Symposium on the Mechanism of Action of Pesticide Chemicals

In our knowledge concerning pesticides there exists a great hiatus where there should be an understanding of the intimate details of the mechanism of the toxic action of several important contact pesticides. The ability finally to overcome the defenses of the arthropod pests may have to await the filling in of this gap.

The Symposium on Research Progress on Insect Resistance, held in Washington, D.C., in October 1959 under the sponsorship of a committee of the National Agricultural Chemicals Association and the Entomological Society of America emphasized the growing need for a greater knowledge of resistance to pesticides.

The chief objective of the Symposium on the Mechanism of Action of Pesticide Chemicals, presented by the Division of Agricultural and Food Chemistry at the 137th meeting of the American Chemical Society in Cleveland, Ohio, in April 1960, was to stimulate efforts to find more effective and more lasting means of chemical pest control. Most of the papers from that symposium are printed in the following pages.

Some of the contributors present results of their recent investigations on the ability of certain chlorinated pesticides somehow to mobilize materials whose source is in and whose effect is on the nerve tissue. Another reviews our present limited knowledge of the metabolism of pesticides, a field whose development is of essential importance to a fuller understanding of both intoxication and detoxication, and hence of toxicity and resistance. The contribution which completes the symposium deals with the action of synergists as they may increase or decrease the toxicity of pesticide chemicals.

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Approaches to Mechanisms of Insecticidal Action

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Insect species and vertebrates differ with respect to response to drug and insecticide action. The insect neuromuscular system appears to be unaffected by insecticides and drugs paralyzing vertebrates. In seeking an explanation for chlorinated hydrocarbon poisoning in the cockroach, no evidence of a biochemical lesion was obtained. Furthermore, biologically active substances found in cockroach blood appear to be a consequence, but not a cause of DDT or dieldrin intoxication.

Editor's Note

The four papers published here deal with mechanisms of action of pesticide chemicals in insects. A fifth paper in the symposium, by E. A. Hosein and P. Proulx, was concerned with some effects of pesticide chemicals in mammals. We hope to present the latter paper in an early issue.

INSECT NERVOUS SYSTEM TISSUE forms an essential integrated system which is a primary target for the action of many insecticides. Certain differences have been found between the insect and vertebrate in the comparative action of drugs and insecticides in muscles and in the nervous system. Differences were found when simple ions such as potassium were used, with pharmacological agents, including acetylcholine, eserine, and atropine, and finally with the organophosphates. Mechanisms of chlorinated hydrocarbon poisoning are discussed.

Potassium

Hoyle (*18*) showed that nerve conduction in the fifth leg nerve of the locust was not affected by concentrations of potassium high enough to block conduction in the vertebrate nerve. This observation led to a hypothesis that the insect nervous and muscle systems are protected by an ion-impermeable barrier. The data given in Table I show that when the effect of potassium upon nervous conduction in the locust, American cockroach, and toad is compared, only the locust nervous system is

resistant. This shows a difference between insect species rather than a general difference between the insect and vertebrate nervous systems. The locust muscle system is even more sensitive to potassium than the nervous system. Although no specific data are available for the cockroach, the muscles of the roach are probably no more insensitive to potassium than the nervous system.

An electron microphotograph of a section of the fifth leg nerve of the cockroach is given in Figure 1 (*15*). In structure the leg nerve is apparently similar to that of the locust (*1*). Hoyle (*18*) suggested that a tracheolated membrane is present around the locust leg nerve, but Ashhurst (*1*) has recently indicated that the tracheolated membrane is absent from the locust. So far no anatomical structure has been found to explain the difference in permeability to potassium in the locust and roach nervous systems. There may be a difference in active regulation of ion transport, which probably occurs in the mitochondrial-rich layer of glial cells underlying the connective tissue sheath. Interference with enzyme action (usually associated with mitochondria) in the nerve sheath may lead to self-intoxication of insects, but this would apply only if the irritable tissues of all insects were similarly protected against ion disruption.

Drug Action

A number of drugs produce different effects in the insect and in the vertebrate (Table II). Acetylcholine (ACh), which disrupts nervous activity in vertebrate ganglia and at the neuromuscular junction, has no apparent effect in the locust or cockroach. Unlike potassium, a difference has been found for ACh in the two insect species. In insects where it can be studied, the nervous system appears to be impervious to ACh and in this respect can be compared only with the vertebrate central nervous system. Although ionization of a drug may determine its ability to penetrate into a tissue, one must also consider effector sites

Figure 1. Electron micrograph of cross section of metathoracic fifth leg nerve of *Periplaneta*

Nerve is covered by a fibrillar neural sheath, *N.S.* Immediately below this is a highly basophilic layer, *G*, composed of dovetailed neuroglial cells containing numerous mitochondria and basophilic granular cytoplosm; these cells are bounded by glial cell membranes, *G.M.* The nerve consists of axons, *Ax*, ranging from a fraction of a micron to several microns in diameter. Each axon is covered by a membrane, *A.M.* The interstices between the axons are filled with neuroglial cells contoining mitochondria and an unknown compound, *U.* The neuroglial cells may surround one or several axons in the manner of Schwann cells. Fixed in permanganate and embedded in Shell Epon 812. × 33,000

Table I. Effect of Potassium Concentration on Nervous Conduction

Organism	K^+ Concn., mM	Connective Tissue Nerve Sheath: Intact — Time to Block, Min.	Connective Tissue Nerve Sheath: Removed — Time to Block, Min.
Locust (*18*), *L. migratoria*	50	No effect	Immediately (injected)
	140	240	
Cockroach (*33*), *P. americana*	180	12	10 sec.
	140	30	60 sec.
Toad (*12*), *R. tigrina*	120	15	3/4 to 1

Table II. Effect of Drugs on Nervous and Muscle Activity in Insects and Vertebrates

Insect: Drug concn.	Insect: Central ganglia	Insect: Axonic	Insect: Muscle	Vertebrate: Drug concn.	Vertebrate: Central nervous system	Vertebrate: Ganglio	Vertebrate: Axonic	Vertebrate: Muscle
ACh, 10^{-2} *M*, roach (*10*), locust (*16*)	None	None	None	ACh 10^{-4} *M*	None	Facilitation block	None	Excitation block
Eserine, 10^{-4} *M*, roach (*10*), locust (*16*)	Excitation block	None	None	Eserine 10^{-4} *M*		Block	Block	Excitation
Eserine, 10^{-3} *M*, locust (*16*)	Excitation block	None	Excitation					
Atropine, 10^{-3} *M*, locust (*16*)	None	None	Tremors	Atropine (therapeutic amounts)	Stimulates depresses	Excitation	None	None
Atropine, 10^{-3} *M* roach (*24*)	None	None						
Curare, 10^{-2} *M*, roach (*24*)	None	None	None	Curare		Blocks effect of ACh	None at ganglion concentration	Blocks effect of ACh

Table III. Effect of Organophosphorus Compounds on Nervous and Muscle Activity in Cockroach and Locust

Compound Concn., *P. americana*	Thoracic Ganglion	6th Abdominal Ganglion
TEPP[a], 10^{-4} to 10^{-5} *M* (*8*)	Block, cycling	Facilitation, block, cycling
DFP, 10^{-3} to 10^{-5} *M* (*24*)		Facilitation, block, cycling
L. migratoria (*16*)	Thoracic ganglion leg reflex	Leg muscle
TEPP, 10^{-3} *M*	...	No effect
TEPP, 10^{-5} *M*	Facilitated, blocked	...
Paraxon, 10^{-3} *M*	...	No effect
Paraxon, 10^{-5} *M*	Facilitated, blocked cycling	...

[a] Abdominal and thoracic connectives, motor and sensory fibers of fifth leg nerve and leg muscles. Not interfered with by TEPP at 10^{-4} to 10^{-5} *M*.

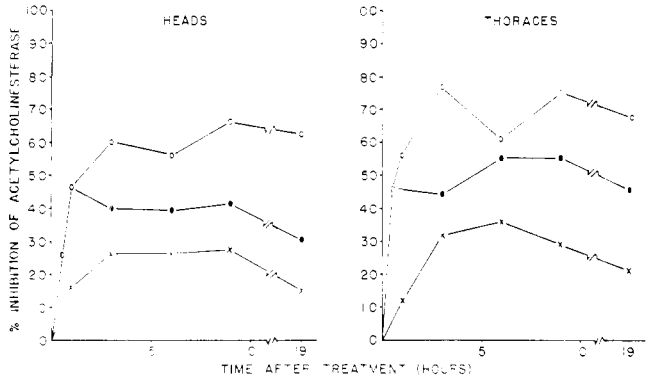

Figure 2. Acetylcholinesterase inhibition in heads and thoraxes of houseflies treated with sublethal dose of TEPP

O Prostrate flies
● Flies recovered from prostration
X Nonprostrate flies

of drug action. Very little is known about insect neuromuscular mechanisms, but it is evident that innervation of insect muscles (*19*) is different from those of vertebrates. Furthermore, the cholinergic system of the vertebrate neuromuscular system is absent in the roach, for Colhoun (*7*) was unable to demonstrate ACh, choline acetylase (ChA), or acetylcholinesterase (AChE) in leg muscles of the cockroach. The absence of the cholinergic system would explain the negative effect of ACh injected into insects better than the hypothesis that ACh is unable to penetrate into the neuromuscular junction.

It is more difficult to find a similar explanation for the different effect of atropine (Table II) in the locust and in man. Harlow (*16*) has shown that atropine stimulated the muscle contraction of the hind leg of the locust but at a higher concentration did not affect ganglionic nervous transmission. In man atropine affects central nervous activity at a concentration which does not interfere with neuromuscular transmission. The brain blood barrier of man is thought to be an effective barrier to certain ions which have easier access at the neuromuscular junction. Finally, eserine seems to have a similar effect in locust and cat. Although pH affects the ability of eserine to penetrate into tissues, eserine penetrates into the nervous system at physiological pH. In the cat eserine blocks ganglionic nervous conduction, but at the same concentration excites neuromuscular nervous transmission. Harlow (*16*) shows that at 2×10^{-4} *M* eserine blocked ganglionic nervous transmission in the locust, but did not affect muscle activity. At a higher concentration eserine affected muscle activity. Eserine may interfere with receptor sites apart from anticholinesterase action. On this basis it may be possible to explain the neuromuscular effect of eserine in the insect in the absence of cholinesterase at the neuromuscular junction.

Organophosphate Action

In the roach a number of organophosphates disrupt central nervous function (Table III) but have no effect upon muscle activity. The absence of the cholinergic system in the roach would explain this difference, as these substances have no apparent effect on muscle activity. The work of O'Brien (*22*) may point the way to devising good insecticides, as un-ionized Tetram has the ability both to penetrate into the nervous system and to inhibit AChE. Indeed, O'Brien's work appears to be further evidence of the importance of AChE in the insect nervous system. However, the introduction of nonionized insecticides would obviously increase their hazard to man, because it would enable the substance to penetrate more easily to all parts of the vertebrate nervous system. O'Brien (*21*) has pointed out the possibility of using insecticides with degrees of ionization such that useful insects with a good ion barrier would be protected. In extensive cropping practices this may be difficult to accomplish, for harmful and beneficial insects may have the same protection. The best insecticide with respect to low hazard to vertebrate life might be one which coupled the weak link theory of O'Brien (*21*) with good ability to penetrate into insect tissue. The weak link would allow the insecticide to be rapidly broken down in the vertebrate.

Recovery from Insecticide Poisoning

Insects are known to recover from prostration induced by insecticide treatment. Stegwee (*28*) showed that houseflies treated with a sublethal dose of tetraethyl pyrophosphate (TEPP) recovered from prostration with about the same degree of AChE inhibition as that determined when they first became prostrate. These results (Figure 2) might indicate that AChE was not involved in organophosphorus poisoning, but this question will best be resolved when the function of AChE in the insect nervous system is more fully understood.

The ability of an insect to recover without therapeutic aid from what appears to be a lethal dose of an insecticide may be explained by the ultimate evaluation of dependency of tissues and metabolic processes in the insect compared with a similar system in man. Although the insect may have less freedom than man with respect to environment, its internal processes may be less susceptible to the action of drugs and poisons. This argument is based on the known direct tracheal respiration of insects, which would eliminate blood as a necessary carrier of oxygen. An insect has the ability to withstand fluctuations of ions and metabolites in the blood and its individual tisseus may have aerobic and anaerobic metabolic mechanisms, enabling them to carry on under adverse conditions with adequate metabolic reserve. In these respects the vertebrate is a great deal more sensitive, for brain activity is intimately dependent upon the levels of sugar, ions, and oxygen in the blood; any interference with these factors by drug action—for instance, respiration in organophosphorus poisoning—results in far-reaching effects that may culminate in death.

The ability of an insect to survive after insecticide treatment for at least

a time without gross physiological disruption provides greater scope for any compensatory or adaptive mechanism to come into effect. For example, Stegwee (*28*) found that by the time the initial toxic effect of TEPP had disappeared, the housefly had been able to overcome the initial disruption of function. The fact that the insect may be able to tolerate adversity in the shape of a poison better than the vertebrate has at least two corollaries. An opportunity for detoxication mechanisms to operate is greater in the insects, and the wider range of individual tolerances among insects permits a more effective selection of resistant strains than in mammals.

Chlorinated Hydrocarbon Poisoning

The mode of action of chlorinated hydrocarbon poisoning in insects is not understood clearly. Recently it has been suggested (*29*) that a toxicant found in the blood of roaches treated with DDT may be the causal factor in DDT prostration. The term "toxicant" would imply that an intoxication agent has been found, but so far no precise quantitative evidence has been obtained that the intoxication of the DDT-treated roach is due to this or any other blood factor. The work of Sternburg, Chang, and Kearns (*29*) followed the earlier findings of Sternburg and Kearns (*31*), that the blood of DDT-prostrate roaches induced high nervous activity of the isolated nerve cord of a normal roach. Subsequently other investigators have reported active biological properties in insect blood. The data given in Table IV show that treatment of roaches with various insecticides of different molecular structure resulted in active blood factors. These were also found in roaches treated by electrical shock or in restrained roaches. The common effect of all these treatments would appear to be some form of stimulation of the roach, and this may in all instances affect the nervous and endocrine system. Because the toxicant of Sternburg, Chang, and Kearns (*30*) has been found in DDT, TEPP, or electrical stimulation, showing no specificity of action, it is reasonable to suppose that it may be found in other insecticide treatment.

While investigating the earlier report of Sternburg and Kearns (*31*) Colhoun (*6, 7, 9*) showed that the blood of roaches treated with DDT and TEPP contained a number of substances in abnormal amounts. Among these was a catechol amine which was biologically active when tested against the isolated heart and nerve cord preparation of a normal roach. The main source of the catechol amine in the roach appears to be the corpus cardiacum gland, although Gersch, Unger, and Fischer (*13*) reported its occurrence in the nerve cord. The presence of this active substance in the blood of DDT- and TEPP-treated roaches led to the correct supposition that more than one factor may be responsible for biological activity when blood is tested against various preparations. The work of Ozbas and Hodgson (*23*) and the very recent report of Milburn, Weiant, and Roeder (*20*) show that corpus cardiacum extracts are biologically active when tested in the roach.

There seems little merit in discussing the importance of one blood substance relative to that of another, for in the blood of a single treated roach all abnormal factors contribute to some degree of biochemical or physiological disturbance. It is important to resolve whether blood factors cause insecticide-like disturbances in roaches where the insecticide is not present. In this way it might be possible to separate primary from secondary insecticide action.

Without carrying out a single experiment it seemed that this was possible, for Spiller (*26*) showed that when a DDT-treated *Rhodnius* was joined in parabiosis to an untreated *Rhodnius*, the untreated insect was unaffected. This clearly showed that the blood of the treated insect was not toxic to the insect to which it was joined. This nontoxicity could scarcely be attributed to a poor interchange of blood, as both insects moulted; furthermore this type of preparation has been repeatedly used by Wigglesworth (*34*) to demonstrate the presence of hormones in *Rhodnius*. Bodenstein (*4*) has also successfully joined two cockroaches together to show hormone effects, and if it were possible for minute amounts of hormones to be translocated from one roach to another, it seemed possible to use this technique (Figure 3) to test the toxicity of blood factors in roaches treated with DDT, dieldrin, or TEPP.

Parabiosis Experiments with the American Cockroach. Roaches intoxicated by topical treatment with TEPP, DDT, or dieldrin were joined to untreated roaches. No toxic effect was observed in the untreated roaches in 48 hours, but they became prostrate at 72 to 120 hours. It is at once evident that the results are different from those found for *Rhodnius*, for after a few days all the untreated roaches died. The physiology of *Rhodnius* may be different from the roach, as *Rhodnius* appears to go into a state of akinesis when exposed to abnormal conditions. If this condition is induced by intoxication, the processes of tissue degeneration may be retarded in *Rhodnius* when they are attached. To learn the effects of the dying roach upon the normal roach, roaches treated with cyanide (which stops respiration and electrical nervous conduction) were joined to normal roaches. The normal roaches died, and their time of death was about the same as in the experiments with DDT, TEPP, or dieldrin.

The dying untreated roaches did not show the typical hyperactive symptoms of poisoning associated with insecticide treatment. They became sluggish, lethargic, and at the most uncoordinated, before they became prostrate. The presence of insecticides in the untreated roaches could not be detected, although DDT and dieldrin were recovered from the treated insects. Almost all of the dieldrin was recovered from treated roaches, showing that this insecticide was not metabolized at a detectable rate in the roach. The difference between symptoms of the treated and untreated

Table IV. Occurrence of Biologically Active Substances in Blood of Cockroaches

	Method of Determining Blood Activity[a]			
Treatment to Cockroach	Chromatogram	Roach isolated nerve cord, electrical activity	Roach isolated heart	Other insects or means
DDT (*5*)	..	..	..	Calliphora flies
DDT (*31*)	..	+	..	Sarcophagid flies
Pyrethrum (*3*)	..	..	..	Sarcophagid flies
Restrained (*3*)	..	..	..	Cockroach
Mechanical (*2*) stimulation (powder mill)	..	..	..	Cockroach
DDT (*7*)	+	+	+	...
TEPP (*7*)	+	+	+	Frog rectus abdominis muscle, Venus heart
Electrical (*10*) grid stimulation	+	+	+	...
Dieldrin (*9*)	..	+	..	...
DDT (*25*) + temp.	..	+	..	...
DDT (*30*)	+	+	..	...
TEPP (*30*)	+	+	..	...
Electrical stimulation (*30*)	+	+	..	...

[a] + Means of detecting biologically active substances.

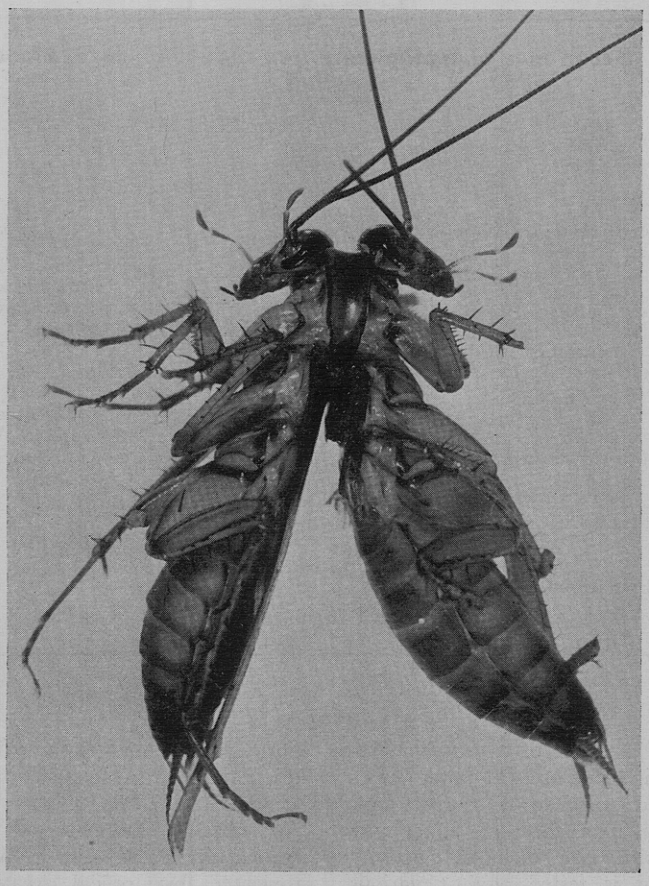

Figure 3. Technique of joining *Periplaneta* in parabiosis to determine toxicity of blood factors resulting from insecticide treatment

Note wax ring at prothoracic shield

roaches indicates that the primary hyperactive and convulsive effects of the insecticides cannot be due to blood factors. In experiments where DDT and TEPP were applied to one of a pair of joined roaches, in sufficient amounts to ensure intoxication of the untreated roach, the insecticidal effect was the same as in a roach treated alone, in both symptoms and the time to prostration. The lack of distinction between the effects, in the normal insect, of union with a dying insect and of union with one treated with either insecticide, makes it impossible to attribute the reaction of the normal insect only to blood factors resulting from insecticide treatment. In these experiments it was expected that a highly intoxicating blood factor would affect the untreated joined roach within the first few hours, for Beament (*2*) has shown that a paralysis factor induced in roaches when pinned to a block could affect a joined roach within 12 to 24 hours.

Central Nervous Effects of Chlorinated Hydrocarbon Poisoning

In seeking a possible explanation of the nervous effects of chlorinated hydrocarbon poisoning, it was established that the amount of acetylcholine increased in the nerve cords of roaches prostrate by DDT treatment (*6*) from the time of early prostration. The occurrence of free ACh in nerve cords would explain nervous excitation caused by DDT treatment, for ACh has this effect when applied to desheathed nerve cords (*32*). However, this explanation ignores the activity of acetylcholinesterase in nervous tissue, for this enzyme rapidly inactivates ACh when it and its substrate are brought together (*6*). Colhoun (*6*) concluded that ACh was a secondary factor in DDT intoxication, as in vivo evidence of free ACh or inhibition of AChE was not obtained. Increase of ACh in the nerve cords of roaches prostrate after treatment with dieldrin has been found, but not during the hyperactive early convulsive phase of intoxication. A common effect has been found in dieldrin and DDT intoxication.

Stegwee (*27*) showed inhibition of AChE in roaches treated with DDT. Until the recent finding of Sternburg, Chang, and Kearns (*30*), no other evidence was available that this enzyme was interfered with in DDT intoxication. These authors injected roaches with tetramethyl pyrophosphate (TMPP) and found that AChE of the nerve cords of DDT-prostrate roaches was only slightly inhibited, whereas the AChE of control roaches was entirely inhibited.

This work was repeated by injecting TMPP and TEPP into roaches prostrate by DDT, dieldrin, or cyanide treatment. The AChE of thoracic nerve cords of roaches prostrate by DDT, dieldrin, or cyanide was less inhibited by injection of TMPP or TEPP than the AChE of normal roaches. TMPP was a less effective inhibitor than TEPP. TMPP has a very short half life, and would be effective in the insect for only a short time after injection. The results confirmed the findings of Sternburg, Chang, and Kearns (*30*) but do they show that AChE is protected or inhibited by DDT treatment? An explanation seems to emerge from the experiments where cyanide was used, for TMPP was no more effective as an inhibitor of the AChE of the nerve cords of cyanide-treated roaches than of DDT-treated roaches. A factor produced by nervous activity in the nerve cord capable of occupying the hydrolysis sites of AChE would be absent in the roaches treated with cyanide, for cyanide blocked the electrical nervous activity of the nerve cords. It would appear more difficult to inhibit the AChE of a prostrate roach regardless of the treatment, and until an adequate explanation is found, it seems unwise to postulate a cholinesterase mechanism of chlorinated hydrocarbon poisoning.

The occurrence of high amounts of ACh in the nerve cords of DDT and dieldrin-treated roaches shows a biochemical abnormality in the nerve cord, for in the roach the ACh titer is remarkably constant under normal conditions. It seemed expedient to determine whether the increase in ACh was due to the peripheral action of DDT or to blood factors. Nerve cords were isolated from roaches treated with DDT or dieldrin at a time when the roaches showed signs of becoming prostrate, and washed and placed in saline. The ACh content of the cords and those left in intact treated roaches was examined at intervals. The ACh content of the isolated nerve cords increased with startling similarity to that of nerve cords of intact treated roaches. The results would appear to show a central nervous effect of chlorinated hydrocarbon poisoning which was not dependent upon the presence of the intact peripheral nervous system or blood factors. The nervous activity of the treated isolated nerve cords failed before that of the controls, which showed only slight reduction in nervous activity at 24 hours. Failure of nervous activity occurred first in the cords isolated from dieldrin-treated roaches and the highest increase of ACh was found in these cords.

Finally, in an attempt to learn the reason for the interference of nervous activity in the isolated nerve cords, tests

were made to determine the possible occurrence of γ-butyrobetaine which Hosein (*17*) concluded was a factor in dieldrin poisoning of the rat. While attempting to find this substance, Colhoun and Spencer (*11*) found that the esters of the betaine were more likely to cause toxic effects if found in the nervous system. However, neither γ-butyrobetaine nor its esters were present in the nerve cords of dieldrin-treated roaches or those of normal roaches. The occurrence of any one of these substances in the nerve cord of treated roaches would suggest that dieldrin is capable of inducing the metabolism of a substance not normally found. This principle, although exciting, has no factual foundation in chlorinated hydrocarbon poisoning in insects at present. However, the possibility suggests a biochemical approach to solving the mechanism of chlorinated hydrocarbon in the central nervous system of insects alongside the search for a physical effect thought to occur in the peripheral nervous system (*14*).

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Effect of Insecticides on Neurophysiological Activity in Insects

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During the course of DDT poisoning, a neuroactive substance accumulates in the blood of American cockroaches and crayfish. Although its structure is unknown, it is not a metabolite of DDT, as its release by nervous tissue can be effected by electrical stimulation alone or by the action of TEPP. Chemical and chromatographic evidence indicates it is not a known neurohumoral agent. The chemical structures of this and other physiologically active compounds must be determined before their role in the mode of action of insecticides is understood.

Much of our understanding of the events occurring during neurotransmission in insects is based on a comparison of the properties of insect nerves with known properties of vertebrate nerves. In many ways, insect nerves respond to chemicals much as do the nerves of vertebrates. For example, physostigmine, a reversible inhibitor of cholinesterase, and the organophosphates, irreversible inhibitors of cholinesterase, disrupt synaptic transmission in insects. These synapses are located in the ganglia of the central nervous system and it is here that high concentrations of cholinesterase are located. Acetylcholine and choline acetylase, the other components required for cholinergic transmission, are also found for the most part within the ganglia (*3*). The presence of these substances clearly indicates that at least some phases of neurotransmission in insects are cholinergic. Disruption of normal neuroactivity by anticholinesterases confirms this. The intense stimulation of central nervous activity by pilocarpine in insects (*13, 16*) also lends support to the presence of cholinergic pathways, as pilocarpine is believed to act by stimulating cholinergic vertebrate effectors.

But there are some disturbing differences. Curare and atropine are known in vertebrates to block neuromuscular junctions and sympathetic ganglia from the action of either