(8) 増量剤としてクレー＋タルク (25%) を用い安定剤を使用せず室温貯蔵試験を15ケ月間実施した場合 1.5% 粉剤で約 12～14% の分解率を示した.

本実験を実施するに当り始終熱心に分析に協力頂いた弊社技術部浅田嬢に深謝する.

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Résumé

We have made an inveatigation about the stabilization and the rate of decomposition of malathion dust formulations in storage days, prepared with various mineral carriers and stabilizers. The results are as fallows :

(1) When clay alone was used as the diluent, the degradation of the active ingredient was remarkably reduced by adition of polyoxyethylene alkyl ether 0.7% or polyoxyethylene alkyl ether 0.5% plus fatty acid 0.5%. When clay 85% plus talc 15%, clay 85% plus silica sand 15%, clay 85% plus kieselguhr 15%, clay 85% plus special kieselguhr 15% were used as diluents, the degradation decreased markedly by addition of polyoxyethlene alkyl ether 0.5% plus fatty acid 0.5% as stabilizers.

(2) We measured surface acidities (pKa) of mineral carriers and malathion dust formulations. In the case of pKa≳3, the rate of degradation was very slow. The value of pKa≳3 is a necessary condition for stabilizing malathion dust formulations. From this result, hydrogen ions (H+) on the surface of diluents are supposed to have large effect upon the degradation.

(3) None of stabilizers used were effective when acid clay was a diluent. Therefore, acid clay should not be used for malathion dust formulation.

(4) The rate of degradation varied with the sort of carriers. Without stabilizer in the next decreasing order : kieselguhr>acid clay>clay>talc ; with polyoxyethlene alkyl ether 0.7% as stabilizer : acid clay>kieselguhr>talc>clay.

(5) In the case of acid clay and kieselguhr, the moisture in them is believed to have some effects on the degradation rate.

(6) As explained, the value of pKa is very important for the stability of malathion dust formulations. Substitution acidity and Thomas acidity must be simultaneously inspected. Furthermore, the effects of surface structure of diluents and their adsorption abilities must be also studied.

(7) Correlation of pH of mineral carriers to the stability of malathion in dust formations was irregular.

(8) In the case of malathion dust formulation (cont. 1.5%), prepared with clay 75% plus talc 25% and no additives, 12～14% of original malathion was decomposed in the storage for 15 months at room temperature (max temp 33.5°C, min temp 1°C, av. temp 20°C).

Nervous Activity as a Factor of Development of Dieldrin Symptoms in the Cockroach. Studies on the Mechanism of Action of Insecticides. XVI. Teruo YAMASAKI and Toshio NARAHASHI (Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Tokyo, Japan). Received Jan. 31, 1958. *Botyu-Kagaku* **23**, 47, 1958.

9. ゴキブリのデイールドリン中毒症状と神経機能 殺虫剤の作用機構に関する研究 第16報

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デイールドリンの作用機構究明の第一段階として, それが神経系にどのように働いて中毒症状を起させるかを調べた. その結果神経系に対する作用は DDT とは質的に著しく異なり, むしろ BHC に共通したところがあるが作用力はより緩慢であることが明らかとなつた.

It is the first step of research for clarifying the mode of action of any insecticide to observe the symptoms of poisoning of the insecticide and to analyze the mechanism of development of the

symptoms[18]. Little has been known concerning the mode of action of dieldrin in spite of its increasing practical use in recent years. The present paper gives the results of experiments dealing with the effects of dieldrin on the nervous activity of insect.

Methods

Insects : Adults of the American cockroach, *Periplaneta americana* L., reared in the laboratory at a constant temperature of about 30°, were used throughout the experiments.

Treatment with dieldrin : The acetone solution of dieldrin was applied topically on the dorsum of the abdomen with the dose of 400 γ/g. Then the symptoms of poisoning were observed and the activity of nerve was examined as described below after various time intervals. The cockroach once excised in order to examine the nervous activity at any stage of the poisoning symptoms was unable to be employed for further observation. Dieldrin was also applied to the nerve preparations excised from the normal or untreated cockroaches. In this case the dieldrin suspension prepared by introducing dieldrin ethanol solution into a Ringer's solution was applied to the nerve. The dieldrin suspension was injected in the leg in order to observe the effects on the sensory neurones as described in the previous report[14]. Ethanol alone at the concentration used had no effect on the nervous activity in question.

Nerve preparations and methods of recording action potentials : The discharges in the abdominal nerve cord, the synaptic transmission through the sixth abdominal ganglion and the sensory discharges in the crural nerve were observed. Synapses in the sixth abdominal ganglion and the pathway of nerve fibres in the abdominal nerve cord have been studied by Roeder[6] and have also been described briefly in our previous papers[12,14,16,20–22]. Sensory neurones in the crural nerve (nerve No. 5) and their discharges have also been described[5,8–10,14,15]. The discharges of the nerve cord were observed *in situ* by recording the action potentials from the nerve cord as described in our previous paper[14]. The synaptic transmission through the sixth abdominal ganglion was observed both *in situ* and with the isolated nerve preparations. In the former case it was observed by recording the action potentials of the giant axons from the abdominal nerve cord between the fourth and the fifth abdominal ganglia while a single shock was being applied to the cercal nerve. In the case of the isolated nerve preparations a different electrode was in contact with the sixth abdominal ganglion, while an indifferent electrode was in contact with the first abdominal ganglion, a small region of the nerve cord between these two electrodes being crushed by forceps to make the action potentials monophasic in most cases. However, the time constant of amplifying circuit was insufficient for recording any synaptic potential which might have been set up by cercal nerve volley[3,19], so that only the propagated action potentials of the giant axons were able to be observed. The method of recording the sensory discharges in the crural nerve was the same as that used previously[14].

Fine silver wires were used as both recording and stimulating electrodes in any case. The recorded action potentials were amplified by a CR-coupled amplifier and observed and photographed by a cathode ray oscilloscope. The action potentials amplified were also fed to a speaker to produce sounds which served as an auditory monitor.

Solution and drug : The Ringer's solution used was the same as that described previously[14,20]. Dieldrin tested was of technical grade.

All the experiments were carried out at room temperatures ranging from 14° to 19°.

Results

Symptoms of poisoning

The course of poisoning symptoms following topical treatment of dieldrin was as follows : latent period, period of ataxia, convulsive period, paralytic period and death. The latent periods following topical application of 400 γ/g dieldrin lay between 9 hours and 13 hours. Then ataxia developed. Although walking was slow and abnormal in this period, the cockroaches were able to stand up when they had been knocked down. The period of ataxia lasted about 10 hours,

after which the cockroaches were knocked down and convulsions developed. The convulsions, lasting as long as two days, were very severe immediately after knockdown and gradually became less severe during that period. The poisoned cockroaches paralyzed after the convulsive period, and death finally followed.

Nervous activity in the poisoned cockroaches

The changes in the discharges in the abdominal nerve cord, in the synaptic transmission through the sixth abdominal ganglion and in the sensory discharges in the crural nerve at various stages of the poisoned cockroaches are given in Table 1, and the oscillograms of the discharges in the abdominal nerve cords of the poisoned cockroaches are shown in Fig. 1.

Period of ataxia : The frequency of discharges in the abdominal nerve cord increased markedly in the period of ataxia (Fig. 1, No. 2). The amputations of the cerci of both sides had a tendency to cause the slight decrease in frequency of the discharges. Prolonged after-discharges of the giant axons were elicited by a single stimulus of the cercal nerve in the poisoned cockroaches showing ataxia. This effect was very easy to detect since only slight after-discharges were elicited by a single volley of preganglionic nerve in the normal or untreated cockroaches. The sensory discharges in the crural nerve did not show any sign of change in the poisoned cockroaches showing ataxia : spontaneous discharges were observed with normal frequency and no train of impulses was detectable.

Convulsive period : In the cockroaches showing severe convulsions immediately after knockdown the frequency of discharges in the abdominal nerve cord was still very high before and after amputating the cerci and the prolonged synaptic after-discharges were also elicited by a single

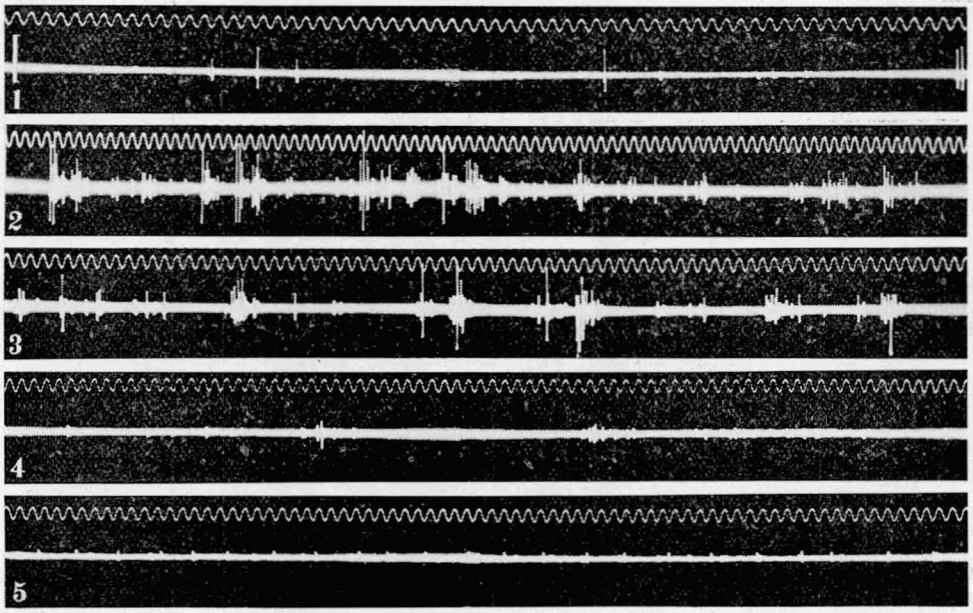

Fig. 1. Discharges in the abdominal nerve cord of the dieldrin-poisoned cockroaches at various stages of symptoms. *In situ* recordings. Each record was taken from different cockroaches. Voltage calibration of 1 mV shown in record *1* is applied to all records. Time marker, 50 c.p.s.

1 : Normal or untreated, with amputating cerci.
2 : 13 hours after treatment, ataxia, with intact cerci.
3 : 24 hours after treatment or 2 hours after knockdown, severe convulsion, with intact cerci.
4 : 75.5 hours after treatment, weak convulsion, with intact cerci.
5 : 76 hours after treatment, paralysis, with intact cerci.

Table 1. Changes in the discharges in the abdominal nerve cord, in the synaptic transmission through the sixth abdominal ganglion and in the sensory discharges in the crural nerve at various stages of the dieldrin-poisoned cockroaches. 400 γ/g.

Symptoms	No.	Time after treatment (hr.)	Time after knockdown (hr.)	Discharges in the nerve cord		Synaptic after-discharges	Sensory discharges
				Before amputating the cerci	After amputating the cerci		
Ataxia	1	13	—	++		+	
	2	13.5	—	++		+	
	3	14	—	++		+	
	4	14.5	—	++		+	
	5	15.5	—	+	+		○
	6	15.5	—	++	+		○
	7	20	—	+	+		
	8	25	—	+	+		○
	9	25	—	++	+		
Severe convulsion	10	13	0	++		+	
	11	17.5	0	+		+	
	12	17.5	0	++		+	
	13	22.5	0	++		++	
	14	14.5	0	+	○		○
	15	15	0	+	+		
	16	20	0	+	+		
	17	24	0	++	+		○
	18	24	0	+	+		○
	19	29	0	++	+		
Severe convulsion	20	22	3	++		+	
	21	23	2	○		+	
	22	23.5	2	○		+	
	23	24	2	++		+	
	24	24	2	○		±	
	25	21	4	+	○		
	26	21	>7	○	○		
	27	21	>7	++	+		
	28	21.5	>7	++	+		
	29	30.5	>7.5	+	○		
Weak convulsion	30	75.5		○		+	
	31	76.5		+		+	
	32	80.5		+	○		
	33	80.5		−			
	34	80.5		+	+		
	35	80.5		+	+		
	36	80.5		+	+		
Paralysis	37	76		○		+	
	38	76		++		+	

±, + or ++ means the increase in nervous activity and its degree.
○ means the unchangeness in nervous activity.
− means the decrease in nervous activity.

cercal nerve volley. In this period the sensory discharges in the crural nerve also showed no sign of abnormality. In the cockroaches which had continued to develop convulsions for a period of two hours or more after knockdown the prolonged synaptic after-discharges were still able to be observed, while the frequency of discharges in the nerve cord decreased to some extent (Fig. 1, No. 3). In this case the tendency to decrease in frequency of the discharges following amputating the cerci was also found. In the cockroaches showing weak convulsions about three days after treatment the prolonged synaptic after-discharges were still elicited, while the frequency of discharges in the nerve cord decreased further (Fig. 1, No. 4).

Paralytic period : In the cockroaches just paralyzed about three days after treatment the prolonged synaptic after-discharges were still elicited, while the frequency of discharges in the abdominal nerve cord was of the extremely low level (Fig. 1, No. 5).

Direct application of dieldrin to the nerve

The applications of 5×10^{-5} dieldrin suspension to the isolated nerve preparation caused the discharges in the abdominal nerve cord to increase in frequency and caused the synapses to produce prolonged after-discharges by a single cercal nerve volley (Fig. 2, Nos. 1~3). Both effects became apparent almost simultaneously several minutes after application. These effects, especially the prolongation of synaptic after-discharges, became more marked with the advance of time lasting as long as more than one hour.

The applications of the same concentration of dieldrin suspension to the exposed preparation whose nerves left intact or whose cercal nerves had been amputated had the same effects as those of dieldrin applied to the isolated nerve preparation on the frequency of discharges in the abdominal nerve cord and on the synaptic transmission

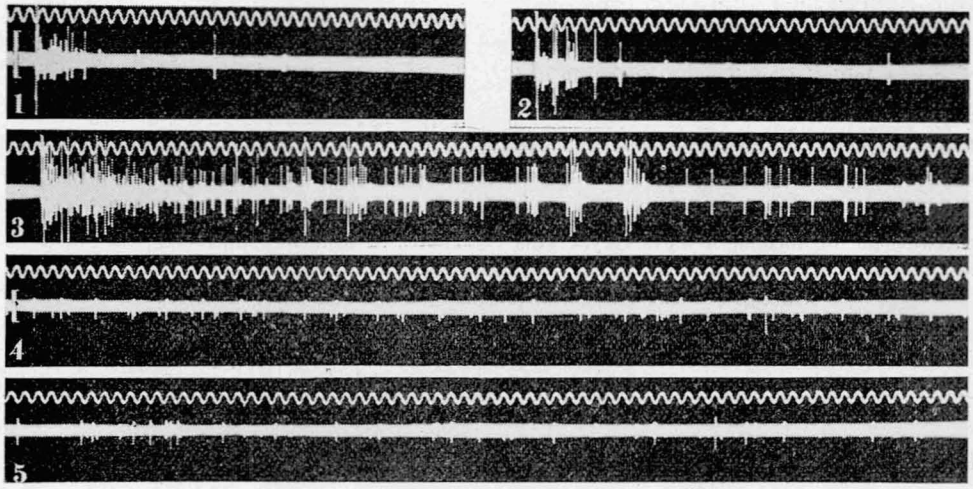

Fig. 2. Responses of the giant axons in the abdominal nerve cord evoked by a single stimulus of the cercal nerve (*1*~*3*), and the sensory discharges in the crural nerve (*4* & *5*). Voltage calibration of 1 mV shown in record *1* is applied to records *1* to *3*, which were taken *in situ* from one and the same cockroach. Voltage calibration of 0.2 mV shown in record *4* is applied to records *4* and *5*, which were taken *in situ* from another one and the same cockroach. Time marker, 50 c.p.s.

1 : Before treatment.
2 : 16 minutes after treatment with 5×10^{-5} dieldrin, slight prolongation of after-discharge.
3 : 79 minutes after treatment, marked prolongation of after-discharge.
4 : Before injection.
5 : 5 minutes after injection of 5×10^{-5} dieldrin in the leg, no effect.

qualitatively similar effects as those of BHC on both the spontaneous activity of the nerve cord and the synaptic transmission. It seems therefore difficult to explain the absence of hyperexcitation in terms of the synaptic after-discharges and the spontaneous activity of the nerve cord. However it is probable that the absence of hyperexcitation in the dieldrin poisoning is partly due to the less striking and more moderate effects of dieldrin on nervous activity which will be discussed later. In the case of DDT poisoning there appears no hyperexcitation probably because the synaptic after-discharges and the increase in spontaneous activity in the central nerve cord are much less marked than in the case of BHC or dieldrin[12,14].

Ataxia, convulsion and paralysis are likely to be explained in terms of the changes in nervous activity as in the case of BHC poisoning[17]. Prolonged synaptic after-discharges and increased spontaneous activity make it difficult for the cockroach to coordinate its movement, causing ataxia. Further progress of such changes in nervous activity makes it quite impossible for the cockroach to coordinate its movement, causing knockdown and convulsion. Paralysis of the cockroach is caused by the progressive decline in nervous activity with the advance of time.

The amputations of the cerci in the poisoned cockroach cause the frequency of discharges in the abdominal nerve cord to decrease slightly. The slight higher level of activity before amputation is considered to be due to the facilitatory synaptic activations of the neurones in the sixth abdominal ganglion by the sensory discharges from the cerci. Similar event also occurred in the case of BHC poisoning[17].

There exists an additional important difference between the effects of dieldrin and BHC upon nerve. Though the prolongation of synaptic after-discharges and increase in the spontaneous activity of the central nerve cord are observable with both dieldrin and BHC, such effects of dieldrin are less striking and more moderate than those of BHC. The advance of the poisoning symptoms of dieldrin is also much slower than that of BHC even the dose of dieldrin applied is as much as sixteen-fold the dose of BHC[17].

The slow advance of the dieldrin symptoms is in accordance with the observations of Harvey & Brown[2] on the respirations of poisoned insects. Such moderate action of dieldrin upon nerve seems partly responsible for so-called "slow insecticidal action" of this insecticide.

One discrepancy between the present observation and the observation made by other authors should be noted here. Although Lalonde and Brown[4] found the short trains of impulses of low voltage in the sensory nerves two hours after painting dieldrin on the cockroach leg, we could not detect any sign of train following injection of dieldrin in the leg or in the cockroaches showing ataxia or convulsion following topical application of dieldrin. However, these two observations cannot be compared with ease, because the method of application, the duration of observation and the purity of dieldrin are different between them.

The mechanisms involved in the prolongation of synaptic after-discharges and the increase in spontaneous activity of the nerve cord are left to be solved. Similar effects are also observed with the treatments of anticholinesterases[3,7,11,19]. In the case of anticholinesterases a prolongation of synaptic after-discharges is usually followed by a synaptic block, but this is not the case with dieldrin. Futhermore it has been demonstrated that dieldrin is without effect *in vitro* on the activity of cholinesterase[1]. Hence it can safely be said that such effects of dieldrin upon nerve are not due to the inhibition of cholinesterase.

Summary

The effects of dieldrin on nervous activity of the cockroach were studied in order to clarify the mechanism of development of dieldrin poisoning symptoms.

The course of poisoning symptoms of dieldrin was as follows : latent period, period of ataxia, convulsive period, paralytic period and death.

Spontaneous activity in the abdominal nerve cord increased markedly and synaptic after-discharges in the sixth abdominal ganglion prolonged remarkably in the cockroach showing ataxia. The synaptic after-discharges were maintained at the prolonged level when the

poisoning symptoms advanced to the initial stage of paralysis, while the enhanced spontaneous activity in the abdominal nerve cord progressively decreased during that period.

The increase in spontaneous activity in the abdominal nerve cord and the prolongation of synaptic after-discharges were also found when the dieldrin suspended Ringer's solution had been applied directly to the nerve.

The sensory discharges in the crural nerve showed no sign of changes in the cockroaches developing ataxia or convulsion or following injection of dieldrin suspension in the leg.

The mechanism of development of dieldrin poisoning symptoms was discussed comparing those of DDT and BHC which had been reported previously. The poisoning symptoms of dieldrin is different from those of both DDT and BHC. Prolonged synaptic after-discharges and increased spontaneous activity make it difficult for the cockroach to coordinate its movement, causing ataxia. Further progress of such changes in nervous activity makes it quite impossible for the cockroach to coordinate its movement, causing knockdown and convulsion. Paralysis of the cockroach is caused by the progressive decline in nervous activity with the advance of time.

The effects of dieldrin upon nerve were less striking and more moderate than those of BHC, which seems to be partly responsible for slow insecticidal action of dieldrin.

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第6回国際熱帯病及びマラリヤ会議

本会議は本年9月5日から13日までポルトガル政府後援のもとにリスボンで開催されます．これについて準備委員長の J. Fraga de Azevedo 教授から，防虫科学研究所宛に協力方および可能ならば代表を派遣するよう依頼して来ております．なお出席申込書とともに会議の内容を記したパンフレットも参つておりますから，その由こゝにお知らせいたします．