

RECENT ADVANCES IN MODE OF ACTION OF INSECTICIDES¹

Richard W. Beeman

US Grain Marketing Research Laboratory, Agricultural Research, Science and Education Administration, US Department of Agriculture, Manhattan, Kansas 66502

The field of insecticide mode of action has become so large that a review of this length cannot hope to be comprehensive. I have therefore attempted to clarify the current status of only a few unresolved or controversial problems and to indicate certain possibilities for future research. I have omitted any discussion of the acetylcholinesterase-inhibitory organophosphate or carbamate insecticides. Many other important topics and novel concepts, including juvenile hormone (JH) mimics (44, 176), anti-JHs (21, 27, 156, 157, 175), cholinergic receptor agonists (98, 172), choline acetyltransferase inhibitors (164), 1-phenylcarbamoyl-2-pyrazolines (73), glutamate analogs (76, 122), and antibodies as insecticides (142) are likewise not discussed.

Recent accounts of various aspects of the mode of action of pyrethroids and DDT analogs can be cited, including effects on sodium and potassium conductance changes (135-137), structure-activity relationships (55, 83, 84, 134, 138), and inhibition of calcium-independent ATPases (33, 45, 50). I have focused my discussion of this group of insecticides on recent attempts to locate their primary sites of action *in vivo*, and to relate their mode of neurotoxic action to calcium function. Other paths traced in the present review are the aminergic theory of formamidine action, the mechanism of synaptic facilitation by cyclodienes and γ -BHC, and the question of chitin synthetase inhibition by benzoylphenyl urea insecticides. It is assumed that the reader is familiar with the most basic principles of neurophysiology.

¹The US Government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

PYRETHROIDS AND DDT ANALOGS

Effects on Axon Membrane Currents

Insecticidal pyrethroids and DDT analogs are well-known to be directly toxic to nerves. Furthermore, while these two groups are chemically dissimilar (Figure 1), they interact with related or identical target sites on the nerve membrane. Both groups of insecticides display a negative temperature correlation with toxicity (62, 63).

This common mechanism of neurotoxicity has been elucidated in terms of transmembrane ion fluxes in isolated, intact axons, with the aid of voltage clamp techniques. Briefly, the following effects on nerve are involved. (a) A prolongation of the falling phase of the action potential and an increase in the "negative after-potential" (NAP)², resulting in a broadening of the wave of depolarization during impulse propagation. These phenomena appear to be largely due to a prolongation of the inward sodium (Na⁺) current and to a lesser extent, a suppression of the outward potassium (K⁺) current. The insecticide molecules are considered to wedge into open (conducting) Na⁺ channels, such that Na⁺ conductance is unimpeded, but the channels cannot return to their nonconducting, "closed-gate" configuration (82). (b) Repetitive firing in response to a single stimulus. This occurs when sodium inactivation is retarded to such an extent that the axon is held in a sustained state of depolarization. (c) Conduction blockade by exhaustion of the membrane potential. In the continued presence of insecticide, the axons will continue to fire repetitively for many hours, presumably until the electrochemical Na⁺ and K⁺ gradients which energize them decay. A number of DDT analogs are of this type, including methoxychlor and DDT itself (193), or (d) Conduction blockade without exhaustion of the membrane potential. Some DDT analogs and pyrethroids block nerve much more rapidly than others. Rapid blockers (e.g. p,p'-OH-DDT, Figure 1) often act without increasing the NAP and without exciting the nerve (193). These compounds block Na⁺ channels in the nonconducting (closed-gate) configuration, such that the Na⁺ inward current is inhibited, the amplitude of the action potential is reduced, and impulse conduction is finally blocked, without significant diminution of the membrane potential. For a more detailed discussion, the reader is directed to several excellent reviews (135-137).

The Role of Calcium

The effects of many pyrethroids and DDT analogs on Na⁺ and K⁺ fluxes in nerve axons have been described in detail, as summarized above. However, another cation, calcium (Ca²⁺), is also intimately associated with the

²This term has caused confusion. See (135) for clarification.

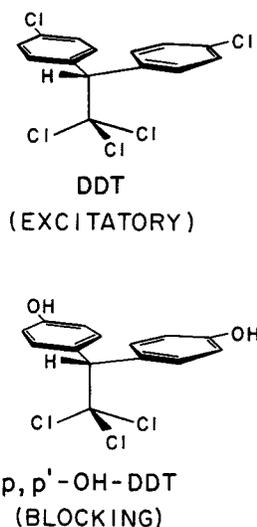


Figure 1 Structures of DDT and one of synthetic pyrethroid, decamethrin.

axon and with the toxic reaction vital to the proper functioning of Ca²⁺ are well-known to antagonize the action of nerve (65, 72, 115). Concentration around arthropods reminiscent of DDT (144). In the DDT-induced repetitive discharge of Na⁺ and K⁺ fluxes as these relative depolarizing current applied across repetitive discharge in the presence (196). In certain neurons Ca²⁺ dependent current. The response of such neurons to that of Na⁺-dependent cells (

Calcium is known to be intimately associated with membrane potential and Na⁺ conductance. Ca²⁺ concentration desensitizes the Na⁺ gate from being "propagated" (60). With respect to the action of the Na⁺ gate from being "propagated"

Actions of other neuroactive Ca²⁺ have been explained in similar terms. It is known to block axonal conduction

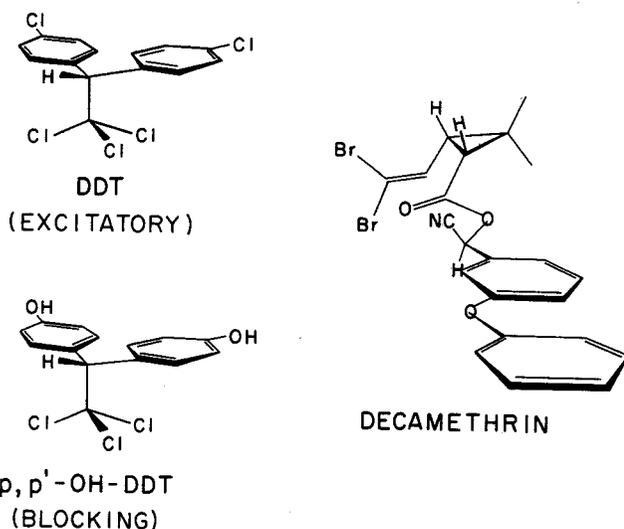


Figure 1 Structures of DDT and one of its nerve-blocking analogs and of the extremely toxic synthetic pyrethroid, decamethrin.

axon and with the toxic reaction between axon and insecticide. Calcium is vital to the proper functioning of all excitable tissues, and in particular it has a stabilizing effect on the nerve axon. High concentrations of external Ca^{2+} are well-known to antagonize the DDT- or pyrethroid-induced excitation of nerve (65, 72, 115). Conversely, reducing the extracellular Ca^{2+} concentration around arthropod nerve axons elicits repetitive discharge reminiscent of DDT (144). In this regard it is relevant to mention that DDT-induced repetitive discharges are difficult to explain solely in terms of Na^+ and K^+ fluxes as these relate to membrane potential, since sustained depolarizing current applied across an unpoisoned axon does not induce repetitive discharge in the presence of normal concentrations of Ca^{2+} (135, 196). In certain neurons Ca^{2+} replaces Na^+ as the carrier of depolarizing current. The response of such neurons to DDT and pyrethroids is similar to that of Na^+ -dependent cells (147a).

Calcium is known to be intimately involved in conductance gating. Specifically, extracellular Ca^{2+} controls the degree of coupling between membrane potential and Na^+ conductance, such that increasing the extracellular Ca^{2+} concentration desensitizes Na^+ conductance to a unit depolarization (60). With respect to the action of DDT, Ca^{2+} can be viewed as protecting the Na^+ gate from being "propped open" by this insecticide.

Actions of other neuroactive substances which are also modulated by Ca^{2+} have been explained in similar terms. Local anesthetics, for example, are known to block axonal conduction by suppressing Na^+ conductance

directly
dissim-
on the
perature

n terms
voltage
ved. (a)
ncrease
g of the
ena ap-
current
current.

lucting)
hannels
m (82).
s when
held in
austion
de, the
ntil the
A num-
1 DDT
mem-
much
gure 1)
e (193).
d-gate)
plitude
locked,
a more
s (135-

fluxes
How-
ith the

(101, 177). Calcium antagonizes this blockade, possibly by competing with the local anesthetic for a binding site on the membrane which regulates the Na^+ gate (78). Perhaps the blocking actions of certain pyrethroids and DDT analogs can be explained in similar terms.

In general our understanding of the role of Ca^{2+} in axonal function lags far behind that for Na^+ and K^+ . However, in recent years several elements of the complex system of Ca^{2+} regulation in axon have been elucidated. These will be briefly discussed in the context of insecticide action.

Like Na^+ , Ca^{2+} shows a large electrochemical gradient across the axon membrane, with high Ca^{2+} outside and low Ca^{2+} inside (47). Recently it has been shown that at least two distinct components of the axon membrane are specialized to effect the transport of Ca^{2+} against both its concentration and potential gradients. These are the $\text{Na}^+-\text{Ca}^{2+}$ exchange pump (powered by the Na^+ gradient), and the ATP-driven Ca^{2+} pump (18, 47).

It is known that a trace of Ca^{2+} enters the axon during an action potential (81, 120). This inward Ca^{2+} flux is triggered by depolarization but is a negligible contribution to the total membrane current. In addition, in certain neurons Ca^{2+} influx triggers K^+ efflux (117). It is therefore possible that the well-known K^+ -mediated repolarization of the axon during an action potential is dependent on Ca^{2+} influx. Calcium influx is known to mediate the posttetanic, hyperpolarizing current (carried by effluxing K^+) which occurs after multiple discharges in certain vertebrate and invertebrate nerve cells (92, 116). It is tempting to infer a relationship between these hyperpolarizing or rectifying effects of Ca^{2+} and its previously discussed stabilizing effect on the nerve membrane.

The impulse-induced Ca^{2+} current across the axon membrane is pharmacologically similar to the Ca^{2+} influx which mediates release of transmitter at the presynaptic terminal (8, 9). Thus the membrane structures of axon and synapse may be regarded as fundamentally alike.

Unlike Na^+ and K^+ , a large portion of axonal Ca^{2+} is membrane-bound. Part of the Ca^{2+} -sequestering activity of the nerve axon resides in the mitochondria, but other major portions reside in nonmitochondrial axoplasmic factors and on the axon membrane itself (7, 23, 77, 132). These Ca^{2+} binding sites, or "receptors," are probably involved in several distinct Ca^{2+} -dependent nerve functions in addition to Na^+ and K^+ conductance gating, including axonal transport (75) and the presynaptic release of neurotransmitters (43). Several Ca^{2+} receptor proteins have been isolated and chemically identified, including calmodulin, troponin C, and parvalbumin (see 34). One of these, calmodulin, is present in both central and peripheral mammalian nerve (95). However, the relationship of these Ca^{2+} receptors to the excitability of the axon membrane is uncertain.

$\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase is also a universal component of nerve tissue (94, 100, 161, 170). DiPolo & Beaugé (48) have expressed the view that a

$\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase in rat brain by Robinson (159), may represent a driven Ca^{2+} pump. Analogous components of erythrocyte membranes, which (109, 163), and the ouabain-sensitive as the *in vitro* manifestation of the Iqbal & Ochs (95) considered the mammalian nerve may be related to these are energy-requiring and calmodulin ATPase in rat brain synaptic membrane for activity, may have a function in regulation of intracellular Ca^{2+} by paracetomyosin-like fibers present in cymes were stimulated by low concentrations of the intracellular environment.

It is not yet possible to precisely state clearly how Ca^{2+} is involved in consensus on the functional significance of the process measured in nerve homogenates.

Nonetheless, several attempts at explanations of the mode of action in terms of Ca^{2+} -related events. (Recently reported that DDT is a potent inhibitor of ATPase in lobster peripheral axons effective. On the basis of a relationship between stimulation at $\sim 100 \mu\text{M}$ Ca^{2+} and intact axon suspensions, these workers proposed an "ecto" ATPase, involved in the surface of the axon. Ecto ATPase is implicated in the regulation of cell and cell aggregation (118). The ATP-driven Ca^{2+} pump, which is sensitive to Ca^{2+} and requires intracellular Ca^{2+} (unpublished data) have recently been reported in squid retinal axons which has a high sensitivity to many pyrethroids were potent inhibitors of methrin, cypermethrin, and decamethrin. The relationship is that inhibition of Ca^{2+} availability for binding to sites on the membrane results in a decrease in the rate of action potential generation.

It is well-known that DDT inhibits Ca^{2+} ATPase including ouabain-sensitive Na^+

$\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase in rat brain with a high affinity for Ca^{2+} , described by Robinson (159), may represent the biochemical counterpart of the ATP-driven Ca^{2+} pump. Analogous cases would be the $\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase of erythrocyte membranes, which is functionally linked with a Ca^{2+} pump (109, 163), and the ouabain-sensitive $\text{Na}^+ + \text{K}^+$ ATPase, which is regarded as the *in vitro* manifestation of the ATP-driven $\text{Na}^+ - \text{K}^+$ exchange pump. Iqbal & Ochs (95) considered that a $\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase from mammalian nerve may be related to axoplasmic transport, since both processes are energy-requiring and calmodulin-dependent. A similar $\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase in rat brain synaptic membranes, which also requires calmodulin for activity, may have a function either in Ca^{2+} extrusion, in the sequestration of intracellular Ca^{2+} by particulate components, or may be related to actomyosin-like fibers present in the synaptic region (170). All these enzymes were stimulated by low concentrations of Ca^{2+} ($\sim 1 \mu\text{M}$), characteristic of the intracellular environment.

It is not yet possible to present a clear picture of how all these Ca^{2+} -regulating elements are integrated into the functioning axon, nor to state clearly how Ca^{2+} is involved in nerve excitability. Also, there is no consensus on the functional significance of any Ca^{2+} -dependent biochemical process measured in nerve homogenates.

Nonetheless, several attempts have been made to construct biochemical explanations of the mode of action of pyrethroids and DDT analogs in terms of Ca^{2+} -related events. Ghiasuddin & Matsumura (69, 70) have recently reported that DDT is a potent inhibitor of a Ca^{2+} -dependent ATPase in lobster peripheral axons. DDE, a nontoxic analog, was less effective. On the basis of a relatively low affinity for Ca^{2+} (half-maximum stimulation at $\sim 100 \mu\text{M}$ Ca^{2+}) and activity towards extracellular ATP in intact axon suspensions, these workers suggested that this enzyme might be an "ecto" ATPase, involved in the regulation of Ca^{2+} binding to the outer surface of the axon. Ecto ATPases from mammalian sources have been implicated in the regulation of cell surface charges, membrane permeability, and cell aggregation (118). The enzyme is presumably different from the ATP-driven Ca^{2+} pump, which is stimulated by submicromolar levels of Ca^{2+} and requires intracellular ATP (47). J. M. Clark & F. Matsumura (unpublished data) have recently studied a labile $\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase in squid retinal axons which has a high affinity for Ca^{2+} . They found that many pyrethroids were potent inhibitors of this enzyme, particularly permethrin, cypermethrin, and decamethrin. In both of these cases the implication is that inhibition of Ca^{2+} -ATPase leads to a condition of reduced Ca^{2+} availability for binding to specific sites involved in membrane stabilization.

It is well-known that DDT inhibits several Ca^{2+} -independent ATPases, including ouabain-sensitive $\text{Na}^+ + \text{K}^+$ ATPase of axon membrane, and

mitochondrial Mg^{2+} ATPase (33, 50). However, as discussed by Brooks (25), inhibition of these enzymes cannot be directly related to nerve excitation. The significance of DDT and pyrethroid inhibition of Ca^{2+} -dependent ATPases will likewise remain in doubt until the functions of these enzymes can be elucidated.

Effects on Intact Tissues

Biochemical studies of nerve homogenates and physiological studies of isolated axons, while indispensable, cannot reveal the complex interactions which define the mode of action of an insecticide in a highly integrated multicellular organism. A question of major interest in regard to the mode of action of pyrethroids and DDT analogs has been, Which nervous structures are the actual sites of toxic action? Distinctions can be made between specific effects on impulse initiation, axonal conduction, and synaptic transmission. At the organismal level, specific actions on the central and peripheral nervous system can be differentiated. Miller and his collaborators (3, 123) have devised a convenient method for distinguishing between the central and peripheral actions of insecticides by recording simultaneously from several separate flight motor units in the intact house fly. These motor units are normally "coupled." That is, they act in concert under the direction of the central nervous system (CNS) to effect coordinated flight. The determination that an insecticide is a central or a peripheral nerve poison is based on the observation that many types of damage to the CNS result in the uncoupling of flight motor activity, but that no amount of damage to peripheral nerves will cause uncoupling (123).

PERIPHERAL EFFECTS It has been claimed that DDT exerts its most potent action on peripheral nerve, particularly sensory nerve, and that even concentrated emulsions of DDT have no effect on the cockroach CNS (160). Furthermore, DDT at lethal doses does not uncouple the house fly flight motor, even after prolonged irritation of the individual motor neurons (124).

Pyrethroids also effect peripheral nerves. Clements & May (35) reported that pyrethroids bearing an α -cyano substituent (such as fenvalerate) had potent stimulatory effects on locust sensory neurons and that the impulse generator region of the cell, rather than the axon, was the primary site of action. Osborne (148) reported that permethrin induced prolonged depolarizations in crayfish sensory cell bodies, which in turn caused repetitive firing in the sensory axons. Van den Bercken et al (17) concluded that vertebrate peripheral nerve terminals are generally more sensitive to irritation by either allethrin or DDT than peripheral axons, since repetitive firing recorded from axons was shown to originate in sense organs. In a similar

study, Adams & Miller (3) recorded neurons minutes after treatment. The discharges were shown to be rather than the axon. The phenomenon (Adams & Miller) also occurred. These workers considered that results to knockdown or death, since several fenvalerate) were potent knockdown backfiring.

CENTRAL EFFECTS In addition, pyrethroids also have significant actions on DDT analogs. Adams & Miller (3) studied the house fly flight motor, in which the effect of DDT (Adams & Goodchild (30) concluded that the effect of pyrethroid intoxication, was rapid and appeared much more rapidly by application. However, Clements (123) concluded that knockdown by pyrethroids was a central nerve effect.

D. W. Gammon has recently reported on the effects of DDT on peripheral actions of DDT. He implanted electrodes in free-walkers and found that the greater toxicity of allethrin was due to a greater sensitivity of the peripheral but not the central nervous system against an LD_{95} dose of allethrin. He reported different results: DDT caused hyperexcitability of peripheral nerves over a broad range of doses, while tetrodotoxin poisoning (63, 64). This is unexpected since DDT is primarily a peripheral poison.

SYNAPTIC EFFECTS If the Ca^{2+} function is fundamentally presynaptic terminal, it is relevant to the synapse, in addition to its well-known effects on the postsynaptic terminal. Adams et al (59) have recently studied the effect of DDT on the postsynaptic membrane potential at a miniature end-plate potential (mEPP).

study, Adams & Miller (3) recorded repetitive discharges in house fly motor neurons minutes after treatment with extremely low doses of tetramethrin. The discharges were shown to arise in the intramuscular nerve terminal rather than the axon. The phenomenon (termed "repetitive backfiring" by Adams & Miller) also occurred during intoxication with DDT analogs (4). These workers considered that repetitive backfiring was not directly related to knockdown or death, since several pyrethroids (kaldethrin, decamethrin, fenvalerate) were potent knockdown and killing agents but did not cause backfiring.

CENTRAL EFFECTS In addition to their peripheral effects, the pyrethroids also have significant actions on the CNS, which are lacking with DDT analogs. Adams & Miller (4) found that most pyrethroids uncoupled the house fly flight motor, in sharp contrast to DDT and its analogs. Burt & Goodchild (30) concluded that rapid knockdown, which is characteristic of pyrethroid intoxication, was related to CNS action, since this symptom appeared much more rapidly by injection of insecticide than by topical application. However, Clements & May (35) observed that in most cases knockdown by pyrethroids was accompanied by hyperexcitation of sensory nerve.

D. W. Gammon has recently shed new light on the question of central vs peripheral actions of DDT and pyrethroids. He used chronically implanted electrodes in free-walking cockroaches to provide direct evidence that the greater toxicity of allethrin at lower temperatures was associated with a greater sensitivity of the peripheral nervous system (62). Moreover, doses of the sodium channel blocker, tetrodotoxin, which blocked the peripheral but not the central nervous system, afforded complete protection against an LD₅₀ dose of allethrin (64). Similar tests with DDT gave quite different results: DDT caused hyperexcitation of both peripheral and central nerves over a broad range of temperatures, and selective blockade of peripheral nerves by tetrodotoxin provided no protection against DDT poisoning (63, 64). This is unexpected in view of previous claims that DDT is primarily a peripheral poison.

SYNAPTIC EFFECTS If the action of DDT is related to Ca²⁺, and if Ca²⁺ function is fundamentally similar both along the axon and at the presynaptic terminal, it is relevant to ask whether DDT has an effect at the synapse, in addition to its well-established action on the nerve axon. Farley et al (59) have recently studied the effect of the DDT analog EDO on the crayfish neuromuscular junction. They found that while EDO had no direct effect on the postsynaptic membrane, it caused a prolonged increase in the miniature end-plate potential (mepp) frequency. An increased rate of firing

in the presynaptic nerve was shown to be a precondition to this mepp facilitation. However, their key observation was that the increased firing in the presynaptic nerve was by itself not sufficient to explain the prolonged increase in mepp frequency, since the mepp increase was sustained even after nerve block by tetrodotoxin. Furthermore, high-frequency stimulation of the unpoisoned nerve to mimic the effect of EDO did not cause a similar increase. This suggests an additional effect of EDO directly on the presynaptic terminal. The mepp frequency increase required Ca^{2+} and was sustained even after the EDO-induced excitation of the axon membrane was blocked by a high concentration of extracellular Ca^{2+} .

Finally, Clements & May (35) reported a direct effect of a pyrethroid on locust muscle. They found that very low concentrations of pyrethroid suppressed the muscle spike induced by a depolarizing current applied directly to the muscle cell. This effect could be related to the increased Ca^{2+} conductance which normally accompanies depolarization of insect muscle cells (190).

CYCLODIENES AND γ -BHC

Just as the pyrethroids and DDT analogs appear to act at similar or identical target sites on the nerve membrane, so the cyclodienes (e.g. aldrin, dieldrin, chlordane, heptachlor) and γ -BHC (lindane) analogs, while comprising two chemically rather dissimilar classes, are believed to share a common mode of action, which is quite distinct from that of the DDT-pyrethroid group (183). Symptoms of intoxication by cyclodienes and γ -BHC in insects are cholinergic in nature and are qualitatively different from those induced by pyrethroids and DDT analogs (24, 183).

Cyclodiene insecticides act both centrally and peripherally, but the central effects appear to be most significant (71, 167, 189). Wang et al (189) reported that dieldrin irritated sensory neurons and also facilitated transmission across central synapses in cockroaches. The metathoracic ganglion was much more sensitive to dieldrin than the last abdominal ganglion. Shankland & Schroeder (167) suggested that dieldrin acts presynaptically at cholinergic junctions in the insect CNS by facilitating both spontaneous and impulse-induced release of acetylcholine. The dieldrin-induced synaptic facilitation was antagonized by cholinergic blocking agents. Enhanced release was inferred, rather than cholinomimetic action, since depletion of endogenous acetylcholine stores by exhaustive electrical stimulation in the presence of hemicholinium-3 rendered the ganglia insensitive to dieldrin but not to the cholinomimetic agent nicotine. When acetylcholine release (which is Ca^{2+} -dependent) was blocked by the Ca^{2+} antagonist, Mg^{2+} , the ganglia similarly became insensitive to dieldrin but not to nicotine. Uchida

et al (183-185) reported that γ -l synaptic facilitation or "after-disc" quickly suppressed by the choline discharge was apparently caused line release.

Akkermans et al (5) investigated by aldrin *trans*-diol (a neuroactive nerve-muscle preparations. They both spontaneous and evoked by dieldrin in the insect CNS. How major portion of the facilitated independent. Aldrin *trans*-diol a action on the frog end-plates.

Yamaguchi et al (194, 195) have a mechanism for cyclodiene-induced transmission by heptachlor epoxide at a concentration of Ca^{2+} -induced and depolarization of transmitter (glutamate) from presynaptic brain. This synaptic facilitation was by several effects of heptachlor epoxide, all of which should tend to increase of intracellular free Ca^{2+} in the presynaptic terminal, inhibition of $\text{Ca}^{2+} + \text{Mg}^{2+}$ ATP-dependent of Ca^{2+} uptake into whole synaptosomes, and synaptosomal membrane fragmentation.

Dieldrin poisoning symptoms in insect ganglia, become apparent only after 167, 189). Wang et al (189) found that *trans*-diol, was as potent as dieldrin and furthermore did not require the activation of dieldrin to aldrin. Dieldrin is known to be metabolized in insects (26, 141, 165, 166).

However, Schroeder et al (167) found that dieldrin is toxic to cockroaches by injection, though all three compounds concluded that the metabolic conversion was not the cause.

The question of the metabolic conversion remains unresolved. For example, Wang et al (189) found that the *trans*-diol-like metabolite of dieldrin, less polar metabolites of dieldrin, less polar

et al (183-185) reported that γ -BHC analogs, like cyclodienes, induced a synaptic facilitation or "after-discharge" in the cockroach CNS, which was quickly suppressed by the cholinergic blocking agent nereistoxin. The after-discharge was apparently caused by presynaptic stimulation of acetylcholine release.

Akkermans et al (5) investigated the mechanism of synaptic facilitation by aldrin *trans*-diol (a neuroactive metabolite of dieldrin) in isolated frog nerve-muscle preparations. They concluded that this substance enhanced both spontaneous and evoked transmitter release, as in the example of dieldrin in the insect CNS. However, in contrast to the case in insects, a major portion of the facilitated transmitter release in frog was Ca^{2+} -independent. Aldrin *trans*-diol also had secondary postsynaptic blocking action on the frog end-plates.

Yamaguchi et al (194, 195) have recently proposed a biochemical mechanism for cyclodiene-induced transmitter release. These workers found that heptachlor epoxide at a concentration of 10^{-7} M stimulated both Ca^{2+} -induced and depolarization (high K^+)-induced release of a neurotransmitter (glutamate) from pre-loaded synaptosomes isolated from rat brain. This synaptic facilitation was considered to be adequately explained by several effects of heptachlor epoxide on synaptosomal calcium regulation, all of which should tend to promote an increase in the concentration of intracellular free Ca^{2+} in the presynaptic terminal. These effects included inhibition of $\text{Ca}^{2+} + \text{Mg}^{2+}$ ATPase both in vitro and in vivo, stimulation of Ca^{2+} uptake into whole synaptosomes, and inhibition of Ca^{2+} binding to synaptosomal membrane fragments.

Dieldrin poisoning symptoms in insects, either in vivo or in isolated ganglia, become apparent only after a lag time of one hour or longer (24, 167, 189). Wang et al (189) found that a metabolite of dieldrin, aldrin *trans*-diol, was as potent as dieldrin in producing synaptic after-discharges, and furthermore did not require a lag time. They suggested that metabolic activation of dieldrin to aldrin *trans*-diol might be required for toxicity. Dieldrin is known to be metabolized to aldrin *trans*-diol and aldrin *cis*-diol in insects (26, 141, 165, 166).

However, Schroeder et al (165) found that dieldrin was about 200X as toxic to cockroaches by injection as either the *cis*- or *trans*-diol, even though all three compounds penetrated the CNS equally well. They concluded that the metabolic conversion of dieldrin to either diol was a detoxification.

The question of the metabolic activation of dieldrin in insects is still unresolved. For example, Wang et al (189) reported that an unidentified *trans*-diol-like metabolite of dieldrin was neurotoxic in cockroaches. Several metabolites of dieldrin, less polar than the diols, have been detected in

to this mepp
eased firing in
he prolonged
stained even
y stimulation
ause a similar
on the presy-
and was sus-
embrane was

pyrethroid on
pyrethroid sup-
plied directly
 Ca^{2+} conduc-
muscle cells

ar or identi-
(e.g. aldrin,
while com-
to share a
the DDT-
dienes and
ly different
)

et the cen-
et al (189)
ated trans-
ic ganglion
ganglion.
naptically
ontaneous
ed synap-
Enhanced
pletion of
ion in the
eldrin but
re release
 Mg^{2+} , the
e. Uchida

insects (141, 166, 168). One of these (structure unconfirmed) appears in significant quantities in the CNS of dieldrin-treated cockroaches and locusts (169). The CNS itself has recently been shown to be a relatively active site of dieldrin metabolism (168).

If dieldrin is intrinsically toxic and requires no metabolic activation, then the pronounced delay which always precedes the appearance of toxic symptoms in dieldrin-treated insects must result either from penetration barriers or from an unusually slow interaction between dieldrin and the nerve target. In this context it would be instructive to determine whether dieldrin itself stimulates transmitter release from isolated synaptosomes. The target membranes in such preparations would be directly exposed to the insecticide, and opportunities for metabolic side-reactions on dieldrin would be minimized.

FORMAMIDINES

The formamidines are a group of compounds with toxicity to a limited range of insects and acarines (86). Active research into the mode of action of formamidines, most notably chlordimeform, has been ongoing for about ten years. However, a universal mechanism of toxicity for this group of chemicals has not been apparent.

The repellent effects of chlordimeform have often been cited (49, 67, 79), and repellency has been regarded as a second mode of action in addition to direct toxicity. However, the distinction between repellency and toxicity has gradually become blurred. On the one hand, it has been pointed out that the acute toxicity of formamidines may result from death by starvation or desiccation following a primary repellent action (12, 80). On the other hand, in many cases what appears to be a repellent effect may actually be a disruption of feeding behavior via a direct sublethal effect on the CNS. For example, Lund et al (112) showed that larvae of *Manduca sexta*, which appeared to be repelled from chlordimeform-treated leaves, in fact suffered a CNS disturbance which caused them to drop off the leaves. Watanabe & Fukami (191) reported a similar phenomenon in armyworm and silkworm larvae. Kono et al (106) found that low concentrations of chlordimeform stimulated probing behavior in leafhoppers. The phenomenon did not appear to be a repellent effect, since the behavior persisted with increased intensity after the insects were subsequently transferred to insecticide-free medium. Beeman & Matsumura (13) showed that chlordimeform was a potent appetite suppressant in cockroaches and that this anorectic effect was independent of the repellent effect of chlordimeform in the same species. Thus, a subacute toxic effect can produce repellent-like behavior, and the subsequent nonfeeding can lead to death by starvation or desiccation. These

observations point to a conclusion for the chemical control of insects: to be lethal to be effective, and to be practical to be practical.

A wide variety of biochemical effects have been reported including neuromuscular effects, aminergic effects [see (86, 112,

The evidence to date suggests that the effects of formamidines in invertebrates may involve (amine-related) nervous or endocrine inhibitors of monoamine oxidase (MAO inhibition is not considered in insects, since MAO is not reported with the exception of the Malpighian tubules in Malpighian tubules). In no one has specifically examined the primary function of Malpighian tubules showed chlordimeform to be a mimicking epinephrine, serotonin, and

In contrast to the potent formamidines in insects and mites, appetite stimulants in mammals and hunger is known to be regulated (89, 173). Although no direct biogenic amines and feeding behavior in Mollusca (192). Furthermore, the structure in the insect brain (61), repellent behavior in insects (52, 93).

The neuroexcitatory effect of chlordimeform in *Manduca* (see above) was shown to be a direct effect on the synapses in the CNS of the insect. The number of biogenic amines (1) suspected in this case, although

In recent years a refinement of action has emerged. This is the case of the receptor agonists. A growing interest in topamine has special importance that octopamine represents the catecholamines, norepinephrine specific receptors have been found

observations point to a conclusion which may have far-reaching implications for the chemical control of insects: Chemical control agents need not be lethal to be effective, and behavior-modifying, "pestistatic" chemicals may represent a practical alternative to biocides.

A wide variety of biochemical and pharmacological actions of formamides have been reported including mitochondrial uncoupling, local anesthesia, neuromuscular effects, monoamine oxidase inhibition, and other aminergic effects [see (86, 112, 114) for reviews].

The evidence to date suggests that most of the potent effects of formamides in invertebrates may involve the disruption of aminergic (i.e. biogenic amine-related) nervous or endocrine functions. Formamides are potent inhibitors of monoamine oxidase (MAO) in mites and ticks, (6, 14, 114). MAO inhibition is not considered to be a significant action of formamides in insects, since MAO is not readily demonstrable in insect tissues (14, 56) with the exception of the Malpighian tubules (19). Unfortunately the function of MAO in acarines or in insect Malpighian tubules is unknown. Also, no one has specifically examined whether formamides disrupt the excretory function of Malpighian tubules in insects. Beeman & Matsumura (11) showed chlordimeform to be a potent cardiostimulant in the American cockroach. This action is mimicked by a number of biogenic amines including epinephrine, serotonin, and octopamine (11, 41, 107).

In contrast to the potent anorectic and feeding deterrent effects of formamides in insects and mites referred to above, formamides are potent appetite stimulants in mammals (152, 153, 197). In mammals the sensation of hunger is known to be regulated by aminergic central nervous structures (89, 173). Although no direct evidence exists for a relationship between biogenic amines and feeding behavior in insects, the opposite is true in Mollusca (192). Furthermore the central body, a catecholamine-rich structure in the insect brain (61), reportedly has a role in the regulation of feeding behavior in insects (52, 93).

The neuroexcitatory effect of formamides observed by Lund et al in *Manduca* (see above) was shown to be a direct effect on noncholinergic synapses in the CNS of the insect (112). The effect was mimicked by a number of biogenic amines (110). Thus, aminergic involvement must be suspected in this case, although definite proof is lacking.

In recent years a refinement of the aminergic theory of formamide action has emerged. This is the concept that formamides are octopamine receptor agonists. A growing accumulation of evidence suggests that octopamine has special importance as a neurotransmitter in invertebrates and that octopamine represents the invertebrate equivalent of the vertebrate catecholamines, norepinephrine, and epinephrine (188). Octopamine-specific receptors have been found in orthopteran leg muscle (58), lepidop-

teran flight muscle (103), the firefly lantern (139, 158), glycogen-mobilizing cells in cockroach fat body (51), and cockroach central nervous tissue (20, 140).

Hollingsworth & Murdock (87) found that *N*-demethylchlordimeform was an extremely potent and long-lasting agonist for octopamine receptors in the firefly lantern. It stimulated octopamine-dependent adenylate cyclase (an indicator of octopamine receptor activation) in the lantern (133) and caused the organ to glow brightly. Chlordimeform also caused the lantern to glow, but only after a 4-hr delay. Furthermore, this compound did not activate octopamine-dependent adenylate cyclase, either in the firefly lantern (133) or in the cockroach central nervous system (114). Metabolic transformation of chlordimeform may be required to produce the active octopamine agonist. In ticks, metabolic activation of chlordimeform is known to occur, probably by *N*-demethylation (104), although there is no evidence of an octopaminergic action of formamidines in acarines.

Evans (57) found that superfusion of chlordimeform or *N*-demethylchlordimeform (threshold $\sim 10^{-6}$ M and 10^{-7} M, respectively) mimicked the modulatory effect of octopamine on neurally evoked contraction and relaxation of locust leg muscle. The aminergic receptors in this tissue had previously been shown to be specific for octopamine, since they did not cross-react with dopamine, norepinephrine, serotonin, or several other related amines (150). The effects of both octopamine and chlordimeform were blocked by the α -adrenergic antagonist phentolamine but not by the β -adrenergic antagonist, propranolol.

Low doses of chlordimeform trigger incessant flight in adult moths (102, 111, 191). It has recently been suggested that the activity of certain flight muscles in adult *Manduca sexta* may be modulated by octopaminergic neurons with cell bodies present in the thoracic ganglia (102, 103; A. E. Kammer, personal communication). Both octopamine and chlordimeform augment the excitatory junction potentials (EJPs) recorded extracellularly from pharate adults of *Manduca* (L. W. Klaassen, personal communication). In this context it is tempting to speculate that the potent motor stimulation by chlordimeform of larvae and adults of *Manduca* and other lepidopterans may reflect octopamine receptor stimulation.

The difficulty with this idea is that octopamine has been studied only as a peripheral modulator of motor activity, whereas the stimulation of moth larvae by chlordimeform is mediated by synapses in the CNS. Chlordimeform-induced motor irritation in adult moths is probably also a central effect, although this has not been proven unequivocally.

Octopamine applied in situ has been shown to augment motor output from both thoracic and abdominal ganglia of *Manduca* larvae (110; A. E. Kammer, personal communication). O'Shea & Evans (150) have suggested

IN

that dorsal, unpaired, median (I) may serve a general function of modulation in insects. A more insight into the mode of action of aminergic neurons in

INHIBITORS OF CHITIN

Insect cuticle is an obviously desiccation-resistant structure. Diflubenzuron and related slow-acting stomach poisons which exert their toxic actions on the cuticle. They exert their toxic actions on the cuticle. Affected larvae appear normal but often cannot free themselves from the cuticle. Sowa & Marks (174) showed that the epidermis in regenerating tissue from benzuron failed to secrete new cuticle.

Sowa & Marks (174) showed that the epidermis in regenerating tissue from benzuron failed to secrete new cuticle. Post et al (155) found that benzuron prevented incorporation of glucosamine into chitin. Other workers have confirmed that benzuron prevents the transfer of monomer from UDPNAG to chitin synthetase (CS). (1, 127, 186) and have shown that acetylglucosamine (UDPNAG) accumulation interferes with the final biosynthesis of chitin.

The recent report of a cell-free system (38) has permitted the direct assessment of the mode of action of diflubenzuron. It has complicated cellular or hormonal regulation. diflubenzuron did not inhibit the synthesis of chitin even though this preparation was of fungal CS.

However, this observation does not rule out the mode of action of diflubenzuron, in vivo as an inhibitor of the synthesis of chitin. Similarly, Mulder & Mulder (190) showed that integument was affected by diflubenzuron. It was affected by diflubenzuron in the integumentary structures (gut and trachea) and has recently been shown to direct the synthesis of microsomes in vitro (91). A crucial

that dorsal, unpaired, median (DUM) cells and other aminergic neurons may serve a general function of both neuromuscular and central nervous modulation in insects. A more detailed knowledge of the structure and function of aminergic neurons in insects will undoubtedly provide clearer insight into the mode of action of formamidine insecticides.

INHIBITORS OF CHITIN

Insect cuticle is an obviously desirable target for potentially selective insecticides. Diflubenzuron and related benzoylphenyl ureas were found to be slow-acting stomach poisons which specifically affect insect cuticle (131). They exert their toxic actions on immature forms, particularly during ecdysis. Affected larvae appear normal until shortly after apolysis, when they often cannot free themselves from their old skins. Histological examination showed that the epidermis in recently apolysed insects treated with diflubenzuron failed to secrete new endocuticle.

Sowa & Marks (174) showed that diflubenzuron was a potent inhibitor of β -ecdysone-dependent *N*-acetylglucosamine (NAG) incorporation into chitin in regenerating tissue from cockroach legs after fourteen days in culture. Post et al (155) found that the diflubenzuron analog DU 19111 prevented incorporation of glucose into *Pieris* larval endocuticle in vivo. Other workers have confirmed this observation using other benzoylphenyl ureas (1, 127, 186) and have shown clearly that uridine diphospho-*N*-acetylglucosamine (UDPNAG) accumulates in treated larvae (46, 53, 74, 186). UDPNAG accumulation suggests that diflubenzuron analogs may interfere with the final biosynthetic step in the pathway to chitin, namely the transfer of monomer from UDPNAG to the growing chitin chain by chitin synthetase (CS).

The recent report of a cell-free extract of *Tribolium* gut with CS activity (38) has permitted the direct assessment of CS inhibition in the absence of complicating cellular or hormonal influences. Contrary to expectation, diflubenzuron did not inhibit the *Tribolium* enzyme, even at 300 μ M (39), even though this preparation was extremely sensitive to known inhibitors of fungal CS.

However, this observation does not rule out CS inhibition as the primary mode of action of diflubenzuron, since this compound was also ineffective in vivo as an inhibitor of the same gut CS preparation. Indeed, it was stimulatory. Similarly, Mulder & Gijswijt (131) stated that only the external integument was affected by diflubenzuron. The cuticular linings of invaginated structures (gut and tracheae) were normal. Also diflubenzuron has recently been shown to directly inhibit a purified CS from brine shrimp microsomes in vitro (91). A crucial test of the hypothesis will be whether

well-known example of this phenomenon is insensitive cholinesterase in strains resistant to organophosphate and carbamate insecticides. The genetic association between resistance and insensitive cholinesterase is perhaps the single most compelling piece of evidence that cholinesterase inhibition is causally related to toxicity for this group of insecticides (154).

Pyrethroids and DDT Analogs

The existence of target insensitivity in strains resistant to DDT and pyrethroids has important implications for mode of action studies, since it allows the possibility of precisely identifying the modified nerve component, and thus the site of insecticide action.

The best known case of specific resistance to pyrethroids and DDT analogs involving target insensitivity is knockdown resistance (*kdr*) in house flies (154, 180). Osborne & Hart (149) reported that sensory nerve in a *kdr* strain of house fly was 1000 times less sensitive to permethrin than sensory nerve from a susceptible strain. However, any differences in the CNS were not investigated. Studies on the intact, integrated nervous system in another *kdr* strain of house fly have shown that nerve insensitivity was distributed throughout the nervous system, involving sensory, motor, and central neural elements (125). Omer et al (146) reported that neuromuscular preparations from a strain of mosquito possessing *kdr*-like target insensitivity were 20-fold resistant to repetitive firing induced by (1R)-*cis*-permethrin.

It has recently been reported that DDT + pyrethroid resistance in *kdr* and super-*kdr* strains of house flies does not extend to carbinol analogs of DDT, such as the acaricide dicofol (162). This was taken as evidence that such compounds may have a different mechanism of toxicity from DDT itself. The carbinols did not show a negative temperature correlation with toxicity, and they also produced different symptoms in house flies from those produced by DDT and its noncarbinol analogs.

Another case of target insensitivity involves a DDT-resistant strain of the German cockroach, described by Matsumura (113). The resistance factor was shown to be genetically linked to low Ca^{2+} -ATPase activity in the whole head. The Ca^{2+} -ATPase in the resistant strain showed a reduced sensitivity to DDT, in addition to reduced total activity (68). Although the enzyme had similar characteristics to the "ecto" Ca^{2+} -ATPase of lobster axon (69, 70), its origin is uncertain, since no attempt was made to separate brain from other tissue.

In a similar study, Patil et al (151) found that a DDT-resistant strain of house fly carrying the *kdr* gene had reduced titers of $\text{Na}^{+} + \text{K}^{+}$ -ATPase in the whole head, compared to a susceptible (S) strain. The $\text{Na}^{+} + \text{K}^{+}$ -ATPase in the *kdr* strain was 20-fold less sensitive to DDT than that in the

e known to be
insects (39).
vidence that the
on, since NAG
t not in larvae
t itself is not a
difficult to re-
G accumulates
is anomaly by
d normally in
as specifically
ed from UDP-
, van Eck (53)
walls of house

ed chitinase in
ve explanation
es and for the
into polymer.
s not begin to
lifubenzuron,
more rapidly.
s 15 min after
t the primary
enzoylphenyl

ods. It inhib-
n of glucose-
ts of connec-

has two dis-
synthesis. As
vivo by the
the develop-
hen adminis-
differentiated

ion has been
sitivity." A

S strain. Unfortunately, the resistant strain used had not been genetically purified by the usual technique of multiple backcross and selection. Thus, the "altered ATPase" in this strain might be unrelated to DDT resistance. The same two strains also differed significantly in the activity of two dehydrogenases.

A case of resistance to permethrin, apparently involving nerve insensitivity, was recently reported by Gammon (65) and Gammon & Holden (66) in a strain of the Egyptian cotton leafworm. However, the resistant strain was also highly resistant to organophosphate insecticides (85). Thus, the insensitive nerve factor may be a nonspecific penetration barrier, rather than a modified, pyrethroid-specific target on the nerve membrane. To resolve this uncertainty, the question of genetic linkage between organophosphate and pyrethroid resistance in this strain must be answered. Interestingly, the resistant strain showed little or no cross-resistance to cypermethrin or decamethrin, two pyrethroids which bear an α -cyano substituent. The authors suggested that α -cyano pyrethroids might have a different mode of action on insect nerve from that of permethrin. This opinion was supported by the observation that permethrin, but not cypermethrin, caused stimulus hypersensitivity in the abdominal nerve cord of *Spodoptera*.

Clements & May (35) reported a very similar phenomenon in peripheral nerves of locust: α -cyano pyrethroid esters never caused repetitive after-discharges in motor axons in response to a single electrical stimulus, whereas most pyrethroids not containing an α -cyano substituent were potent inducers of repetitive after-discharges in these axons. Conversely, α -cyano pyrethroids had potent stimulatory actions on sensory neurons, whereas most other pyrethroids did not. However permethrin itself (both *cis*- and *trans*-isomers), along with the natural pyrethrins, was a conspicuous exception to these generalizations, since it had typical α -cyano-type activity.

Cyclodienes and γ -BHC

A type of cyclodiene resistance which may involve target insensitivity is well-known, particularly in the Diptera. Busvine (31) has noted that cyclodiene-resistant insects are always cross-resistant to γ -BHC but not always to other classes of insecticide. Resistant strains do not show reduced penetration, or increased detoxification or excretion. Oppenoorth & Nasrat (147) showed that a single genetic factor conferred resistance to both dieldrin and γ -BHC in the house fly. Target site resistance has not been applied to studies of cyclodiene and γ -BHC mode of action.

In order to correctly utilize target site resistance as a rigorous test for theories of insecticide mode of action, it is necessary to carefully purify the resistance factor to eliminate nonspecific genetic differences between the

strains and thus to quell any doubt as to whether the altered target and the resistance. This factor has seldom been taken in published reports as a target for a chlorinated hydrocarbon or pyrethroid.

OPTICAL ISOMERISM

Optical isomerism provides another factor which may influence insecticide mode of action. Similar to many chemical and physical processes, insecticide uptake, storage, and translocation may be bypassed. Metabolism would have to be frequently differentiated between enantiomers.

The use of chirality as a tool is well suited to the pyrethroids, since their activity is greatly influenced by insecticide activity (54). It is reported a difference in toxicity of *o*- and *p*- (1S) enantiomer, although there was no difference in metabolism. This suggests that with the *trans*-configuration about the ester group, an equally impressive dependence on configuration on toxicity. For example, Ackermann (55) reported that *trans*-cypermethrin is 1400-fold more toxic to *Heliothis* larvae of \sim 1400-fold more toxic than the *cis*-isomer.

Miyazaki et al (128-130) have demonstrated that the *trans*-isomer influences the toxicities of several insecticides. A greater difference in insecticide activity between the *cis*- and *trans*-enantiomers of *cis*-chlordane and (+) enantiomers of *cis*-chlordane, chlordene, chlordene epoxide, and permethrin remains to be seen whether this optical activity is observed in *in vitro* studies on the presumed biological target.

PROSPECTS FOR FUTURE RESEARCH

Inhibitors of Tanning

For insects, cuticular chitin is essential for survival. It is not tanned, in addition to hardening, by the deposition of epicuticular lipids, which in turn are waterproofed (10).

Normal tanning requires the presence of tyrosine, although the precise nature of the reaction is not known.

strains and thus to quell any doubt about the genetic linkage between the altered target and the resistance. Unfortunately, these precautions have seldom been taken in published reports concerning target site resistance to chlorinated hydrocarbon or pyrethroid insecticides.

OPTICAL ISOMERISM

Optical isomerism provides another potentially useful tool for investigations into insecticide mode of action. Since enantiomers are identical with respect to many chemical and physical properties which may influence insecticide uptake, storage, and translocation, these variables could be successfully bypassed. Metabolism would have to be carefully considered, since enzymes frequently differentiate between enantiomers.

The use of chirality as a tool in mode of action studies is particularly suited to the pyrethroids, since their optical isomers are well-known to differ greatly in insecticidal activity (54, 55). For instance, Soderlund (171) reported a difference in toxicity of over 200-fold between NRDC 157 and its (1S) enantiomer, although there were only slight differences in penetration and metabolism. This suggests that the target site itself is chiral. Pyrethroids with the *trans*-configuration about the dihalovinyl cyclopropane ring show an equally impressive dependence on absolute configuration for maximum toxicity. For example, Ackermann et al (2) measured a difference in toxicity to *Heliothis* larvae of ~ 1400-fold between the (1R)-(aS) and (1S)-(aR) enantiomers of *trans*-cypermethrin (Figure 2).

Miyazaki et al (128-130) have determined that absolute stereochemistry influences the toxicities of several chiral cyclodiene insecticides. A twofold or greater difference in insecticidal activity was observed between the (-) and (+) enantiomers of *cis*-chlordan, *trans*-chlordan, dichlorochlordene, chlordan, chlordan epoxide, and heptachlor epoxide (Figure 2). It remains to be seen whether this optical specificity will be observed during *in vitro* studies on the presumed biochemical targets.

PROSPECTS FOR FUTURE INSECTICIDES

Inhibitors of Tanning

For insects, cuticular chitin is essential to life. But insects will die just as surely if the new cuticle is not tanned (sclerotized). A vital function of tanning, in addition to hardening, is to facilitate the proper orientation of epicuticular lipids, which in turn assures that the cuticle is adequately waterproofed (10).

Normal tanning requires the biosynthesis of *N*-acetyldopamine from tyrosine, although the precise nature of the interaction between the un-

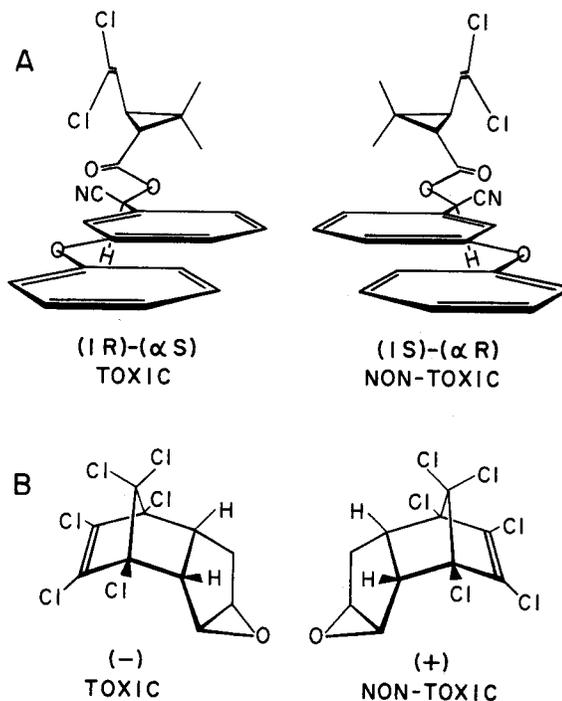


Figure 2 Toxic and non-toxic enantiomers of *trans*-cypermethrin (A) and chlordane epoxide (B).

tanned cuticle and quinones derived from *N*-acetyldopamine is still a subject of debate (187). Thus, tyrosine hydroxylase, DOPA decarboxylase, dopamine *N*-acetyl transferase, and phenol oxidase constitute potential targets of insecticide action (28).

In some insects DOPA decarboxylase is a key enzyme in the biosynthetic pathway from tyrosine to tanning quinones, and its activity in hemolymph and integument rises dramatically during the molting cycle (90). Turnbull & co-workers (181, 182) recently reported that inhibitors of DOPA decarboxylase are toxic to blowfly larvae, apparently by a specific interference with sclerotization. This conclusion was based on the observation that larvae exposed to toxic doses of α -methylDOPA or other DOPA decarboxylase inhibitors appear normal until the time of molt, but desiccate and die without tanning, immediately after ecdysis. The epicuticle in affected larvae was abnormal in its ultrastructure and was defective in its role as a water permeability barrier. Also, the toxicity of α -methylDOPA was completely antagonized by the sclerotizing agent *N*-acetyldopamine.

Kollonitsch et al (105) reported a very potent, specific, and irreversible inhibitor of DOPA decarboxylase. Jung et al (99) found that this inhibitor produced long-lasting depletion of DOPA in mammalian tissues. Other less potent inhibitors have not yet been tested. It is not clear whether this inhibitor has significant effects in vivo in the pathway of biogenic amine synthesis. The use of α -methylDOPA has not yet been tested.

None of the DOPA decarboxylase inhibitors are cheap or effective enough to have been used in the field. Nevertheless, specific disruption of sclerotization in the search for biorational insecticides is a promising area.

GABA Synapses

Among the proven or suspected targets of insecticides are at least two, the cholinergic and GABAergic synapses. γ -aminobutyric acid, respectively, is a major inhibitory neurotransmitter. With regard to the GABA synapse, attention has been focused on the possibility of exploiting this synapse as a potential target of insecticide action.

Picrotoxinin is well-known as a GABA antagonist. It blocks GABA transmission (97). This blockade is thought to occur at the site on the postsynaptic membrane where GABA binds to its receptor (179).

Miller et al (126) found that picrotoxinin is highly toxic to house flies by topical application when applied directly to the integument. Picrotoxinin became extremely potent when it was combined with a penetration enhancer. (145) found that another GABA antagonist, bicuculline, is also toxic to house flies but not excitatory neuromuscularly. GABA antagonism is a promising target for insecticide development. Structural modifications must be made to overcome penetration barriers with these compounds.

Miller et al (126) and Kuwana (145) have been successful in attempts to discover new GABA antagonists which would retain activity in the presence of lactones with weak toxic and cholinergic activity.

Bellet & Casida (15) and Casida (16) have reported on organophosphates which were effective against house flies but did not poison by a cholinergic mechanism.

Kollonitsch et al (105) reported that (S)- α -fluoromethyl-dopa is an extremely potent, specific, and irreversible inhibitor of mammalian DOPA decarboxylase. Jung et al (99) found that low doses of this inhibitor in vivo produced long-lasting depletion of catecholamines and serotonin in various mammalian tissues. Other less potent inhibitors of DOPA decarboxylase do not have significant effects in vivo, because this enzyme is not rate limiting in the pathway of biogenic amine biosynthesis in mammals. α -Fluoromethyl-dopa has not yet been tested in insects.

None of the DOPA decarboxylase-inhibitors tested in insects to date are cheap or effective enough to have economic potential as insecticides. Nonetheless, specific disruption of sclerotization remains an appealing strategy in the search for biorational insecticides.

GABA Synapses

Among the proven or suspected types of mammalian chemical synapses, at least two, the cholinergic and GABAergic types (utilizing acetylcholine and γ -aminobutyric acid, respectively), are known to be extremely prone to lethal disruption. With regard to insects, the cholinergic system has been exploited by insecticide chemists with phenomenal success. Within the past several years, attention has begun to focus on GABAergic synapses in insects as a potential target of insecticides.

Picrotoxinin is well-known as a potent convulsant and toxicant in mammals by virtue of its specific blocking action on GABAergic (inhibitory) transmission (97). This blockade is not competitive, but rather involves a site on the postsynaptic membrane which is distinct from the GABA receptor (179).

Miller et al (126) found that synergized picrotoxinin was only slightly toxic to house flies by topical application and was a rather weak convulsant when applied directly to the intact thoracic ganglion in situ. However, it became extremely potent when the ganglion was desheathed. Olsen et al (145) found that another GABA antagonist, bicuculline, suppressed inhibitory but not excitatory neuromuscular transmission in cockroaches. Apparently, GABA antagonism is a feasible strategy in insecticide design, but structural modifications must be introduced into active substances to overcome penetration barriers without loss of biological activity.

Miller et al (126) and Kuwano et al (108) prepared simplified analogs of picrotoxinin in attempts to discover smaller, more easily synthesized partial structures which would retain activity. Both groups found several bicyclic lactones with weak toxic and convulsant actions in insects.

Bellet & Casida (15) and Casida et al (32) described a class of bicyclic organophosphates which were extremely potent convulsants in mammals but did not poison by a cholinergic mechanism. These substances and

ordene epox-

still a sub-
rboxylase,
potential

osynthetic
molymp
Turnbull
PA decar-
terference
ation that
ecarboxy-
e and die
ted larvae
s a water
mpletely

several related *ortho*-carboxylates and silatranes were subsequently shown to be GABA antagonists, which bound selectively to the picrotoxinin rather than the GABA receptor site (22, 179). Certain analogs were moderately toxic to insects, but because of their extreme potency against mammals, they must be considered to be selective mammalicides (121).

Benzodiazepines (anti-anxiety agents) are another class of neuroactive substances which exert specific actions at GABAergic synapses. They facilitate GABAergic transmission and protect against the convulsant effects of the GABA antagonists picrotoxinin and bicuculline in mammals (97). A few convulsant benzodiazepines seem to have the opposite action, namely a picrotoxinin-like GABA blockade (143). Clifford and his collaborators (36, 37) described a series of benzodiazepine analogs and their carbamate derivatives which showed significant insecticidal and acaricidal activity, but their mode of toxic action is unknown.

In recent years a search for an endogenous benzodiazepine ligand in mammals (i.e. a naturally occurring anti-anxiety substance whose actions are supposedly mimicked by the synthetic benzodiazepines) has been mounted by several research groups. This had led to the discovery of the possible purinergic nature of the benzodiazepine receptor (42, 178). Inhibitory purinergic neurons have been known in mammals for some time (29), but a link between GABAergic and purinergic synapses was not previously suspected. Interestingly, Bellet & Casida (15) had speculated on a possible relationship between the bicyclic phosphate or "cage" convulsants and a purine nucleotide (cyclic AMP) based on structural similarities, even before any link was suspected between the cage convulsants and GABA or between GABAergic and purinergic neurons.

Purine analogs which affect purine titers in flukes can be numbered among the economically important anthelmintics. For example, Coles (40) has suggested that the anthelmintic mebendazole may act by a purinergic mechanism. However, there is no evidence for the involvement of GABA in this instance nor is there a similar case known among the insecticides. Thus, a GABAergic insecticide has not yet been discovered.

The possibility that GABAergic inhibitors (or indeed any candidate insecticide) could effect crop protection by sublethal actions on pest species should be considered in future evaluations. This concept has already been alluded to with respect to the formamidines, which can provide a significant level of crop protection at sublethal doses. Other types of insecticides may also have important effects in the field at sublethal application rates, as recently discussed by Hollingworth & Murdock (88). If such a concept is to be implemented, new types of chemical screening procedures must be developed to detect sublethal effects, and thus reveal candidate pestitatic substances.

SUMMARY

1. Excitatory pyrethroids and DDT on the nerve axon and thereby
2. The sensitivity of the sodium gradient to DDT depends upon the extent of
3. Calcium-dependent ATPases related to maintenance of the membrane components, the resistance gating, axonal transport,
4. Certain calcium-dependent ATPases. Such an effect in vivo might or to a decalcification of the membrane might disrupt the normal requirement for monovalent cations.
5. Some observers have found that the sensitive to DDT than the CNS has been claimed. Pyrethroids excite cell bodies and motor nerve terminals, uncouple the house fly flight muscles,
6. Cyclodienes and γ -BHC act upon the CNS, by stimulating excessive stores. This effect is mediated at the level of intracellular free calcium in the CNS.
7. There is some evidence that diethyl DDT expression of toxicity, but the active principle is positively identified.
8. Formamidines are both acutely and chronically toxic. A primary effect may be localized utilization of octopamine.
9. Diflubenzuron and related benzodiazepines cuticle by preventing the incorporation of chitin into the chitin polymer and by epidermal cells.
10. Target insensitivity is a type of insensitivity in mode of action studies. The chemical mechanisms of target insensitivity of analogs in strains of house fly and Egyptian cotton leafworm. γ -BHC has also been reported

SUMMARY

1. Excitatory pyrethroids and DDT analogs retard closure of sodium gates on the nerve axon and thereby oppose membrane repolarization.
2. The sensitivity of the sodium gate both to the membrane potential and to DDT depends upon the extracellular concentration of calcium.
3. Calcium-dependent ATPases detected in nerve homogenates may be related to maintenance of the calcium gradient, calcium binding to membrane components, the regulation of sodium and potassium conductance gating, axonal transport, and transmitter release.
4. Certain calcium-dependent ATPases are inhibited by DDT and pyrethroids. Such an effect in vivo might lead to calcium pump dysfunction or to a decalcification of the external surface of the axon, which in turn might disrupt the normal regulation of the conductance gating of monovalent cations.
5. Some observers have found that peripheral nerve is considerably more sensitive to DDT than the CNS, but significant central effects have also been claimed. Pyrethroids excite peripheral nerves, particularly sensory cell bodies and motor nerve terminals. Pyrethroids, but not DDT analogs, uncouple the house fly flight motor by a central action.
6. Cyclodienes and γ -BHC act primarily at cholinergic synapses in the CNS, by stimulating excessive release of transmitter from presynaptic stores. This effect is mediated at least in part by increased concentrations of intracellular free calcium in the presynaptic terminal.
7. There is some evidence that dieldrin requires metabolic activation for the expression of toxicity, but the active metabolite, if it exists, has not been positively identified.
8. Formamidines are both acutely toxic and antifeedant, but these actions may be two manifestations of a single, primary effect on the CNS. This primary effect may be localized at aminergic synapses, particularly those utilizing octopamine.
9. Diflubenzuron and related benzoylphenyl ureas act specifically on insect cuticle by preventing the incorporation of *N*-acetylglucosamine units into the chitin polymer and by a cytostatic action on chitin-producing epidermal cells.
10. Target insensitivity is a type of resistance which can be a valuable tool in mode of action studies. There has been recent interest in the biochemical mechanisms of target insensitivity to pyrethroids and DDT analogs in strains of house flies, mosquitoes, cockroaches, and the Egyptian cotton leafworm. Target insensitivity to cyclodienes and γ -BHC has also been reported.

11. Chirality is another potentially useful tool in mode of action research. There is evidence that the biochemical targets for both the DDT-pyrethroid group and the cyclodiene- γ -BHC group may be chiral.
12. Many vital processes remain to be exploited in the design of new types of insecticides. Two of these, sclerotization and GABA synapses, are discussed.

ACKNOWLEDGMENTS

I thank L. W. Klaassen, A. E. Kammer, F. Matsumura, J. M. Clark, R. M. Hollingworth, A. E. Lund, and G. R. Needham for supplying preprints or for permission to cite unpublished results. I also thank A. Oppenlander for typing this manuscript.

Literature Cited

1. Abdel-Monem, A. H., Cameron, E. A., Mumma, R. O. 1980. Toxicological studies on the molt-inhibiting insecticide (EL-494) against the gypsy moth and effect on chitin biosynthesis. *J. Econ. Entomol.* 73:22-25
2. Ackermann, P., Bourgeois, G., Drabek, J. 1980. The optical isomers of α -cyano-3-phenoxybenzyl 3-(1,2-dibromo-2,2-dichloroethyl)-2,2-dimethylcyclopropanecarboxylate and their insecticidal activities. *Pestic. Sci.* 11:169-79
3. Adams, M. E., Miller, T. A. 1979. Site of action of pyrethroids: repetitive "backfiring" in flight motor units of house fly. *Pestic. Biochem. Physiol.* 11:218-31
4. Adams, M. E., Miller, T. A. 1980. Neural and behavioral correlates of pyrethroid and DDT-type poisoning in the house fly, *Musca domestica* L. *Pestic. Biochem. Physiol.* 13:137-47
5. Akkermans, L. M. A., van den Bercken, J., van der Zalm, J. M., van Straaten, H. W. M. 1974. Effects of dieldrin (HEOD) and some of its metabolites on synaptic transmission in the frog motor end-plate. *Pestic. Biochem. Physiol.* 4:313-24
6. Atkinson, P. W., Binnington, K. C., Roulston, W. J. 1974. High monoamine oxidase activity in the tick *Boophilus microplus*, and inhibition by chlordimeform and related pesticides. *J. Aust. Entomol. Soc.* 13:207-10
7. Baker, P. F. 1976. Regulation of intracellular Ca and Mg in squid axons. *Fed. Proc.* 35:2589-95
8. Baker, P. F., Hodgkin, A. L., Ridgway, E. B. 1971. Depolarization and calcium entry in squid giant axons. *J. Physiol.* 218:709-55
9. Baker, P. F., Meves, H., Ridgway, E. B. 1973. Effects of manganese and other agents on the calcium uptake that follows depolarization of squid axons. *J. Physiol.* 231:511-26
10. Beament, J. W. L. 1976. The ecology of cuticle. In *The Insect Integument*, ed. H. R. Hepburn, pp. 359-74. Amsterdam: Elsevier
11. Beeman, R. W., Matsumura, F. 1974. Studies on the action of chlordimeform in cockroaches. *Pestic. Biochem. Physiol.* 4:325-36
12. Beeman, R. W., Matsumura, F. 1978. Formamidin pesticides—actions in insects and acarines. In *Pesticide and Venom Neurotoxicity*, ed. D. L. Shankland, R. M. Hollingworth, T. Smyth, Jr., pp. 179-88. New York: Plenum
13. Beeman, R. W., Matsumura, F. 1978. Anorectic effect of chlordimeform in the American cockroach. *J. Econ. Entomol.* 71:859-61
14. Beeman, R. W., Matsumura, F., Kikukawa, T. 1979. Monoamine oxidase in the cheese mite, *Tyrophagus putrescentiae*. *Comp. Biochem. Physiol. C* 64:149-52
15. Bellet, E. M., Casida, J. E. 1973. Bicyclic phosphorus esters: high toxicity without cholinesterase inhibition. *Science* 182:1135-36
16. Bentley, J. P., Weber, G. H., Gould, D. 1979. The effect of diflubenzuron feeding on glycosaminoglycan and sulfhemoglobin biosynthesis in mice. *Pestic. Biochem. Physiol.* 10:162-67
17. Bercken, J. van den, Kroese, A. B. A., Akkermans, L. M. A. 1979. Effects of insecticides on the sensory nervous system. See Ref. 137, pp. 183-210
18. Blaustein, M. P. 1976. The ins and outs of calcium transport in squid axons: internal and external ion activation of calcium efflux. *Fed. Proc.* 35:2574-78
19. Boadle, M. C., Blaschko, H. 1968. Cockroach amine oxidase: classification and substrate specificity. *Comp. Biochem. Physiol.* 25:129-38
20. Bodnaryk, R. P. 1979. Identification of specific dopamine- and octopamine-sensitive adenylate cyclases in the brain of *Mamestra configurata* Wlk. *Insect Biochem.* 9:155-62
21. Bowers, W. S., Ohta, T., Cleere, J. S., Marsella, P. A. 1976. Discovery of insect anti-juvenile hormones in plants. *Science* 193:542-47
22. Bowery, N. G., Collins, J. F., Hill, R. G. 1976. Bicyclic phosphorus esters that are potent convulsants and GABA antagonists. *Nature* 261:601-3
23. Brinley, F. J. Jr. 1980. Regulation of intracellular calcium in squid axon. *Fed. Proc.* 39:2778-82
24. Brooks, G. T. 1974. *Chlorinated Insecticides, Volume II, Biological and Chemical Aspects*. Cleveland: CRC Press, 197 pp.
25. Brooks, G. T. 1980. Biochemical target and insecticide action. In *Insect Neurobiology and Pesticide Action, Proc. Soc. Chem. Ind. Symp.*, pp. 41-55. London: Soc. Chem. Ind.
26. Brooks, G. T., Harrison, A., Lewis, S. E. 1970. Cyclodiene epoxide ring hydration by microsomes from mammalian liver and houseflies. *Biochem. Pharmacol.* 19:255-65
27. Brooks, G. T., Pratt, G. E., Jennings, F. C. 1979. The action of precocenes in milkweed bugs (*Oncopeltus fasciatus*) and locusts (*Locusta migratoria*). *Nature* 281:570-72
28. Brunet, P. C. J. 1980. The metabolism of the aromatic amino acids concerns in the cross-linking of insect cuticle. *Insect Biochem.* 10:467-500
29. Burnstock, G. 1972. Purinergic nerve. *Pharmacol. Rev.* 24:509-81
30. Burt, P. E., Goodchild, R. E. 1974. Knockdown by pyrethroids: its role in the intoxication process. *Pestic. Sci.* 5:625-33
31. Busvine, J. R. 1964. The insecticidal potency of γ -BHC and the chlorinated cyclodiene compounds and the significance of resistance to them. *Bull. Entomol. Res.* 55:271-88
32. Casida, J. E., Eto, M., Mosconi, A. D., Engel, J. L., Milbrath, D. S., Verkade

action research.
both the DDT
may be chiral.
ign of new types
A synapses, are

f. M. Clark, R.
flying preprints
A. Oppenlander

axons. *J. Physiol.*

H., Ridgway, E. B.
ganese and other
n uptake that fol-
of squid axons. *J.*

76. The ecology of
t Integument, ed.
359-74. Amster-

sumura, F. 1974.
of chlordimeform
Pestic. Biochem.

sumura, F. 1978.
es—actions in in-
in *Pesticide and*
ed. D. L. Shank-
orth, T. Smyth,
York: Plenum
umura, F. 1978.
umura, F. 1978.
chlordimeform in
ch. *J. Econ. En-*

Matsumura, F.,
Monoamine oxi-
Tyrophagus pu-
chem. Physiol. C

. E. 1973. Bicy-
: high toxicity
se inhibition.

f. H., Gould, D.
benzuron feed-
can and sulf-
in mice. *Pestic.*
2-67

oese, A. B. A.,
1979. Effects of

- insecticides on the sensory nervous system. See Ref. 137, pp. 183-210
18. Blaustein, M. P. 1976. The ins and outs of calcium transport in squid axons: internal and external ion activation of calcium efflux. *Fed. Proc.* 35:2574-78
 19. Boadle, M. C., Blaschko, H. 1968. Cockroach amine oxidase: classification and substrate specificity. *Comp. Biochem. Physiol.* 25:129-38
 20. Bodnaryk, R. P. 1979. Identification of specific dopamine- and octopamine-sensitive adenylate cyclases in the brain of *Mamestra configurata* Wlk. *Insect Biochem.* 9:155-62
 21. Bowers, W. S., Ohta, T., Cleere, J. S., Marsella, P. A. 1976. Discovery of insect anti-juvenile hormones in plants. *Science* 193:542-47
 22. Bowery, N. G., Collins, J. F., Hill, R. G. 1976. Bicyclic phosphorus esters that are potent convulsants and GABA antagonists. *Nature* 261:601-3
 23. Brinley, F. J. Jr. 1980. Regulation of intracellular calcium in squid axons. *Fed. Proc.* 39:2778-82
 24. Brooks, G. T. 1974. *Chlorinated Insecticides, Volume II, Biological and Chemical Aspects*. Cleveland: CRC Press. 197 pp.
 25. Brooks, G. T. 1980. Biochemical targets and insecticide action. In *Insect Neurobiology and Pesticide Action, Proc. Soc. Chem. Ind. Symp.*, pp. 41-55. London: Soc. Chem. Ind.
 26. Brooks, G. T., Harrison, A., Lewis, S. E. 1970. Cyclodiene epoxide ring hydration by microsomes from mammalian liver and houseflies. *Biochem. Pharmacol.* 19:255-65
 27. Brooks, G. T., Pratt, G. E., Jennings, R. C. 1979. The action of precocenes in milkweed bugs (*Oncopeltus fasciatus*) and locusts (*Locusta migratoria*). *Nature* 281:570-72
 28. Brunet, P. C. J. 1980. The metabolism of the aromatic amino acids concerned in the cross-linking of insect cuticle. *Insect Biochem.* 10:467-500
 29. Burnstock, G. 1972. Purinergic nerves. *Pharmacol. Rev.* 24:509-81
 30. Burt, P. E., Goodchild, R. E. 1974. Knockdown by pyrethroids: its role in the intoxication process. *Pestic. Sci.* 5:625-33
 31. Busvine, J. R. 1964. The insecticidal potency of γ -BHC and the chlorinated cyclodiene compounds and the significance of resistance to them. *Bull. Entomol. Res.* 55:271-88
 32. Casida, J. E., Eto, M., Moscioni, A. D., Engel, J. L., Milbrath, D. S., Verkade, J. G. 1976. Structure-toxicity relationships of 2,6,7-trioxabicyclo[2.2.2]-octanes and related compounds. *Toxicol. Appl. Pharmacol.* 36:261-79
 33. Cheng, E. Y., Cutkomp, L. K. 1977. The sensitivity of mitochondrial Mg^{2+} -ATPase to DDT and analogs at different temperatures. *Pestic. Biochem. Physiol.* 7:360-66
 34. Cheung, W. Y. 1979. Calmodulin plays a pivotal role in cellular regulation. *Science* 207:19-27
 35. Clements, A. N., May, T. E. 1977. The actions of pyrethroids upon the peripheral nervous system and associated organs in the locust. *Pestic. Sci.* 8:661-80
 36. Clifford, D. P., Jackson, D., Edwards, R. V., Jeffrey, P. 1976. Herbicidal and pesticidal properties of some 1,5-benzodiazepines, 1,3,5-benzotriazepines, and 3,1,5-benzothiadiazepines. *Pestic. Sci.* 7:453-58
 37. Clifford, D. P., Jeffrey, P. 1977. The insecticidal and acaricidal properties of some 3-alkylcarbamoyloximino-2,4-dimethyl-1,5-benzodiazepines. *Pestic. Sci.* 8:446-48
 38. Cohen, E., Casida, J. E. 1980. Properties of *Triboleum* gut chitin synthetase. *Pestic. Biochem. Physiol.* 13:121-28
 39. Cohen, E., Casida, J. E. 1980. Inhibition of *Triboleum* gut chitin synthetase. *Pestic. Biochem. Physiol.* 13:129-36
 40. Coles, G. C. 1977. The biochemical mode of action of action of some modern anthelmintics. *Pestic. Sci.* 8:536-43
 41. Collins, C., Miller, T. 1977. Studies on the action of biogenic amines on cockroach heart. *J. Exp. Biol.* 67:1-15
 42. Crawley, J. N., Marangos, P. J., Paul, S. M., Skolnick, P., Goodwin, F. K. 1981. Interaction between purine and benzodiazepine: inosine reverses diazepam-induced stimulation of mouse exploratory behavior. *Science* 211:725-27
 43. DeLorenzo, R. J., Freedman, S. D., Yohe, W. B., Maurer, S. C. 1979. Stimulation of Ca^{++} -dependent neurotransmitter release and presynaptic nerve terminal protein phosphorylation by calmodulin and a calmodulin-like protein isolated from synaptic vesicles. *Proc. Natl. Acad. Sci.* 76:1838-42
 44. DeMilo, A. B., Redfern, R. E. 1979. New insect juvenile hormone mimics: aromatic Schiff bases and related compounds against the large milkweed bug and yellow mealworm. *J. Agric. Food Chem.* 27:760-62
 45. Desai, D., Cutkomp, L. K., Koch, R. B. 1974. A comparison of DDT and its biodegradable analogues tested on AT-

- Pase enzymes in cockroaches. *Pestic. Biochem. Physiol.* 4:232-38
46. Deul, D. H., de Jong, B. J., Kortenbach, J. A. M. 1978. Inhibition of chitin synthesis by two 1-(2,6-disubstituted benzoyl)-3-phenylurea insecticides. II. *Pestic. Biochem. Physiol.* 8:98-105
 47. DiPolo, R. 1978. Ca pump driven by ATP in squid axons. *Nature* 274:390-92
 48. DiPolo, R., Beaugé, L. 1979. Physiological role of ATP-driven calcium pump in squid axon. *Nature* 278:271-73
 49. Doane, C. C., Dunbar, D. M. 1973. Field evaluation of insecticides against the gypsy moth and the elm spanworm and repellent action of chlordimeform. *J. Econ. Entomol.* 66:1187-89
 50. Doherty, J. D., Matsumura, F. 1975. DDT effects on certain ATP related systems in the peripheral nervous system of the lobster *Homarus americanus*. *Pestic. Biochem. Physiol.* 5:242-52
 51. Downer, R. G. H. 1980. Short-term hypertrehalosemia induced by octopamine in the American cockroach, *Periplaneta americana* L. See Ref. 25, pp. 335-39
 52. Drescher, W. 1960. Regenerationsversuche am Gehirn von *Periplaneta americana* unter Berücksichtigung von Verhaltensänderung und Neurosekretion. *Z. Morphol. Oekol. Tiere* 48:576-649
 53. Eck, W. H. van. 1979. Mode of action of two benzoylphenyl ureas as inhibitors of chitin synthesis in insects. *Insect Biochem.* 9:295-300
 54. Elliott, M., Farnham, A. W., Janes, N. F., Needham, P. H., Pulman, D. A. 1974. Insecticidally active conformations of pyrethroids. In *Mechanism of Pesticide Action ACS Symp. Ser. 2*, ed. G. K. Kohn, pp. 80-91. Washington DC: Am. Chem. Soc.
 55. Elliott, M., Janes, N. F. 1978. Synthetic pyrethroids—a new class of insecticide. *Chem. Soc. Rev.* 7:473-505
 56. Evans, P. D. 1980. Biogenic amines in the insect nervous system. *Adv. Insect Physiol.* 15:317-473
 57. Evans, P. D. 1980. Action of formamidine pesticides on octopamine receptors. *Nature* 287:60-62
 58. Evans, P. D., O'Shea, M. 1977. The identification of an octopaminergic neurone which modulates neuromuscular transmission in the locust. *Nature* 270:257-59
 59. Farley, J. M., Narahashi, T., Holan, G. 1979. The mechanism of action of a DDT analog on the crayfish neuromuscular junction. *Neurotoxicology* 1:191-207
 60. Frankenhaeuser, B., Hodgkin, A. L. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol.* 137:218-44
 61. Frontali, N. 1968. Histochemical localization of catecholamines in the brain of normal and drug-treated cockroaches. *J. Insect Physiol.* 14:881-86
 62. Gammon, D. W. 1978. Neural effects of allethrin on the free walking cockroach, *Periplaneta americana*: an investigation using defined doses at 15 and 32°C. *Pestic. Sci.* 9:79-91
 63. Gammon, D. W. 1978. Effects of DDT on the cockroach nervous system at three temperatures. *Pestic. Sci.* 9:95-104
 64. Gammon, D. W. 1979. An analysis of the temperature-dependence of the toxicity of allethrin to the cockroach. See Ref. 137, pp. 97-117
 65. Gammon, D. W. 1980. Pyrethroid resistance in a strain of *Spodoptera littoralis* is correlated with decreased sensitivity of the CNS *in vitro*. *Pestic. Biochem. Physiol.* 13:53-62
 66. Gammon, D. W., Holden, J. S. 1980. A neural basis for pyrethroid resistance in larvae of *Spodoptera littoralis*. See Ref. 25, pp. 481-88
 67. Gemrich, E. G. II, Lee, B. L., Tripp, M. L., VandeStreek, E. 1976. Relationship between formamidine structure and insecticidal, miticidal, and ovicidal activity. *J. Econ. Entomol.* 69:301-6
 68. Ghiasuddin, S. M., Kadous, A. A., Matsumura, F. 1981. Reduced sensitivity of a Ca-ATPase in the DDT-resistant strains of the German cockroach. *Comp. Biochem. Physiol. C* 68:15-20
 69. Ghiasuddin, S. M., Matsumura, F. 1979. DDT inhibition of Ca-ATPase in the peripheral nerves of the American lobster. *Pestic. Biochem. Physiol.* 10:151-61
 70. Ghiasuddin, S. M., Matsumura, F. 1979. Ca²⁺ regulation by Ca-ATPase in relation to DDT's action on the lobster nerve. *Comp. Biochem. Physiol. C* 64:29-36
 71. Gianotti, O., Metcalf, R. L., March, R. B. 1956. The mode of action of aldrin and dieldrin in *Periplaneta americana* (L.). *Ann. Entomol. Soc. Am.* 49:588-92
 72. Gordon, H. T., Welsh, J. H. 1948. The role of ions in axon surface reactions to toxic organic compounds. *J. Cell. Comp. Physiol.* 31:395-419
 73. Grosscurt, A. C., van Hes, R., Wellinga, K. 1979. 1-Phenyl-carbamoyl-2-pyrazolines, a new class of insecticides. 3. Synthesis and insecticidal properties of 3,4-diphenyl-1-phenyl-carbamoyl-2-pyrazolines. *J. Agric. Food Chem.* 27:406-9
 74. Hajjar, N. P., Casida, J. E. 1979. Structure-activity relationships of benzoylphenyl ureas as toxicants and chitin synthesis inhibitors in *Oncopeltus fasciatus*. *Pestic. Biochem. Physiol.* 11:33-45
 75. Hammerschlag, R. 1980. The role of calcium in the initiation of fast axonal transport. *Fed. Proc.* 39:2809-14
 76. Hart, R. J., Potter, C., Wright, R. A., Lea, P. J. 1978. Relationship between the *in vivo* and *in vitro* activity of some naturally occurring glutamate analogues on the somatic neuromuscular junction of *Lucilia sericata*. *Physiol. Entomol.* 3:289-95
 77. Henkart, M. 1980. Identification and function of intracellular calcium stores in axons and cell bodies of neurons. *Fed. Proc.* 39:2783-89
 78. Hille, B. 1977. Local anaesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69:497-515
 79. Hirano, T., Kawasaki, H., Shinohara, H., Kitagaki, T., Wakamori, S. 1972. Studies on some biological activities of N-(2-methyl-4-chlorophenyl)-N',N'-dimethyl formamidine (Galecron) to the rice stem borer, *Chilo suppressalis* Walker. *Botyu Kagaku* 37:135-41
 80. Hirata, M., Sogawa, K. 1976. Antifeeding activity of chlordimeform for plant-sucking insects. *Appl. Entomol. Zool.* 11:94-99
 81. Hodgkin, A. L., Keynes, R. D. 1957. Movement of labelled calcium in squid giant axons. *J. Physiol.* 138:253-81
 82. Holan, G. 1971. Rational design of degradable insecticides. *Nature* 232:644-47
 83. Holan, G. 1975. Mode of action of DDT-new aryl-alicyclic and heterocyclic insecticides. In *Pesticides, 3rd Int. IUPAC Congr. Pestic. Chem.*, ed. F. Coulston, F. Korte, pp. 359-64. Stuttgart: G. Thieme
 84. Holan, G., O'Keefe, D. F., Rihs, K., Walser, R., Virgona, C. T. 1979. New insecticides. Combined DDT-isostere and pyrethroid structures. In *Advances in Pesticide Science*, Pt. 2, ed. H. Geissbuehler, pp. 201-5. Oxford: Pergamon
 85. Holden, J. S. 1979. Absorption and metabolism of permethrin and cypermethrin

- rotoxicology 1:191-
- , Hodgkin, A. L. Calcium on the elec- f squid axons. *J. istochemical localities in the brain of eated cockroaches. 1:881-86*
78. Neural effects of walking cockroach, *na*: an investigation it 15 and 32°C. *Pes-*
78. Effects of DDT nervous system at *Pestic. Sci.* 9:95-
79. An analysis of endence of the tox- the cockroach. See 7
80. Pyrethroid re- f *Spodoptera littor-* ith decreased sen- *in vitro. Pestic. Bio-* 1-62
- Holden, J. S. 1980. A throid resistance in *littoralis*. See Ref.
- ee, B. L., Tripp, M. 1976. Relationship e structure and in- and ovidical activ- *l.* 69:301-6
- Kadous, A. A., 31. Reduced sen- Pase in the DDT- the German cock- *chem. Physiol. C*
- , Matsumura, F. n of Ca-ATPase in s of the american *chem. Physiol.* 10:
- , Matsumura, F. 1 by Ca-ATPase in tion on the lobster *chem. Physiol. C*
- , R. L., March, R. of action of aldrin *laneta americana* *oc. Am.* 49:588-92
- h, J. H. 1948. The surface reactions to pounds. *J. Cell.* 35-419
73. Grosscurt, A. C., van Hes, R., Wel- linga, K. 1979. 1-Phenyl-carbamoyl-2- pyrazolines, a new class of insecticides. 3. Synthesis and insecticidal properties of 3,4-diphenyl-1-phenyl-carbamoyl-2- pyrazolines. *J. Agric. Food Chem.* 27:406-9
74. Hajjar, N. P., Casida, J. E. 1979. Struc- ture-activity relationships of benzoyl- phenyl ureas as toxicants and chitin synthesis inhibitors in *Oncopeltus fas- ciatus*. *Pestic. Biochem. Physiol.* 11: 33-45
75. Hammerschlag, R. 1980. The role of calcium in the initiation of fast axonal transport. *Fed. Proc.* 39:2809-14
76. Hart, R. J., Potter, C., Wright, R. A., Lea, P. J. 1978. Relationship between the *in vivo* and *in vitro* activity of some naturally occurring glutamate ana- logues on the somatic neuromuscular junction of *Lucilia sericata*. *Physiol. Entomol.* 3:289-95
77. Henkart, M. 1980. Identification and function of intracellular calcium stores in axons and cell bodies of neurons. *Fed. Proc.* 39:2783-89
78. Hille, B. 1977. Local anaesthetics: hy- drophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69:497-515
79. Hirano, T., Kawasaki, H., Shinohara, H., Kitagaki, T., Wakamori, S. 1972. Studies on some biological activities of N-(2-methyl-4-chlorophenyl)-N',N'-di- methyl formamide (Galecron) to the rice stem borer, *Chilo suppressalis* Walker. *Botyu Kagaku* 37:135-41
80. Hirata, M., Sogawa, K. 1976. Antifeed- ing activity of chlordimeform for plant- sucking insects. *Appl. Entomol. Zool.* 11:94-99
81. Hodgkin, A. L., Keynes, R. D. 1957. Movement of labelled calcium in squid giant axons. *J. Physiol.* 138:253-81
82. Holan, G. 1971. Rational design of de- gradable insecticides. *Nature* 232: 644-47
83. Holan, G. 1975. Mode of action of DDT-new aryl-alicyclic and heterocy- clic insecticides. In *Pesticides, 3rd Int. IUPAC Congr. Pestic. Chem.*, ed. F. Coulston, F. Korte, pp. 359-64. Stutt- gart: G. Thieme
84. Holan, G., O'Keefe, D. F., Rihs, K., Walser, R., Virgona, C. T. 1979. New insecticides. Combined DDT-isosteres and pyrethroid structures. In *Advances in Pesticide Science*, Pt. 2, ed. H. Geiss- buchler, pp. 201-5. Oxford: Pergamon
85. Holden, J. S. 1979. Absorption and me- tabolism of permethrin and cypermeth- rin in the cockroach and the cotton-leaf- worm larvae. *Pestic. Sci.* 10:295-307
86. Hollingworth, R. M. 1976. Chemistry, biological activity, and uses of formami- dine pesticides. *Environ. Health Per- spect.* 14:57-69
87. Hollingworth, R. M., Murdock, L. L. 1980. Formamidine pesticides: octopa- mine-like actions in a firefly. *Science* 208:74-76
88. Hollingworth, R. M., Murdock, L. L. 1981. Behavioral effects of formami- dines and related compounds in insects and acarines. In *Regulation of Insect Development and Behavior*, Pt. 2, ed. M. Kloza, pp. 1023-32. Wroclaw: Wro- claw Tech. Univ.
89. Holtzmann, S. G., Jewett, R. E. 1971. The role of brain norepinephrine in the anorexic effects of dextroamphetamine and monoamine oxidase inhibitors in the rat. *Psychopharmacologia* 22:151-61
90. Hopkins, T. L., Wirtz, R. A. 1976. DOPA and tyrosine decarboxylase activity in tissues of *Periplaneta americana* in relation to cuticle forma- tion and ecdysis. *J. Insect Physiol.* 22:1167-71
91. Horst, M. N. 1980. The biosynthesis of crustacean chitin by a microsomal en- zyme from larval brine shrimp. *Fed. Proc.* 39:1634
92. Hotson, J. R., Prince, D. A. 1980. A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. *J. Neurophysiol.* 43:409-19
93. Howse, P. E. 1975. Brain structure and behavior in insects. *Ann. Rev. Entomol.* 20:359-79
94. Iqbal, Z., Garg, B., Ochs, S. 1979. Cal- modulin activation of brain and nerve Ca⁺⁺ + Mg⁺⁺ ATPase. *Soc. Neurosci. Abstr.* 5:60
95. Iqbal, Z., Ochs, S. 1980. Calmodulin in mammalian nerve. *J. Neurobiol.* 11: 311-18
96. Ishaaya, I., Casida, J. E. 1974. Dietary TH 6040 alters composition and en- zyme activity of housefly larval cuticle. *Pestic. Biochem. Physiol.* 4:484-90
97. Johnston, G. A. R. 1978. Neurophar- macology of amino acid inhibitory transmitters. *Ann. Rev. Pharmacol. Toxicol.* 18:269-89
98. Jones, S. W., Sudershan, P., O'Brien, R. D. 1979. Interaction of insecticides with acetylcholine receptors. See Ref. 137, pp. 259-75
99. Jung, M. J., Palfreyman, M. G., Wag- ner, J., Bey, P., Ribereau-Gayon, G., Zraika, M., Koch-Weser, J. 1979. Inhi- bition of monoamine synthesis by irre-

- versible blockade of aromatic aminoacid decarboxylase with α -monofluoromethyl-dopa. *Life Sci.* 24:1037-42
100. Khan, M. A., Ochs, S. 1974. Magnesium or calcium activated ATPase in mammalian nerve. *Brain Res.* 81: 413-26
 101. Khodorov, B., Shishkova, L., Peganov, E., Revenko, S. 1976. Inhibition of sodium currents in frog Ranvier node treated with local anaesthetics. Role of slow sodium inactivation. *Biochim. Biophys. Acta* 433:409-35
 102. Kinnamon, S. C., Klaassen, L. W., Kammer, A. E. 1980. Habituation and effects of an octopamine agonist in the developing moth flight control system. *Soc. Neurosci. Abstr.* 6:627
 103. Klaassen, L. W., Kammer, A. E. 1980. Modulation of neuromuscular transmission by octopamine in developing and adult moths (*Manduca sexta*). *Soc. Neurosci. Abstr.* 6:627
 104. Knowles, C. O., Roulston, W. J. 1973. Toxicity to *Boophilus microplus* of formamidine acaricides and related compounds, and modification of toxicity by certain insecticide synergists. *J. Econ. Entomol.* 66:1245-51
 105. Kollonitsch, J., Patchett, A. A., Marburg, S., Maycock, A. L., Perkins, L. M., Doldouras, G. A., Duggan, D. E., Aster, S. D. 1978. Selective inhibitors of biosynthesis of aminergic neurotransmitters. *Nature* 274:906-8
 106. Kono, Y., Nagaarashi, D., Sakai, M. 1975. Effects of cartap, chlordimeform and diazinon on the probing frequency of the green rice leafhopper (Hemiptera: Deltocephalidae). *Appl. Entomol. Zool.* 10:58-60
 107. Krijgsman, B. J., Krijgsman-Berger, N. E. 1951. Physiological investigations into the heart function of arthropods. *Bull. Entomol. Res.* 42:143-54
 108. Kuwano, E., Ohshima, K., Eto, M. 1980. Synthesis and insecticidal activity of 8-isopropyl-6-oxabicyclo (3.2.1)octan-7-one, a partial skeleton of picrotoxinin, and related compounds. *Agric. Biol. Chem.* 44:383-86
 109. Lee, K. S., Shin, B. C. 1969. Studies on active transport of calcium in human red cells. *J. Gen. Physiol.* 54:713-23
 110. Lund, A. E., Hollingworth, R. M., Murdock, L. L. 1979. Formamidine pesticides: a novel mechanism of action in lepidopterous larvae. In *Advances in Pesticide Science*, p. 3, ed. H. Geissbuehler, pp. 465-69. Oxford: Pergamon
 111. Lund, A. E., Hollingworth, R. M., Shankland, D. 1979. Chlordimeform: plant protection by a sublethal, noncholinergic action on the central nervous system. *Pestic. Biochem. Physiol.* 11: 117-28
 112. Lund, A. E., Hollingworth, R. M., Yim, G. K. W. 1979. The comparative neurotoxicity of formamidine pesticides. See Ref. 137, pp. 119-37
 113. Matsumura, F. 1971. Studies on the biochemical mechanisms of resistance in strains of the German cockroach. *Proc. 2nd Int. Congr. Pestic. Chem.* 2:95-116
 114. Matsumura, F., Beeman, R. W. 1976. Biochemical and physiological effects of chlordimeform. *Environ. Health Perspect.* 14:71-82
 115. Matsumura, F., Narahashi, T. 1971. ATPase inhibition and electrophysiological change caused by DDT and related neuroactive agents in lobster nerve. *Biochem. Pharmacol.* 20:825-37
 116. Meech, R. W. 1974. Calcium influx induces a post-tetanic hyperpolarization in *Aplysia* neurones. *Comp. Biochem. Physiol. A* 48:387-95
 117. Meech, R. W., Standen, N. B. 1975. Potassium activation in *Helix aspersa* neurones under voltage clamp: a component mediated by calcium influx. *J. Physiol.* 249:211-39
 118. Medzhradsky, F., Cullen, E. I., Lin, H., Bole, G. G. 1980. Drug-sensitive external-ATPase in human leukocytes. *Biochem. Pharmacol.* 29:2285-90
 119. Meola, S. M., Mayer, R. T. 1980. Inhibition of cellular proliferation of imaginal epidermal cells by diflubenzuron in pupae of the stable fly. *Science* 207:985-87
 120. Meves, H., Vogel, W. 1973. Calcium inward currents in internally perfused giant axons. *J. Physiol.* 235:225-65
 121. Milbrath, D. S., Engel, J. L., Verkade, J. G., Casida, J. E. 1979. Structure-toxicity relationships of 1-substituted-4-alkyl-2,6,7-trioxabicyclo(1.2.2)octanes. *Toxicol. Appl. Pharmacol.* 47:287-93
 122. Miller, T. A. 1978. The insect neuromuscular system as a site of insecticide action. See Ref. 12, pp. 95-111
 123. Miller, T. A. 1979. Mode of action of insecticides: insights gained from neurophysiological preparations of intact or dissected insects. See Ref. 137, pp. 79-96
 124. Miller, T., Kennedy, J. M. 1972. Flight motor activity of house flies as affected by temperature and insecticides. *Pestic. Biochem. Physiol.* 2:206-22
 125. Miller, T. A., Kennedy, J. M., Collins, C. 1979. CNS insensitivity to pyrethroids in the resistant kdr strain of house flies. *Pestic. Biochem. Physiol.* 12:224-30
 126. Miller, T. A., Maynard, M., Kennedy, J. M. 1979. Structure and insecticidal activity of picrotoxinin analogs. *Pestic. Biochem. Physiol.* 10:128-36
 127. Mitsui, T., Nobusawa, C., Fukami, J., Colins, J., Riddiford, L. M. 1980. Inhibition of chitin synthesis by diflubenzuron in *Manduca* larvae. *J. Pestic. Sci.* 5:335-41
 128. Miyazaki, A., Hotta, T., Marumo, S., Sakai, M. 1978. Synthesis, absolute stereochemistry, and biological activity of optically active cyclodiene insecticides. *J. Agric. Food Chem.* 26:975-77
 129. Miyazaki, A., Sakai, M., Marumo, S. 1979. Comparative metabolism of enantiomers of chlordane and chlordane epoxide in German cockroaches, in relation to their remarkably different insecticidal activity. *J. Agric. Food Chem.* 27:1403-5
 130. Miyazaki, A., Sakai, M., Marumo, S. 1980. Synthesis and biological activity of optically active heptachlor, 2-chloroheptachlor, and 3-chloroheptachlor. *J. Agric. Food Chem.* 28:1310-1
 131. Mulder, R., Gijswijt, M. J. 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. *Pestic. Sci.* 4:737-4
 132. Mullins, L. J. 1976. Steady-state calcium fluxes: membrane versus mitochondrial control of ionized calcium in axoplasm. *Fed. Proc.* 35:2583-88
 133. Murdock, L. L., Hollingworth, R. M. 1980. Octopamine-like actions of formamidines in the firefly light organ. See Ref. 25, pp. 415-22
 134. Najyo, K., Katsuyama, N., Kariya, A., Yamamura, T., Hyeon, S., Suzuki, A., Tamura, S. 1980. New insecticidal pyrethroid-like oximes. *Agric. Biol. Chem.* 44:217-18
 135. Narahashi, T. 1971. Effects of insecticides on excitable tissues. *Adv. Insect Physiol.* 8:1-93
 136. Narahashi, T. 1976. Effects of insecticides on nervous conduction and synaptic transmission. In *Insecticide Biochemistry and Physiology*, ed. C. F. Wilkinson, pp. 327-96. New York: Plenum
 137. Narahashi, T. 1979. Nerve membrane ionic channels as the target site of insecticides. In *Neurotoxicology of Insecticides and Pheromones*, ed. T. Narahashi, pp. 211-43. New York: Plenum
 138. Narahashi, T., Nishimura, K., Parmentier, J. L., Takeno, K. 1977. Neurophysiological study of the structural activity relation of pyrethroids. In *Sy.*

- ingworth, R. M., Yim, H. 1971. Comparative neurophysiological effects of insecticide pesticides. See Ref. 137, pp. 37-71. Studies on the biochemistry of resistance in man cockroach. *Proc. Entomol. Soc. India* 2:95-116
- Jeeman, R. W. 1976. Physiological effects of insecticides on cockroaches. *Environ. Health Perspect.* 19:1-11
- Narahashi, T. 1971. Neurophysiological and electrophysiological effects of DDT and related insecticides on lobster. *Pharmacol.* 20:825-37
14. Calcium influx in cockroach hyperpolarization. *Comp. Biochem. Physiol.* 72:95-105
15. Tandem, N. B. 1975. Calcium channel in *Helix aspersa*. *J. Gen. Physiol.* 48:1-11
16. Cullen, E. I., Lin, S. H. 1970. Drug-sensitive exanthematous leukocytes. *Biochem. Biophys. Res. Commun.* 39:2285-90
17. Cullen, R. T. 1980. Inhibition of imago maturation by diflubenzuron in stable fly. *Science* 209:1028-29
18. W. 1973. Calcium in cockroach. *Physiol.* 235:225-65
19. Engel, J. L., Verkade, J. 1979. Structure-toxicity of 1-substituted-4-cyclo(1.2.2)octanes. *Pharmacol.* 47:287-93
20. The insect neurophysiology: a site of insecticide action. *Pharmacol.* 47:95-111
21. Mode of action of insecticides gained from experiments on preparations of insects. See Ref. 137, pp. 37-71
22. J. M. 1972. Flightless house flies as affected by insecticides. *Pestic. Biochem. Physiol.* 11:206-22
23. Cullen, J. M., Collins, J. M., Collins, J. M. 1977. Neurophysiological study of the structure-activity relation of pyrethroids. *Pestic. Biochem. Physiol.* 12:224-30
126. Miller, T. A., Maynard, M., Kennedy, J. M. 1979. Structure and insecticidal activity of picrotoxinin analogs. *Pestic. Biochem. Physiol.* 10:128-36
127. Mitsui, T., Nobusawa, C., Fukami, J., Collins, J., Riddiford, L. M. 1980. Inhibition of chitin synthesis by diflubenzuron in *Manduca* larvae. *J. Pestic. Sci.* 5:335-41
128. Miyazaki, A., Hotta, T., Marumo, S., Sakai, M. 1978. Synthesis, absolute stereochemistry, and biological activity of optically active cyclodiene insecticides. *J. Agric. Food Chem.* 26:975-77
129. Miyazaki, A., Sakai, M., Marumo, S. 1979. Comparative metabolism of enantiomers of chlordane and chlordane epoxide in German cockroaches, in relation to their remarkably different insecticidal activity. *J. Agric. Food Chem.* 27:1403-5
130. Miyazaki, A., Sakai, M., Marumo, S. 1980. Synthesis and biological activity of optically active heptachlor, 2-chloroheptachlor, and 3-chloroheptachlor. *J. Agric. Food Chem.* 28:1310-11
131. Mulder, R., Gijswijt, M. J. 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. *Pestic. Sci.* 4:737-45
132. Mullins, L. J. 1976. Steady-state calcium fluxes: membrane versus mitochondrial control of ionized calcium in axoplasm. *Fed. Proc.* 35:2583-88
133. Murdock, L. L., Hollingworth, R. M. 1980. Octopamine-like actions of formamidines in the firefly light organ. See Ref. 25, pp. 415-22
134. Najyo, K., Katsuyama, N., Kariya, A., Yamamura, T., Hyeon, S., Suzuki, A., Tamura, S. 1980. New insecticidal pyrethroid-like oximes. *Agric. Biol. Chem.* 44:217-18
135. Narahashi, T. 1971. Effects of insecticides on excitable tissues. *Adv. Insect Physiol.* 8:1-93
136. Narahashi, T. 1976. Effects of insecticides on nervous conduction and synaptic transmission. In *Insecticide Biochemistry and Physiology*, ed. C. F. Wilkinson, pp. 327-96. New York: Plenum Press
137. Narahashi, T. 1979. Nerve membrane ionic channels as the target site of insecticides. In *Neurotoxicology of Insecticides and Pheromones*, ed. T. Narahashi, pp. 211-43. New York: Plenum Press
138. Narahashi, T., Nishimura, K., Parentier, J. L., Takeno, K. 1977. Neurophysiological study of the structure-activity relation of pyrethroids. In *Synthetic Pyrethroids*, ACS Symp. Ser. 42, ed. M. Elliot, pp. 85-97. Washington DC: Am. Chem. Soc.
139. Nathanson, J. A. 1979. Octopamine receptors, adenosine 3',5'-mono-phosphate, and neural control of firefly flashing. *Science* 203:65-68
140. Nathanson, J. A., Greengard, P. 1973. Octopamine-sensitive adenylate cyclase: evidence for a biological role of octopamine in nervous tissue. *Science* 180:308-10
141. Nelson, J. O., Matsumura, F. 1973. Dieldrin (HEOD) metabolism in cockroaches and houseflies. *Arch. Environ. Contam. Toxicol.* 1:224-44
142. Nogge, G., Giannetti, M. 1980. Specific antibodies: a potential insecticide. *Science* 209:1028-29
143. O'Brien, R. A., Spirt, N. M. 1980. The inhibition of GABA-stimulated benzodiazepine binding by a convulsant benzodiazepine. *Life Sci.* 26:1441-45
144. O'Brien, R. D. 1966. Mode of action of insecticides. *Ann. Rev. Entomol.* 11:369-402
145. Olsen, R. W., Ban, M., Miller, T. 1976. Studies on the neuropharmacological activity of bicuculline and related compounds. *Brain Res.* 102:283-99
146. Omer, S. M., Georghiou, G. P., Irving, S. N. 1980. DDT/pyrethroid resistance inter-relationships in *Anopheles stephensi*. *Mosq. News* 40:200-9
147. Oppenoorth, F. J., Nasrat, G. E. 1966. Genetics of dieldrin and γ -BHC resistance in the housefly. *Entomol. Exp. Appl.* 9:223-31
- 147a. Orchard, I., Osborne, M. P. 1979. The action of insecticides on neurosecretory neurons in the stick insect, *Carausius morosus*. *Pestic. Biochem. Physiol.* 10:197-202
148. Osborne, M. P. 1980. The insect synapse: structural functional aspects in relation to insecticidal action. See Ref. 25, pp. 29-40
149. Osborne, M. P., Hart, R. J. 1979. Neurophysiological studies of the effects of permethrin upon pyrethroid resistant (kdr) and susceptible strains of Dipteran larvae. *Pestic. Sci.* 10:407-13
150. O'Shea, M., Evans, P. D. 1979. Potentiation of neuromuscular transmission by an octopaminergic neurone in the locust. *J. Exp. Biol.* 79:169-90
151. Patil, T. N., Abd-El-Fattah, A. A., Plapp, F. W., Koch, R. B. 1980. AT-Pase and dehydrogenase activities from house flies susceptible and resistant to organochlorine insecticides. *Pestic. Biochem. Physiol.* 13:5-12

152. Pfister, W. R., Yim, G. K. W. 1977. Formamidinium induced feeding and behavioral alteration in the rat. *Fed. Proc.* 36:352
153. Pfister, W. R., Yim, G. K. W. 1978. Appetite stimulation in rats by diverse pharmacological agents. *Pharmacologist* 20:172
154. Plapp, F. W. 1976. Biochemical genetics of insecticide resistance. *Ann. Rev. Entomol.* 21:179-97
155. Post, L. C., de Jong, B. J., Vincent, W. R. 1974. 1-(2,6-Disubstituted benzoyl)-3-phenylurea insecticides: inhibitors of chitin synthesis. *Pestic. Biochem. Physiol.* 4:473-83
156. Pratt, G. E., Jennings, R. C., Hamnett, A. F., Brooks, G. T. 1980. Lethal metabolism of precocene-I to a reactive epoxide by locust corpora allata. *Nature* 284:320-23
157. Quistad, G. B., Cerf, D. C., Schooley, D. A., Staal, G. B. 1981. Fluoromevalonate acts as an inhibitor of insect juvenile hormone biosynthesis. *Nature* 289:176-77
158. Robertson, H. A. 1975. Octopamine: presence in firefly lantern suggests transmitter role. *Proc. Can. Fed. Biol. Sci.* 18:66
159. Robinson, J. D. 1976. (Ca + Mg)-stimulated ATPase activity of a rat brain microsomal preparation. *Arch. Biochem. Biophys.* 176:366-74
160. Roeder, K. D., Weiant, E. A. 1946. The site of action of DDT in the cockroach. *Science* 103:304-6
161. Roufogalis, B. D. 1973. Properties of a (Mg²⁺ + Ca²⁺)-dependent ATPase of bovine brain cortex: effects of detergents, freezing, cations and local anesthetics. *Biochim. Biophys. Acta* 318:360-70
162. Sawicki, R. M. 1978. Unusual response of DDT-resistant houseflies to carbinol analogs of DDT. *Nature* 275:443-44
163. Schatzmann, H. J., Vincenzi, F. F. 1969. Calcium movements across the membrane of human red cells. *J. Physiol.* 201:369-95
164. Schroeder, M. E., Boyer, A. C., Flatum, R. F., Sundelin, K. G. R. 1978. Novel inhibitors of insect choline acetyltransferase and their effects on synaptic transmission at an insect cholinergic synapse. See Ref. 12, pp. 63-82
165. Schroeder, M. E., Shankland, D. L., Hollingworth, R. M. 1977. The effects of dieldrin and isomeric aldrin diols on synaptic transmission in the American cockroach and their relevance to the dieldrin poisoning syndrome. *Pestic. Biochem. Physiol.* 7:403-15
166. Sellers, L. G., Guthrie, F. E. 1972. Distribution and metabolism of ¹⁴C-dieldrin in the resistant and susceptible housefly. *J. Econ. Entomol.* 65:378-85
167. Shankland, D. L., Schroeder, M. E. 1973. Pharmacological evidence for a discrete neurotoxic action of dieldrin (HEOD) in the American cockroach, *Periplaneta americana* (L.). *Pestic. Biochem. Physiol.* 3:77-86
168. Singh, G. J. P., Thornhill, R. A. 1980. The metabolism of [¹⁴C]dieldrin by microsomal preparations of different tissues of cockroaches and locusts. *Comp. Biochem. Physiol. C* 67:79-82
169. Singh, G. J. P., Thornhill, R. A. 1980. Metabolic fate of [¹⁴C]dieldrin in *Schistocerca gregaria* with particular reference to the nervous system. *Xenobiotica* 10:57-63
170. Sobue, K., Ichida, S., Yoshida, H., Yamazaki, R., Kakiuchi, S. 1979. Occurrence of a Ca²⁺- and modulator protein-activatable ATPase in the synaptic plasma membranes of brain. *FEBS Lett.* 99:199-202
171. Soderlund, D. M. 1979. Pharmacokinetic behavior of enantiomeric pyrethroid esters in the cockroach, *Periplaneta americana* L. *Pestic. Biochem. Physiol.* 12:38-48
172. Soloway, S. B., Henry, A. C., Kollmeyer, W. D., Padgett, W. M., Powell, J. E., Roman, S. A., Tieman, C. H., Corey, R. A., Horne, C. A. 1978. Nitromethylene heterocycles as insecticides. See Ref. 12, pp. 153-58
173. Soullairac, A. 1969. The adrenergic and cholinergic control of food and water intake. *Ann. NY Acad. Sci.* 157:934-61
174. Sowa, B. A., Marks, E. P. 1975. An *in vitro* system for the quantitative measurement of chitin synthesis in the cockroach: inhibition by TH 6040 and polyoxin D. *Insect Biochem.* 5:855-59
175. Sparks, T. C., Wing, K. D., Hammock, B. D. 1979. Effects of the anti hormone-hormone mimic ETB on the induction of insect juvenile hormone esterase in *Trichoplusia ni*. *Life Sci.* 25:445-50
176. Staal, G. B. 1975. Insect growth regulators with juvenile hormone activity. *Ann. Rev. Entomol.* 20:417-60
177. Strichartz, G. R. 1973. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.* 62:37-57
178. Ticku, M. K., Burch, T. 1980. Purine inhibition of [³H]- γ -aminobutyric acid receptor binding to rat brain membranes. *Biochem. Pharmacol.* 29:1217-20
179. Ticku, M. K., Olsen, R. W. 1979. Cag convulsants inhibit picrotoxinin binding. *Neuropharmacology* 18:315-18
180. Tsukamoto, M., Narahashi, T., Yamasaki, T. 1965. Genetic control of low nerve sensitivity to DDT in insecticide-resistant houseflies. *Botyu Kagak.* 30:128-32
181. Turnbull, I. F., Howells, A. J. 1980. Larvicidal activity of inhibitors of DOPA decarboxylase on the Australian sheep blowfly, *Lucilia cuprina*. *Aust. J. Biol. Sci.* 33:169-81
182. Turnbull, I. F., Pyltiotis, N. A., Howells, A. J. 1980. The effects of DOPA decarboxylase inhibitors on the permeability and ultrastructure of the larval cuticle of the Australian sheep blowfly, *Lucilia cuprina*. *J. Insect Physiol.* 26:525-32
183. Uchida, M., Fujita, T., Kurihara, N., Nakajima, M. 1978. Toxicities of γ -BHC and related compounds. See Ref. 12, pp. 133-51. New York: Plenum
184. Uchida, M., Irie, Y., Fujita, T., Nakajima, M. 1975. Effects of noreis toxin on the neuroexcitatory action of insecticides. *Pestic. Biochem. Physiol.* 5:253-57
185. Uchida, M., Irie, Y., Kurihara, N., Fujita, T., Nakajima, M. 1975. The neuroexcitatory, convulsive and lethal effects of lindane analogs on *Periplaneta americana* (L.). *Pestic. Biochem. Physiol.* 5:258-64
186. Verloop, A., Ferrell, C. D. 1977. Benzoylphenyl ureas—a new group of larvicides interfering with chitin depositor. In *Pesticide Chemistry in the 20th Century*, ACS Symp. Ser., ed. J. R. Plimmer, pp. 237-70. Washington DC: Am. Chem. Soc.
187. Vincent, J. F. V., Hillerton, J. E. 1979. The tanning of insect cuticle—a critical review and a revised mechanism. *J. Insect Physiol.* 25:653-58
188. Walker, R. J., Kerkut, G. A. 1978. The first family (adrenaline, noradrenaline, dopamine, octopamine, tyramine

- g syndrome. *Pestic. Biochem. Physiol.* 7:403-15
- Juthrie, F. E. 1972. Dis-metabolism of ^{14}C -diel-dristant and susceptible *m. Entomol.* 65:378-85
- L., Schroeder, M. E. 1972. Physiological evidence for a toxic action of dieldrin on American cockroach, *Periplaneta americana* (L.). *Pestic. Biochem. Physiol.* 3:77-86
- Thornhill, R. A. 1980. Metabolism of ^{14}C -dieldrin by migrations of different tissues and locusts. *Comp. Biochem. Physiol.* 67:79-82
- Thornhill, R. A. 1980. Metabolism of ^{14}C -dieldrin in *Schistocerca gregaria* with particular reference to the nervous system. *Xenobiotica* 10:1-10
- Ueda, S., Yoshida, H., Kakiuchi, S. 1979. Occurrence and modulator properties of ATPase in the synaptic vesicles of brain. *FEBS Lett.* 104:1-4
- Ueda, S. 1979. Pharmacokinetics of enantiomeric pyrethroids in the cockroach, *Periplaneta americana* L. *Pestic. Biochem. Physiol.* 8:1-8
- Henry, A. C., Kolligatt, W. M., Powell, J. A., Tieman, C. H., Orme, C. A. 1978. Nitroterocycles as insecticides. pp. 153-58
9. The adrenergic and role of food and water. *Acad. Sci.* 157:934-61
10. E. P. 1975. An in vivo quantitative measurement of synthesis in the cockroach by TH 6040 and piperonyl BtM. *Biochem. Physiol.* 5:855-59
11. K. D., Hammock, B. D. 1978. Effects of the anti-hormone, TB on the induction of hormone esterase in the cockroach. *Life Sci.* 25:445-50
12. Insect growth regulable hormone activity. *J. Insect Physiol.* 20:417-60
13. 1973. The inhibition of conduction in myelinated nerve by derivatives of lidocaine. *J. Neurophysiol.* 36:7-57
14. T. 1980. Purine metabolism of γ -aminobutyric acid in rat brain mem-branes. *Biochem. Pharmacol.* 29:1217-20
179. Ticku, M. K., Olsen, R. W. 1979. Cage convulsants inhibit picrotoxinin binding. *Neuropharmacology* 18:315-18
180. Tsukamoto, M., Narahashi, T., Yamasaki, T. 1965. Genetic control of low nerve sensitivity to DDT in insecticide-resistant houseflies. *Botyu Kagaku* 30:128-32
181. Turnbull, I. F., Howells, A. J. 1980. Larvicidal activity of inhibitors of DOPA decarboxylase on the Australian sheep blowfly, *Lucilia cuprina*. *Aust. J. Biol. Sci.* 33:169-81
182. Turnbull, I. F., Pyliotis, N. A., Howells, A. J. 1980. The effects of DOPA decarboxylase inhibitors on the permeability and ultrastructure of the larval cuticle of the Australian sheep blowfly, *Lucilia cuprina*. *J. Insect Physiol.* 26:525-32
183. Uchida, M., Fujita, T., Kurihara, N., Nakajima, M. 1978. Toxicities of γ -BHC and related compounds. See Ref. 12, pp. 133-51. New York: Plenum
184. Uchida, M., Irie, Y., Fujita, T., Nakajima, M. 1975. Effects of nereis-toxin on the neuroexcitatory action of insecticides. *Pestic. Biochem. Physiol.* 5:253-57
185. Uchida, M., Irie, Y., Kurihara, N., Fujita, T., Nakajima, M. 1975. The neuroexcitatory, convulsive and lethal effects of lindane analogs on *Periplaneta americana* (L.). *Pestic. Biochem. Physiol.* 5:258-64
186. Verloop, A., Ferrell, C. D. 1977. Benzoylphenyl ureas—a new group of larvicides interfering with chitin deposition. In *Pesticide Chemistry in the 20th Century, ACS Symp. Ser.*, ed. J. R. Plimmer, pp. 237-70. Washington DC: Am. Chem. Soc.
187. Vincent, J. F. V., Hillerton, J. E. 1979. The tanning of insect cuticle—a critical review and a revised mechanism. *J. Insect Physiol.* 25:653-58
188. Walker, R. J., Kerkut, G. A. 1978. The first family (adrenaline, noradrenaline, dopamine, octopamine, tyramine, phenylethanolamine and phenylethylamine). *Comp. Biochem. Physiol. C* 61:261-66
189. Wang, C. M., Narahashi, T., Yamada, M. 1971. The neurotoxic action of diel-drin and its derivatives in the cockroach. *Pestic. Biochem. Physiol.* 1:84-91
190. Washio, H. 1972. The ionic requirements for the initiation of action potentials in insect muscle fibers. *J. Gen. Physiol.* 59:121-34
191. Watanabe, H., Fukami, J. 1977. Stimulating action of chlordimeform and demethylchlordimeform on motor discharges of armyworm, *Leucania separata* Walker (Lepidoptera: Noctuidae). *J. Pestic. Sci.* 2:297-302
192. Weiss, K. R., Cohen, J. L., Kupfermann, I. 1978. Modulatory control of buccal musculature by a serotonergic neuron (metacerebral cell) in *Aplysia*. *J. Neurophysiol.* 41:181-203
193. Wu, C. H., van den Bercken, J., Narahashi, T. 1975. The structure-activity relationship of DDT analogs in crayfish giant axons. *Pestic. Biochem. Physiol.* 5:142-49
194. Yamaguchi, I., Matsumura, F., Kadous, A. A. 1979. Inhibition of synaptic ATPases by heptachlorepoxyde in rat brain. *Pestic. Biochem. Physiol.* 11:285-93
195. Yamaguchi, I., Matsumura, F., Kadous, A. A. 1980. Heptachlor epoxide: effects on calcium-mediated transmitter release from brain synaptosomes in rat. *Biochem. Pharmacol.* 29:1815-23
196. Yamasaki, T., Narahashi, T. 1959. Electrical properties of the cockroach giant axon. *J. Insect Physiol.* 3:230-42
197. Yim, G. K. W., Pfister, W. R., Yau, E. T., Mennear, J. H. 1978. Comparison of appetite stimulation by chlordiazepoxide, chlordimeform, clonidine and cyproheptadine in rats. *Fed. Proc.* 37:860
198. Yu, S. J., Terriere, L. C. 1977. Ecdysone metabolism by soluble enzymes from three species of Diptera and its inhibition by the insect growth regulator TH-6040. *Pestic. Biochem. Physiol.* 7:48-55