preparation, venom concentrations from 0.3 to 1.4 B.U. (in most cases ~0.5 B.U.) ml^{-1} were needed to paralyse the preparation completely during continuous stimulation at a frequency of 0.1 Hz. The effect was semi-irreversible; several hours wash were needed for recovery. However, if the stimulation was interrupted for 5 to 15 min, the first stimulus given after the period of rest resulted in a twitch contraction of a force that often equalled the control value. Subsequent stimuli caused a rapidly progressive decrease in contraction force. This phenomenon, which could be repeated many times, was observed in preparations bathing in venom solution as well as in preparations already washed off for several hours (Fig. 46) (Piek and Spanjer, 1978). Incompletely paralysed preparations stimulated with frequencies higher than 0.1 Hz showed two different and competing phenomena: the activation-induced progressive blockade already described for the twitch, and a facilitation caused by increase in stimulus frequency. Figure 47a shows a representative experiment. Without venom the facilitation is visible from 6 Hz to higher frequency. If the venom (0.5 B.U. ml^{-1}) is added during a period of 5 min, and the preparation is subsequently washed, in the first minutes during wash a depression of contraction force is seen at lower frequencies (Fig. 47b). This progressive or activation-induced depression depends on the stimulation frequency. In the range from 0.1 to 3 Hz the exhaustion was more intensive at 3 Hz than at 0.1 Hz. At frequencies higher than 3 Hz the apparent facilitation obscured the activation-induced depression.

To show that the relatively high tetanus/twitch ratio was not caused by the gradual restoration of the preparation during washing, the series of stimulus trains was repeated (Fig. 47c). At 30 Hz the tetanic contraction is well maintained for several seconds, as shown in the inset of Fig. 47c. The

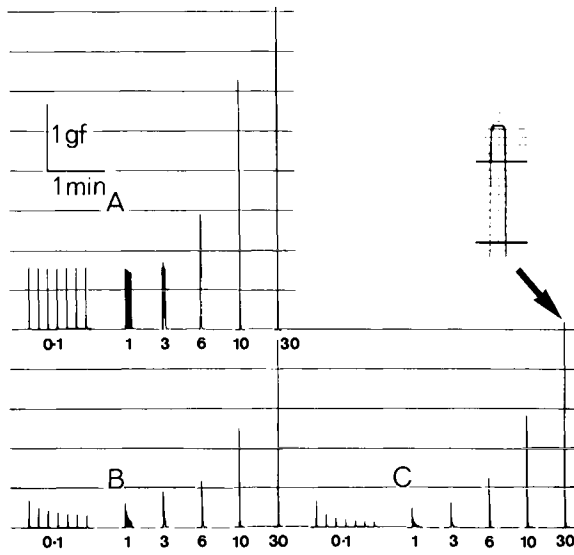


Fig. 47 Effects of 0.5 B.U. ml^{-1} of venom of *Philanthus triangulum* on the force of the fast twitch and tetanus (see Figure 46). (A) Before administration of venom; (B) immediately after the beginning of washing of the preparation, which during 5 min had been pretreated with the venom solution; (C) after 50 min of wash. The intervals of the pulse trains are 5 min. Every pulse train consists of seven pulses. Numbers indicate the frequency in Hz of the trains of seven pulses. Inset: top of the tetanic contraction, recorded with eight times faster paper speed, indicating that the tetanic contraction can be fully maintained. gf, Gram force. From Piek and Spanjer (1978).

above-described phenomena were reported in part for the first time by Lepeletier de Saint-Fargeau (1841) who observed that honey-bee workers stung by *Philanthus triangulum* from time to time slowly move their antennae, their legs or only their tarsae. A comparable phenomenon has been observed in *Halictus* bees stung by *Philanthus denticollis* (Janvier, 1928). This activation-induced paralysis has been explained as a presynaptic effect by Piek and Spanjer (1978), but recent results discussed in the subsequent section provide evidence for a postsynaptic origin of this phenomenon. Despite the overwhelming evidence for a postsynaptic effect (May and Piek, 1979; Piek *et al.*, 1980a,b; Clark *et al.*, 1982), an experiment such as that presented in Fig. 47 (Piek and Spanjer, 1978) can still not be fully explained postsynaptically.

May and Piek (1979) carried out experiments with the isolated metathoracic retractor unguis muscle of the desert locust *Schistocerca gregaria*. This small muscle can be isolated from the insect together with its innervating axons

and therefore has many of the advantages of a larger *in situ* nerve-muscle preparation (Usherwood and Machili, 1968). The normal excitatory process in insect muscle fibres starts with a depolarization of the plasma membrane. Depolarization and the subsequent contraction can, in the retractor muscle, be brought about by suddenly raising the external potassium ion concentration. In phasic muscle fibres, such as the majority of the fibres of the metathoracic extensor tibiae and all fibres of the retractor unguis of locusts (Cochrane *et al.*, 1972), the tension of the contractions returns to zero after a few seconds in high potassium ionic solution. Generation of contractions can also be evoked by addition of L-glutamate (Usherwood and Machili, 1968; Usherwood and Cull-Candy, 1975). These authors conclude that the contractions recorded from insect muscles exposed to L-glutamate are due to the direct activation of glutamate receptors on the excitatory postsynaptic muscle fibre membrane rather than by a presynaptic action. Clements and May (1974) found that in the isolated locust retractor unguis the force of the glutamate-induced contraction was proportional to the concentration of glutamate.

At a dose of 5 B.U. ml^{-1} the venom of *Philanthus triangulum* caused a rapid decrease of neurally evoked twitches but did not significantly affect the contractions induced by potassium ions (Fig. 48). This indicates that the venom does not affect the excitatory–contraction coupling or the contraction mechanism itself. One B.U. ml^{-1} of the venom caused a slower decrease in the neurally evoked twitch force, while the glutamate contraction was more affected than the twitch (Fig. 48). This suggests that the venom may have a postsynaptic action in addition to the presynaptic effect discussed in the following paragraphs.

In a study of the effect of *Philanthus triangulum* venom on skeletal muscle fibres of several insects Piek *et al.* (1971) found that the clearest picture of the peripheral action of the venom was obtained at 3 B.U. ml^{-1} venom in the bath. They found that after an initial increase in the frequency of miniature excitatory postsynaptic potentials, the MEPSP frequency as well as the MIPSP frequency was decreased to zero in about 10 min, without a concurrent decrease in amplitude. In a later study Piek and Njio (1975) observed a similar effect of *P. triangulum* venom on the amplitude distribution of MEPSPs in a flight muscle fibre of the honey-bee worker. At a concentration of 2 B.U. ml^{-1} a distinct decrease in frequency by a factor of 3.85 is seen in Fig. 49. In the same study Piek and Njio (1975) concluded that the venom did not affect the number and distribution of presynaptic vesicles. However, in these experiments the fixation of the muscles was preceded by a period of rest, and the blockade is activation-dependent (see earlier paragraphs).

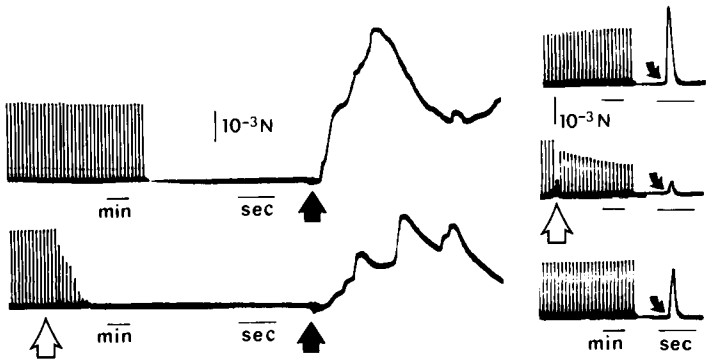


Fig. 48 Effects of the venom preparation from *Philanthus triangulum* on neurally evoked contractions and K^+- and glutamate-induced contractions of the isolated retractor unguis muscle of *Schistocerca gregaria*. Left, top: twitches (low paper speed) and a contraction initiated by a 2 ml pulse of 30 mmol litre^{-1} K^+ to the flowing saline (filled arrow, 100 times faster paper speed). Left, bottom: effect of 5 B.U. ml^{-1} (open arrow) on the twitches and on the K^+ contraction. In this experiment the potassium contraction during exposure to the venom is slightly smaller than in the control situation. This is within the expected experimental variation and is not considered significant. Right, top: neurally evoked contractions (low paper speed) and a contraction induced by a 2 ml pulse of 10^{-4} mol litre^{-1} L-glutamate (filled arrow). Right, middle: effect of 1 B.U. ml^{-1} (open arrow) on the neurally evoked and on the glutamate contractions. Right, bottom: full recovery of neurally evoked and partial recovery of glutamate-evoked contractions after 30-min wash. From May and Piek (1979).

This indicated a reexamination, in which synaptic vesicles were studied and the neuromuscular activity was controlled by recording the contraction as the muscles were stimulated continuously. Using a high-magnesium, zero calcium saline, Njio and Piek (1979) found that despite the fact that under these circumstances the transmitter release may have been inhibited in part, the nerve terminals in the venom-treated preparations contained significantly fewer vesicles per square micrometer than those in the control preparations (Fig. 50). It was concluded that the venom directly or indirectly inhibits the supply of transmitter substance to the terminals. The next question is whether the depletion is caused by a transport inhibition, a block of the synthesis of vesicle membrane or of the synthesis or the reuptake of the transmitter. Using electron microscope autoradiography, van Marle *et al.* (1982) showed that in the neuromuscular junction of locust skeletal muscles the sodium-dependent uptake (van Marle *et al.*, 1983) of labelled glutamate in both glial cells and axon terminals was reduced under influence of the venom of *Philanthus triangulum*. The ratio of the glutamate accumulation of glia and

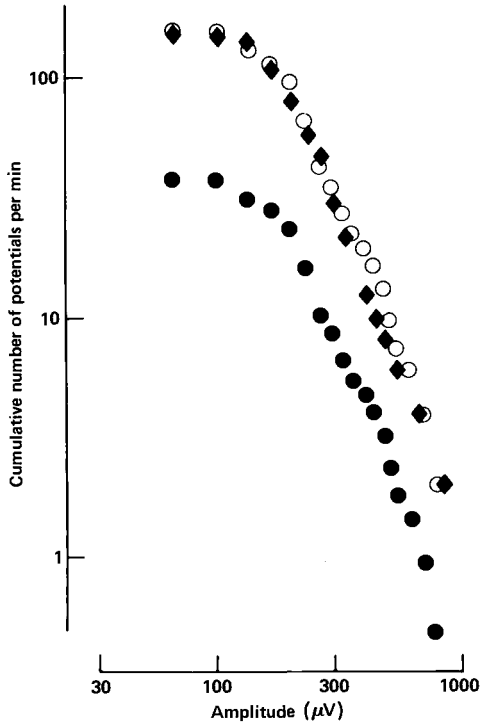
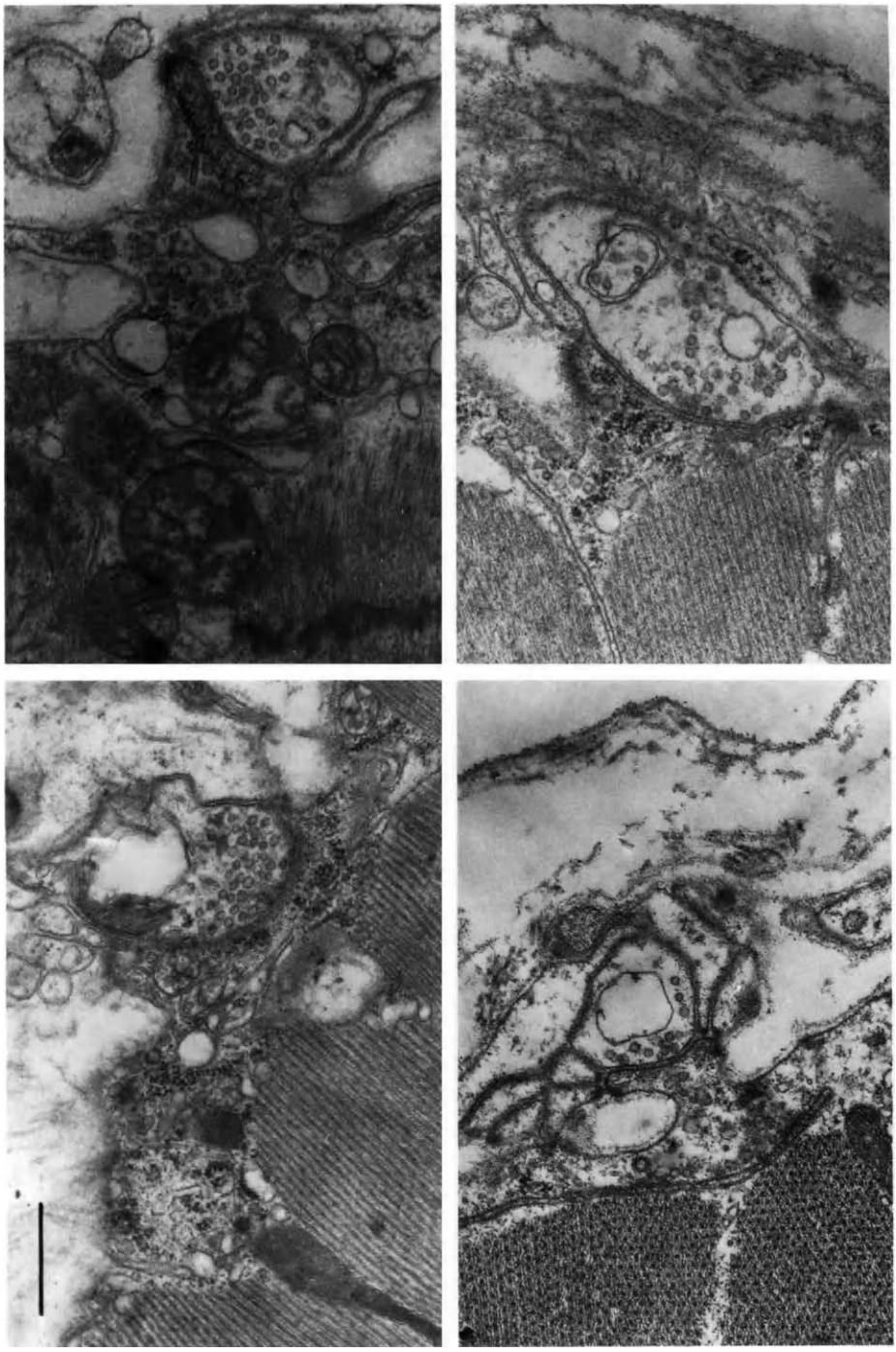


Fig. 49 The effect of the venom of *Philanthus triangulum* on the cumulative amplitude distribution of excitatory miniature potentials in a skeletal muscle fibre of *Apis mellifera*. Control, ◆; 4 min after administration of 2 B.U. ml^{-1} venom, ●. The plot of records taken 4 min after administration of the venom has been shifted along the ordinate by multiplying all values by a factor of 3.85, ○. The excellent fit obtained by this shift along the ordinate shows that the venom affected predominantly the frequency of the MEPSPs. After Piek and Njio (1975).

nerves remained identical. These results suggest that the venom causes a reuptake inhibition in this glutamatergic neuromuscular transmission system. Thus the reuptake inhibition may cause desensitization of the glutamatergic receptor as well as enhanced activation of the receptor ionophore complex. Since one of the toxins (δ-PTX) blocks ion channels only during glutamate activation (Clark *et al.*, 1982) (see following paragraphs), van Marle *et al.* (1982) found it conceivable that the inhibition of transmitter uptake may act

Fig. 50 Electron micrographs showing the effect of the venom of *Philanthus triangulum* (1 B.U. ml^{-1}) on synaptic vesicle density in extensor tibiae muscle of *Schistocerca gregaria*, treated with a high magnesium–zero calcium saline. Left: control preparations. Right: venom-treated preparations. Scale, 0.5 μm. From Njio and Piek (1979).



synergistically with the postsynaptic effect. From the analysis of autoradiographs of muscles treated with either β-PTX, δ-PTX or γ-PTX (see Section II,B,4) it appeared that only in the presence of δ-PTX a reduction of the glutamate uptake was obtained in both glial cells and nerve terminals (van Marle *et al.*, 1984). Similar to what has been described for the whole venom, the ratio of grain densities above glial cells and nerve terminals remained constant. This indicates that δ-PTX may be the toxin responsible for the supposed reuptake inhibition.

Earlier experiments (described above) with 1 to 3 B.U. ml^{-1} venom of *Philanthus triangulum* wasps from Europe showed that it caused a predominant decrease in frequency and only incidentally a small decrease in amplitude. It was concluded (Piek *et al.*, 1971) that these effects indicated that the venom caused a presynaptic rather than a postsynaptic block of the spontaneous neuromuscular transmission. However, it might be premature to explain paralysis as being presynaptic, only by the observation of an inhibition of spontaneous quantal transmitter release. Although an effect on the miniature frequency is often used as an argument for presynaptic action, Fig. 51 demonstrates the danger. In the case of *Microbracon hebetor* venom a good correlation exists between the rate of depression of the frequency of MEPSPs and the rate of decrease in EPSP amplitude (Fig. 51a). This has been found at all venom concentrations used (Piek and Spanjer, 1978). However, in muscle fibres treated with the venom of *P. triangulum* such a correlation has been found only at venom concentrations higher than 2 B.U. ml^{-1}. At 1 B.U. ml^{-1} there is no good correlation in the rates of decreases of both parameters (Fig. 51b). This provided the first indication that at least at low concentrations the effect might not be entirely presynaptic. A second indication appeared when the experiments done with venom from *Philanthus triangulum* collected in Europe were repeated with venom from *Philanthus triangulum* collected in Egypt. At a relatively high concentration of 4 B.U. ml^{-1} of Egyptian wasps Piek *et al.* (1980b) found a predominant decrease in amplitude of the miniature potentials rather than on the frequency. This suggested that the presynaptic effect found with European bee-wolf venom was not present or was less pronounced with Egyptian bee-wolf venom. Piek *et al.* (1980b) now reinvestigated the European bee-wolf venom at much lower concentrations. The results show that at a concentration of 1 B.U. ml^{-1} the venom caused a pronounced decrease in frequency and a small decrease in amplitude. At 0.3 to 0.5 B.U. ml^{-1}, however, both amplitude and frequency decreased distinctly, and at concentrations of 0.20 or 0.15 B.U. ml^{-1} only a decrease in amplitude was recorded (Fig. 52). These experiments indicated that the venom may have a postsynaptic as well as a presynaptic effect, and that with respect to the presynaptic effect the venom of the

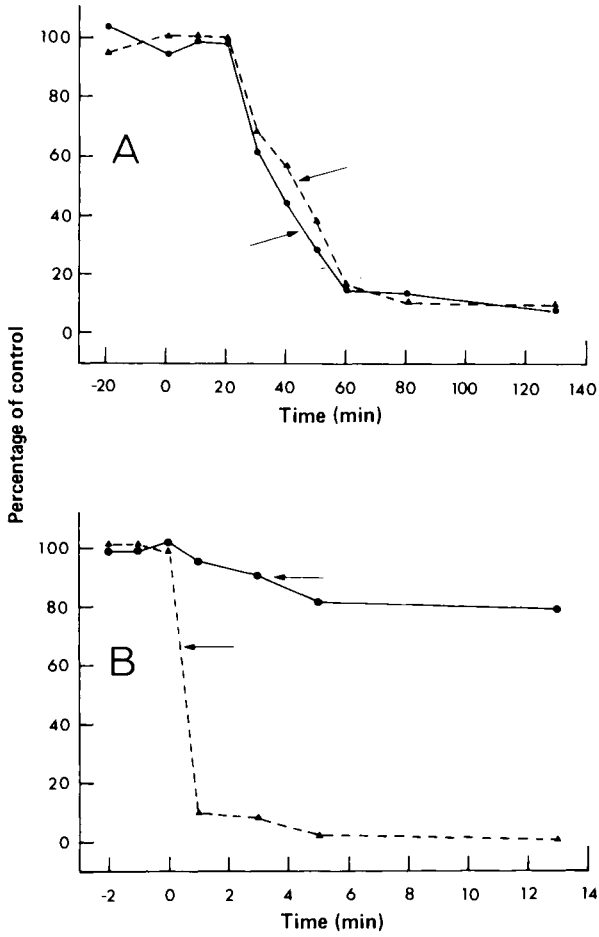


Fig. 51 Time relationship of the effects of two solitary wasp venoms on two different electrical phenomena in insect muscle fibres. (A) Effect of 50 *Galleria* units (G.U.) of venom of *Microbracon hebetor* per ml perfusion fluid on a flight muscle fibre of *Pieris brassicae*. (B) Effect of 1 B.U. (at $t = 0$) venom of *Philanthus triangulum* per ml on a coxal muscle of *Schistocerca gregaria*. MEPSP frequency, ▲; EPSP amplitude, ●. From Piek and Spanjer (1978).

Egyptian bee wolves may differ quantitatively from that of European bee wolves.

It has already been demonstrated that the venom of *Philanthus triangulum* could depress the glutamate-induced contraction (Fig. 48). The contractions brought about by bath-applied glutamate are preceded by depolarizations.

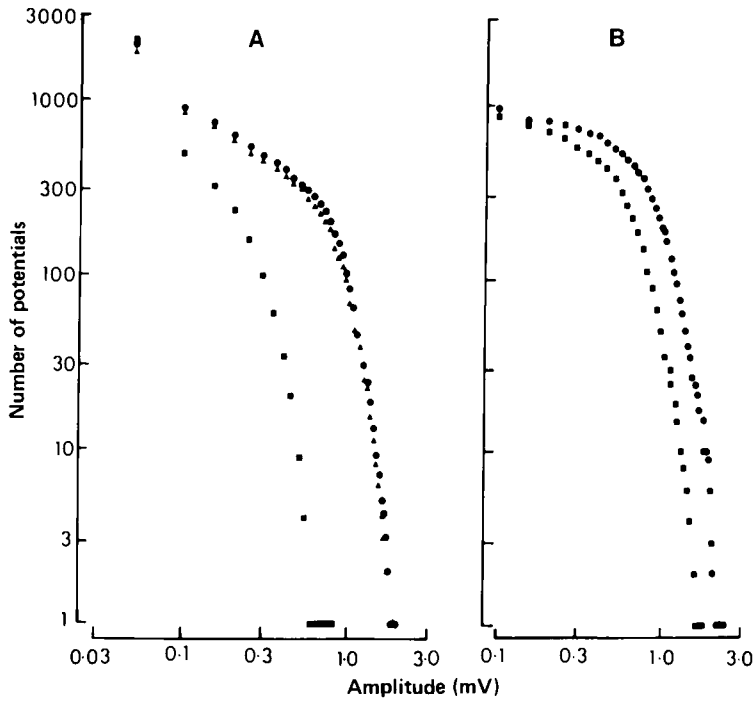


Fig. 52 Effect of the extracts from venom reservoirs of *Philanthus triangulum* from Egypt (A) and from Belgium (B) on the reverse cumulative amplitude plot of miniature EPSPs in a flight muscle fibre of *Pieris brassicae*. Note the shift along the *x* axis, indicating a change in amplitude. Egyptian wasps: control, ●; 5 min after treatment with 4 B.U. ml^{-1} venom, ■; after 40 min wash, ▲. Belgian wasps: control, ●; 15 min after treatment with 0.15 B.U. ml^{-1} venom, ■. From Piek *et al.* (1980b).

The amplitude of the transient depolarizations, called bath-applied glutamate potential, depends on the glutamate concentration used. Doses of *P. triangulum* venom increasing from 0.1 to 2.0 B.U. ml^{-1} progressively flattened the log-dose curve (Fig. 53, left). It is clear that the effect of the venom is a noncompetitive inhibition of the glutamate response (May and Piek, 1979). Depolarizing responses were also evoked by iontophoretic application of glutamate to very localized spots, which closely correspond with the position of excitatory axon terminals. Decrease in amplitude of the glutamate responses by the venom was preceded by a decrease in half-decay time (Fig. 53, right). This fact and the noncompetitive character of the block were reasons for May and Piek (1979) to suppose that the postsynaptic block might be caused by a decrease in open ion channel lifetime in the postsynaptic membrane. This view has been supported by Piek *et al.* (1980a), who studied

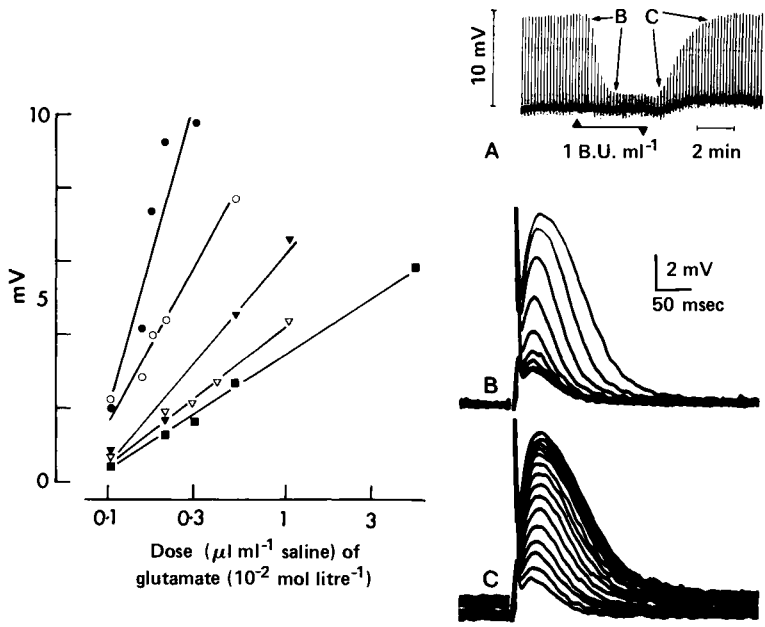


Fig. 53 Effects of the venom of *Philanthus triangulum* on L-glutamate potentials from fibres of the locust retractor unguis muscle. Left: effect of different doses of venom (●, 0.0; ○, 0.1; ▼, 0.5; ▽, 1.0; ■, 2.0 B.U. ml^{-1}) on the log dose–response curve of bath-applied glutamate potentials. Right: effects of 1 B.U. ml^{-1} of the venom on iontophoretically evoked glutamate potentials induced by 0.6-nC pulses. (A) Pen record; (B–C) superimposed oscilloscope records of corresponding parts in (A). From May and Piek (1979).

the effect of *P. triangulum* venom on extracellularly recorded miniature potentials from fibres of the metathoracic retractor unguis muscle of the locust *Schistocerca gregaria*. These extracellular potentials are often called extracellular currents, because in their time course they follow postsynaptic currents. At concentrations from 0.4 to 0.5 B.U. ml^{-1} the venom of European bee wolves causes a decrease in amplitude and in half-decay time of the miniature potentials. Piek *et al.* (1980a) suggested that the venom partially blocks the postsynaptic current by shortening, in the postsynaptic membrane, the ion channel open lifetime. The venom of *P. triangulum* contains at least three different toxins with a blocking effect on iontophoretically applied glutamate potentials evoked in the locust skeletal muscle (Spanjer *et al.*, 1982a). The blocking activity on glutamate potentials of β-, γ- and δ-philanthotoxin showed ratios of about 1:30:100 (Fig. 54).

δ-Philanthotoxin (δ-PTX) and β-philanthotoxin (β-PTX) have been investigated in more detail.

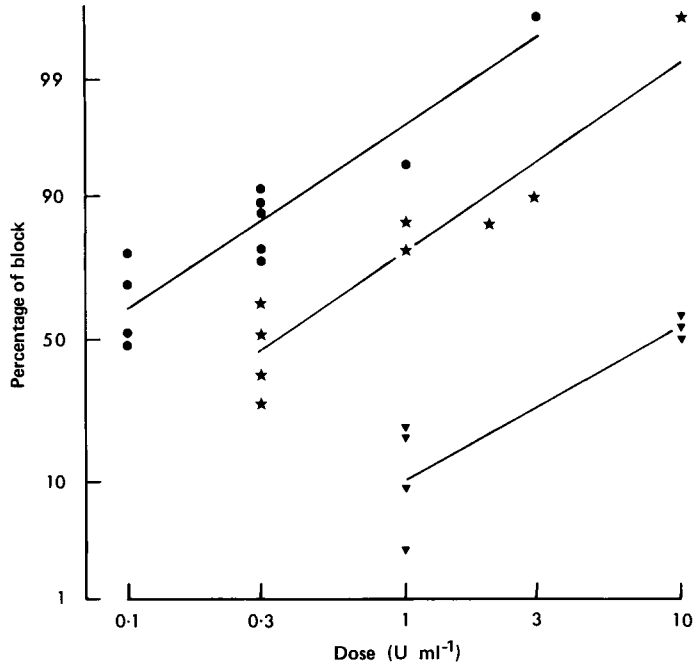


Fig. 54 Dose-response curves of the effect of β-, γ- and δ-PTX on the amplitude of iontophoretically applied glutamate potentials evoked in fibres of the locust (*Schistocerca gregaria*) retractor unguis muscle: ●, δ; ★, γ; ▼, β. The amplitudes have been plotted on a normal probability scale against log doses. From Spanjer *et al.* (1982a).

Like the whole venom, δ-PTX causes a reversible decrease in the amplitude of transient depolarisations of the muscle fibre membrane evoked by iontophoretically applied glutamate pulses close to a nerve muscle junction. As is the case for treatment with the whole venom, the potentials are depressed to a plateau value: the 'steady-state' response ratio (r_∞), which depends on the toxin concentration but only slightly depends on stimulus frequency (Figs. 55 and 56). The rate of decline in amplitude of the glutamate potentials in the presence of δ-PTX is dependent on the toxin concentration (Fig. 55). The amplitudes of the successive responses in a train of pulses declines in an approximately exponential manner to a seemingly constant steady-state level, the amplitude of which decreases with increasing toxin concentration (Clark *et al.*, 1982).

In agreement with what has been found for the whole venom (Fig. 55), the effect of δ-PTX on the amplitude of glutamate potentials is reversible when glutamate activation is continued. If, however, in the presence of the

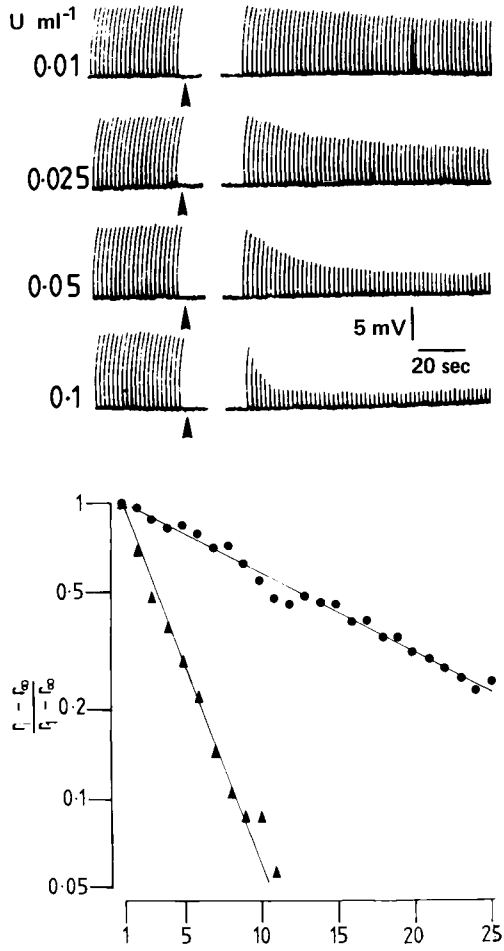


Fig. 55 Effect of the concentration of δ-PTX on the amplitude of iontophoretically evoked L-glutamate potentials recorded from a nerve-muscle junction of the locust extensor tibiae muscle fibre (see below). Top: glutamate doses of 1 nC were applied at a frequency of 0.56 Hz. The glutamate pulses were terminated immediately prior to introduction of the toxin (arrow). All recordings were made at the same junction. Note the decrease in steady-state amplitude with increasing toxin concentrations. Bottom: exponential decay of response amplitude in two of the pulse trains shown above. Toxin concentration (U ml^{-1}: ●, 0.025; ▲, 0.1). The steady-state response ratio (r_∞) has been subtracted from each response (r_i) and this difference has been normalized to the difference for the first pulse in the train (r_1). The linear relationship between $r_i - r_\infty$ (log scale) and the pulse number indicates an exponential decline. Since paralysis of honey-bee workers is caused by a synergistic effect of the different toxins present in the crude venom of *Philanthus triangulum*, the bee unit (B.U.) is of little value in determining the biological activity of the individual components. Therefore, the dose of δ-PTX is expressed in units (U). One U is the amount of toxin separated from one B.U. of crude venom (Spanjer *et al.*, 1982a). From Clark *et al.* (1982).

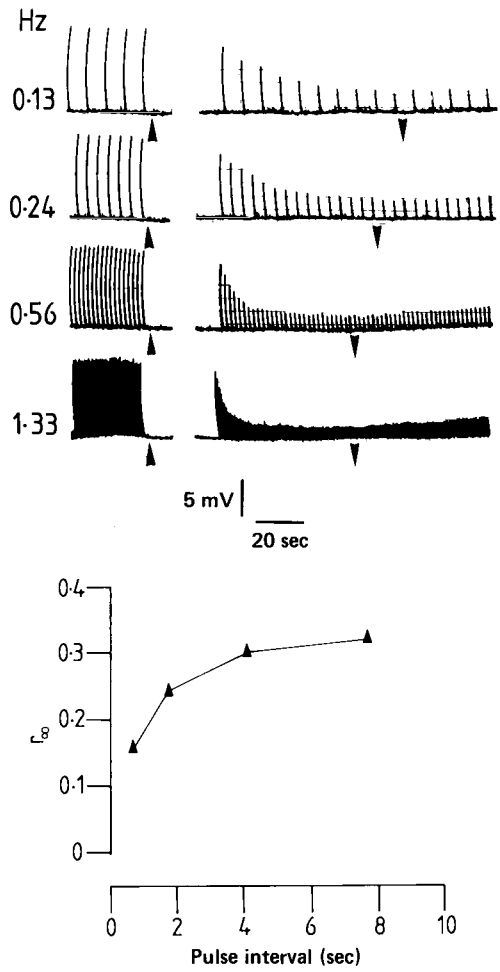


Fig. 56 Top: effect of stimulus frequency on activation-induced decrease in amplitude of iontophoretically evoked L-glutamate potentials recorded from a nerve–muscle junction of the locust extensor tibiae muscle fibre, before and after addition (▲) of 0.1 U ml^{-1} δ-philanthotoxin. All recordings were made at the same junction as that of Fig. 55. The steady-state amplitudes are only slightly dependent on the frequency, especially at lower frequencies (pulse interval between 4 and 8 sec). ▼, Wash. Bottom: steady-state amplitude (r_∞) plotted against pulse interval duration.

toxin the glutamate pulses are interrupted, the amplitude of the glutamate responses increases slightly but does not recover to its control value, even when the time between single pulses of glutamate is 40 sec (Fig. 57). Experiments with interruptions of longer duration indicate that the recovery

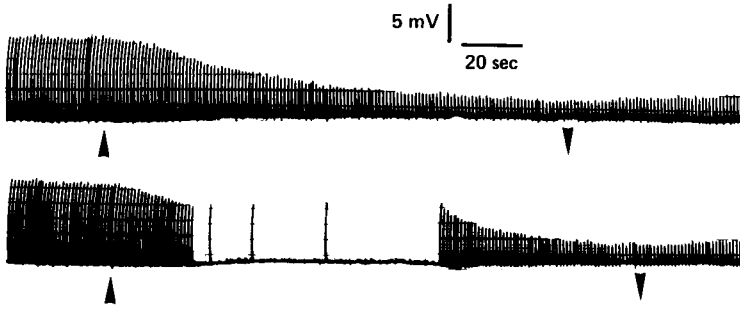


Fig. 57 Effect of δ-PTX on L-glutamate potentials evoked by iontophoresis (1 nC, 0.74 Hz) at the excitatory neuromuscular junction of the locust extensor tibiae muscle. Top: during bath application of 0.05 U ml^{-1} of δ-PTX (between arrows) the glutamate potentials were depressed to a plateau, and they recovered only slowly after removal of the toxin. Bottom: recording of glutamate potentials at the same junction as above. The toxin (0.05 U ml^{-1}) was applied for the period between arrows. In this experiment application of glutamate pulses was interrupted for periods of 6, 12, 24 and 40 sec. Note the slight recovery of response amplitude following these brief periods of rest. From Clark *et al.* (1982).

time constants did not result in reproducible values. This could be explained by supposing that, without glutamate- or transmitter-induced activation of the receptor–ion channel complex, the block is irreversible. Variations in spontaneous transmitter release and leakage of glutamate from the pipette may cause a large variation in recovery time. Comparable results were obtained in experiments in which trains of nerve-evoked postsynaptic currents were interrupted for different intervals. Figure 58 shows an example of recovery of the junctional current, plotted as fractional inhibition against pulse interval. In this particular experiment the recovery time constant was 440 sec, but again in these experiments, the values obtained from different junctions varied considerably.

The above results suggest that the activation-induced block of ion channels by δ-PTX is semi-irreversible; that is, without activation of the receptor–ion channel complex, blocked channels probably cannot be unblocked. As previously indicated, a situation with absolutely no agonist activation cannot be created easily. In the opposite situation, that is, in the continuous presence of agonist, however, it appeared possible to estimate the rate of unblocking. Using the patch-clamp technique of Neher *et al.* (1978), channel kinetics were studied in the extrajunctional membrane of the locust extensor tibiae muscle in the absence and in the presence of δ-PTX (0.2 U ml^{-1}), and in both cases in the presence of 10^{-4} mol $litre^{-1}$ glutamate. Clark *et al.* (1982) estimated the unblocking rates from the distribution of closed times. In the absence of toxin the distribution of closed times can be fitted approximately by a

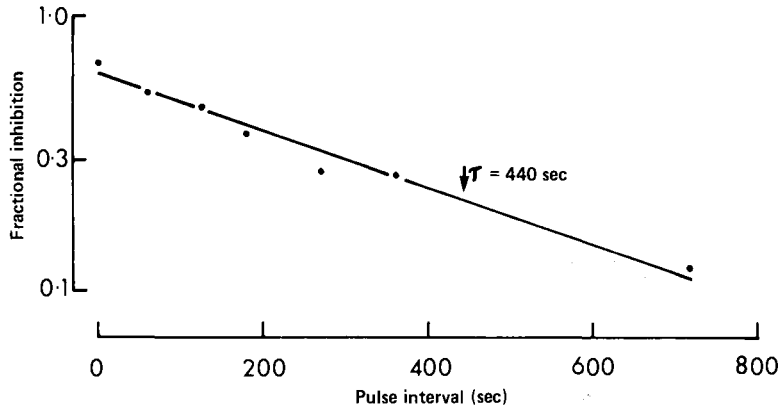


Fig. 58 Effect of pulse interval on the recovery of the junctional current recorded from the excitatory neuromuscular junction of the locust extensor tibiae muscle fibre. The muscle was bathed for a long time in 0.8 U ml^{-1} δ-PTX. The time constant of recovery (here, 440 sec) varied considerably at different junctions. From Piek (1982b).

single exponential function with a time constant of 40 to 100 msec (Fig. 59, left). In the presence of toxin the distribution is doubly exponential; the second and larger time constant is seven times that of the first (Fig. 59, right). It seems most likely that the long intervals underlying this larger time constant represent the periods during which the individual glutamate channels are blocked by the toxin.

Analysis of the open time of single glutamate channels in the presence of 10^{-4} mol $litre^{-1}$ glutamate and with or without toxin (0.2 U ml^{-1}) resulted in a reduction of the mean open time from 2.90 ± 0.39 (SD) msec to 2.24 ± 0.42 (SD) msec caused by the toxin. Analysis of the closed time of single ion channels suggests that, at a glutamate concentration of 10^{-4} mol $litre^{-1}$ the unblocking time constant could have a value on the order of 300 to 700 msec (Clark *et al.*, 1982). Since the mean open time was only decreased by some 20%, the time constant of blocking might be considerably larger than the mean open time, thus more than 3 msec.

A different approach to an approximation of the ratio of both time constants is to consider the steady-state situation, as shown by the plateaus in Figs. 56 and 57, as the result of an equilibrium between blocking and unblocking reactions. Supposing that the association and dissocation of the toxin molecule with the channel structure is only possible if the channel is in its open conformation, then the steady-state condition could be described by the equilibrium condition

$$C^* + T \underset{1/\tau_u}{\overset{1/\tau_b}{\rightleftharpoons}} C^*T \qquad (1)$$

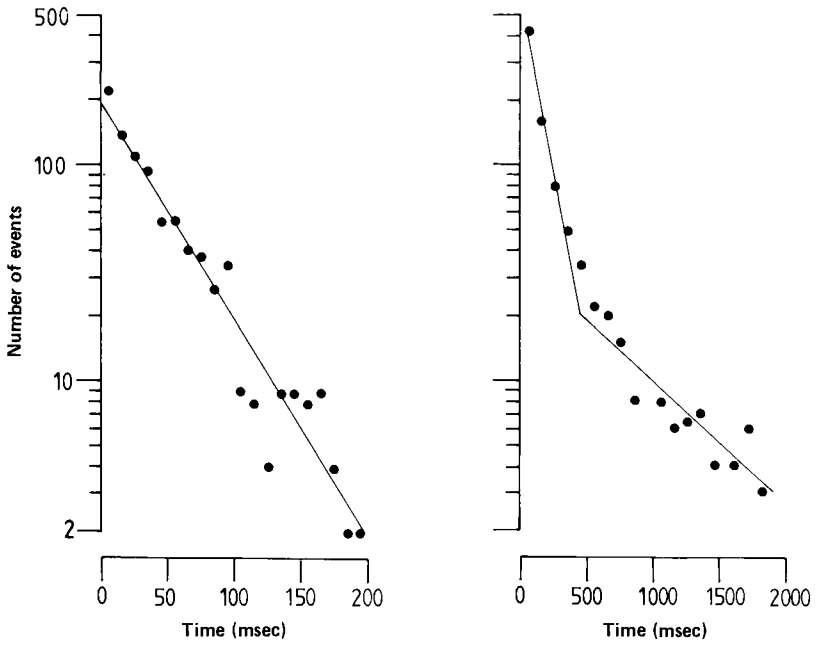


Fig. 59 Comparison of the frequency distribution of glutamate channel closed times measured in the absence (left) and the presence (right) of δ-PTX (0.2 U ml^{-1}). The preparations were pretreated with concanavalin A in order to block desensitization (Mathers and Usherwood, 1976, 1978). Each experiment represents pooled data from three experiments. The data are plotted on semi-log axes. The solid lines were fitted to the data points by the method of least squares. The time constant of the left figure was 43 msec, the time constants of the right figure were τ_{fast}, 100 msec and τ_{slow}, 758 msec. From Clark *et al.* (1982).

in which C^* is the channel structure in its open conformation, T the toxin molecule and τ_b and τ_u are the time constants for blocking and unblocking, respectively.

Hence

$$\frac{\tau_b}{\tau_u} = \frac{[T]\,[C^*]}{[TC^*]}. \tag{2}$$

If one considers the amplitude of the glutamate potentials as a measure of the degree of blocking, the equation can be written as

$$\frac{\tau_b}{\tau_u} = [\delta\text{-PTX}]\,\frac{r_\infty}{1 - r_\infty} \tag{3}$$

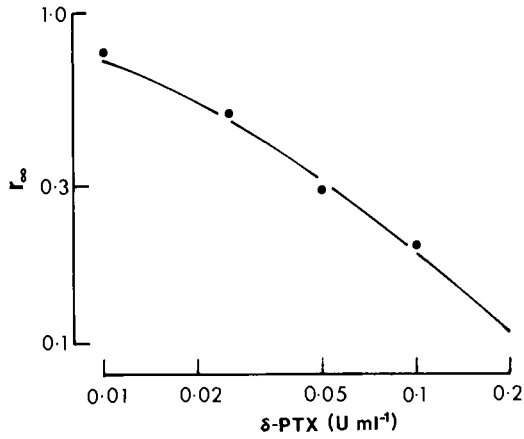


Fig. 60 Relationship between the steady-state ratios, as shown in Fig. 55, and the concentration of δ-PTX. The experimental points fit the line according to Eq. (5). From Piek (1982b).

or as

$$r_\infty = \frac{\tau_b/\tau_u}{\tau_b/\tau_u + [\delta\text{-PTX}]}, \tag{4}$$

in which r_∞ is the steady-state response ratio (see Fig. 56). The relationship between r_∞ and [δ-PTX] is shown in Fig. 60 on a double log scale. The experimental data fit the line

$$r_\infty = \frac{0.024}{0.024\ [\delta\text{-PTX}]}, \tag{5}$$

indicating that the time constant of unblocking is ~40 times larger than the time constant of blocking. This agrees, more or less, with the results obtained from the analysis of patch-clamp recordings, from which the ratio was estimated at less than 100.

In conclusion, δ-PTX probably causes a use-dependent block of glutamate channels in locust muscle fibre membranes, with a blocking time constant roughly estimated at 10 msec and an unblocking time constant of about several hundreds of msec. These values must, however, be considered to be very rough approximations. A comparable ion channel block has been demonstrated in the rat diaphragm treated with δ-PTX (Van Wilgenburg *et al.*, 1984).

The efficacy of δ-PTX as a channel blocker arises from its relatively low unblocking rate rather than from its blocking rate.

β-Philanthotoxin, described in Section II,B,4, was discovered by testing fractions from the venom of *Philanthus triangulum* on intact honey-bee workers. After separation of the active principles considerable loss of total activity occurred. This loss was nearly completely restored after pooling part of the fractions. The β fraction was not active when injected into honey-bees, but it caused potentiation of the paralysing effects of the γ and δ fractions. (Piek and Spanjer, 1978). The inefficacy of the β fraction alone on intact honey-bee was confirmed by Spanjer *et al.* (1982b) who found that in the locust muscle the amplitude of excitatory postsynaptic potentials was not sensitive to the β fraction. However, iontophoretically evoked glutamate potentials, recorded alternatively with the synaptic potentials in the same muscle fibre, showed a decrease in amplitude in a dose-dependent manner (Fig. 54).

K. S. Kits and T. Piek (in preparation) found evidence for a role of β-PTX quite different from that of δ-PTX. Contrary to what has been found for the latter toxin, the action of β-PTX on iontophoretically induced postsynaptic glutamate potentials was abolished by pretreatment of the muscle by concanavalin A (10^{-6} *M*). This suggests that the site of action of the toxin may be a glutamate binding site involved in desensitization, as concanavalin A blocks the glutamate receptor desensitization (Mathers and Usherwood, 1976, 1978). Moreover, K. S. Kits and T. Piek (in preparation) found that β-PTX did not change the distribution of closed times of patch-clamp recorded extra-junctional glutamate channels in the concanavalin A-pretreated muscle of the locust. In muscle fibres that were not treated with concanavalin A an apparent desensitization occurred, starting, in the experiments, at a frequency of ~0.05 Hz and being nearly completed at a frequency of ~0.2 Hz (Fig. 61). β-PTX caused an increase in desensitization; that is, it occurred to a certain level at a lower frequency than in the control. At a toxin concentration of 2.5 μg ml^{-1} the frequency causing a desensitization to a level of 1% decreased from 0.05 Hz in the control to ~0.01 Hz in the presence of the toxin (Fig. 61d). However, the effect of β-PTX on desensitization seems to be frequency-dependent. At 98% desensitization no differences were found. The results suggest that β-PTX inhibits the recovery from desensitization of glutamate receptors and that its action may be competitive to the desensitizing effect of glutamate.

IV. CONCLUSION

The introduction of this book (Chapter 1) described how humans became aware of the presence of paralysing venoms in solitary wasps. After Fabre's work (1855) it was generally accepted that the sting of many solitary wasps

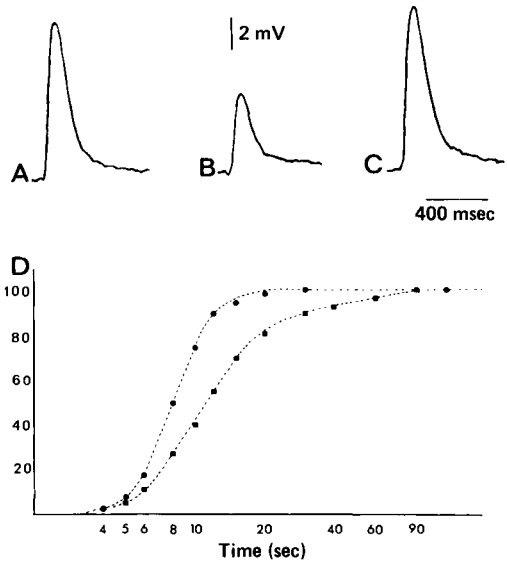


Fig. 61 Effect of 2.5 μg ml^{-1} of β-PTX on iontophoretically evoked glutamate potentials recorded from the locust metathoracic extensor tibiae muscle. (A) control, (B) in the presence of the toxin, (C) after wash. All records show the steady-state amplitude at frequency 0.1 Hz. (D) Frequency dependency of glutamate potentials in the absence (●) and in the presence (■) of the toxin. The steady-state amplitudes are plotted as a percentage of the maximal amplitude, recorded at low frequency, against pulse interval in seconds. From K. S. Kits and T. Piek (in preparation).

caused paralysis in their prey. However, confusion existed about the location to which the sting of aculeate wasps was directed up to recent times. A. L. Steiner, in Chapter 4, provides many arguments in favour of the notion, originally put forward by Fabre (1855, 1879–1910), that these wasps sting into the ganglia, at least into those involved in locomotion and defense.

Although the sphecid wasp *Philanthus triangulum* obviously stings in the direction of the thoracic ganglia of the honey-bee worker, its venom is not only active on synaptic transmission in insect ganglia, but also on insect (and vertebrate) neuromuscular transmission. The philanthotoxins block neuromuscular transmission by blocking in the postsynaptic membrane cationic channels, by inhibiting the recovery of glutamate receptor desensitization, and by inhibiting glutamate reuptake by nerve endings and glia. It is by no means probable that these findings can be generalized to all aculeate wasps. We were unable to demonstrate peripheral effects of venom preparations from other, often closely related, aculeate wasps. It could be that some wasp species that prey on (for them) dangerous insects, such as

other Hymenoptera, have developed peripherally acting toxins in addition to centrally active toxins. It is also conceivable that when the toxins that cause block of transmission in the central nervous system are also active in the periphery, the wasp is able to attack other Hymenoptera without danger to itself.

In addition to paralysing the prey some aculeate wasps change (by stinging) their behaviour in a more or less irreversible way. If this phenomenon, which is called deactivation by Steiner (Chapter 4), might be present in prey of many aculeate wasp species, it is very difficult to discover it when the prey do not completely recover from paralysis, in a reasonably short time. It is also evident that the venoms of certain Terebrantia may have functions quite different from the induction of paralysis. Within the group of Aculeata we can distinguish four different situations enabling the wasp's larva to eat a living prey: (1) the prey is permanently paralysed, (2) the prey is transiently paralysed, but the recovery is so slow that the wasp's larva has time to consume it, (3) the quickly recovered prey is deprived of its legs and/or is imprisoned in a nest in a way that incapacitates the prey from escaping, and (4) the paralysis is very short-lasting and a deactivation follows. In that case the prey may be physically able to escape but shows no initiative unless disturbed. Such a deactivation is described in Chapter 4 (Section II,D) and in this chapter (section III,B,1). It is evident that in these cases the deactivation is caused by a sting in the suboesophageal ganglion.

Since the wasps investigated are such a small sample of all members of living wasp species it can be expected that further studies will reveal quite different entities, useful as tools for the study of experimental entomology and maybe also for developing new drugs or new classes of pesticides. Moreover, it could be that understanding the properties of venoms of solitary wasps is crucial in understanding the evolution of paralysing behaviour.

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