

Studies on the Insecticidal Action of Nereistoxin, 4-*N,N*-dimethylamino-1, 2-dithiolane. II. Symptomatology. Michihiko SAKAI (Kyoto Herbal Garden, Research and Development Division, Takeda Chemical Industries, Ltd. Ichijoji, Sakyo-ku, Kyoto). Received January 21, 1966. *Botyu-Kagaku* 31, 53. 1966

8. イソメ毒 (Nereistoxin, 4-*N,N*-dimethylamino-1, 2-dithiolane) の殺虫作用に関する研究 II. 中毒症状 坂井 道彦 (武田薬品工業株式会社 研究開発本部 京都試験農園) 41. 1. 21 受理

イソメ毒の殺虫効果については既に報告した¹⁾が、その作用機構についての知見は得られていないので、これを解析するための第一段階として中毒症状の様相を検討した。

チャバネゴキブリにイソメ毒を処理すると、やや運動が激しくなり、麻痺前に軽度であるが脚の痙れんが発生するなど、一種の興奮症状が認められた (Fig. 1)。イエバエでも脚の tremor が麻痺前に起った。

イエバエ脚筋より誘導した自発性活動電位はイソメ毒処理急激に spike の頻度と振幅が増大し、神経系に異常な興奮が起ったことを示した (Fig. 3)。この spike 発生の過程は中毒症状の進行過程と平行性が見られた (Table 1)。

チャバネゴキブリの呼吸量はイソメ毒処理後増大するが、速やかに平常値に戻った (Fig. 5)。筋肉組織呼吸に対してはイソメ毒は何らの作用も示さないことから、虫体呼吸量の増大は興奮による運動量の増大にともなうものとみなされる。

このように、イソメ毒の殺虫作用は神経系に対する興奮作用と関連していることが明らかであるが、その興奮の様相は一般の殺虫剤のそれと異なっていて (Fig. 1, Fig. 3)、イソメ毒特有の殺虫作用機構があることが推察される。

In the previous report on the insect toxicological properties of nereistoxin, 4-*N,N*-dimethylamino-1, 2-dithiolane, the author noticed that the symptoms of the poisoned insect by nereistoxin were quite dissimilar to those of known insecticides, such as organophosphates or chlorinated hydrocarbons¹⁾.

Although the preliminary observations were incidental to the toxicological experiments, it was found that the insects poisoned by nereistoxin manifest little hypersensitivity, convulsion or agony. These symptoms which are likely to be specific to nereistoxin are presumed to be derived from a particular mechanism of insecticidal action of this compound.

The experiments reported herein show the results of the symptomatological investigations of nereistoxin-poisoned insects.

The results would be valuable as a step to approach the mechanism of insecticidal action of nereistoxin.

Materials and methods

Insects The insects used in the experiments

were the adult male German cockroach (*Blattella germanica* L.), the adult female house fly (*Musca domestica* L.) which was susceptible to insecticides (lab-em-7-em strain) and the adult male American cockroach (*Periplaneta americana* L.). These insects were reared at the temperature of 25°C.

Chemicals Pure nereistoxin hydrogen oxalate which was synthesized²⁾ was used throughout the experiments. The term "nereistoxin" designates the hydrogen oxalate in this paper. The insecticides employed for comparison were DDVP and lindane. The purity of these compounds were 92% and 99% respectively.

Observations of toxic symptoms To observe the general symptoms of the poisoned German cockroach, the insect was topically treated with acetone solution of nereistoxin on its ventral side of the abdomen. In another test the solution of the compound dissolved in 0.9% NaCl was injected into the thorax of cockroach.

The symptoms were also observed in the house fly injected with nereistoxin. Since the injection

of nereistoxin caused paralysis promptly in the fly, the early symptoms would be masked by the prior anaesthesia with carbon dioxide, if the insect was kept free. Therefore, to observe the symptoms accurately, the fly which was anaesthetized with carbon dioxide was mounted at the tip of a tooth-pick by fixing the tergum with the aid of bees wax. After the insects recovered from the anaesthesia, the nereistoxin dissolved in 0.9% NaCl solution was injected into the thorax.

Entomography of German cockroach The leg-jerk entomogram was recorded kymographically³⁾. Namely the insect was fixed with an adhesive tape ventral side up on a small glass plate. Then the left hind leg was connected to the lever with a fine silk thread and the movement of the leg was magnified and recorded on a smoked paper on a kymographion. Insecticidal solution prepared with acetone were applied topically on the ventral side of the abdomen.

Effects on the nervous action potentials The effects of nereistoxin on the nervous activity were investigated with house fly and the German cockroach. For the house fly, the method of Yamasaki and Narahashi⁴⁾ was resorted to; viz. the action potentials were led off by a pair of electrodes, one electrode being inserted in the femur of left hind leg while the other one in the abdomen. The insecticidal solution prepared with a Ringer solution was perfused onto the exposed thoracic ganglion.

To investigate the relationship between the symptoms and the aspects of nervous discharges, the house fly which was injected with nereistoxin was settled for leading off the discharges from the left hind leg at a certain time after the injection.

In the experiments with the German cockroach the electrodes were inserted as same as in the test with the house fly. The sternum of thorax was carefully removed leaving the right hind leg intact for leading off the action potentials. The exposed thoracic nerve cord was treated with the insecticidal solution.

Throughout the above-mentioned experiments, the action potentials were observed and recorded by a cathode ray oscilloscope. The experiments were conducted at the temperature of 25°C. The

Ringer solution used in the experiments consisted of 214 mM Na⁺, 3.1 mM K⁺ and 1.8 mM Ca⁺⁺, its pH was kept at 7.4 by phosphate buffer⁴⁾.

Measurement of the respiratory rate The effect of nereistoxin on the respiratory rate was determined by the standard Warburg's method at the temperature of 30°C and expressed as the oxygen uptake. To test the effects in the intact insects, three German cockroaches were kept in a flask. Immediately before putting the insects into the flask, the acetone solution of nereistoxin was topically applied on the ventral side of abdomen. For the determination of the effects of nereistoxin on the tissue respiration, the femur of American cockroach weighing approximately 200 mg was floated in 1 ml of the Ringer solution in the flask. The muscle was exposed to the nereistoxin by removing a small portion of the cuticle. The solution of nereistoxin (1 ml) prepared with the Ringer solution was tipped into the flask from the side arm.

Results

Symptoms of German cockroach When the cockroach was treated topically on the ventral side of abdomen with nereistoxin, for example at the dosage of 100 µg/g, the first symptom was a restless locomotion though it was a rather sluggish motion; viz. the insect walked around a little actively in the glass jar keeping the insects. This stage set in after a latent period of about 5 min. Besides the cleaning of the abdomen or the antennae with the legs was observed. About 15 min after the treatment, the locomotion was gradually depressed and the normal posture could not be sustained. However, the cockroach could move in response to a mechanical stimulus such as a touch with a forceps.

In the course of passing over to the complete prostration, weak twitches of the legs manifested in a low frequency. Thus the prostration set in; the insect was immobilized or paralysed. Some of the insects lay on back with continuous slow movement of legs and antennae. This movement of the appendages disappeared in the late period of the prostration stage. The antennae lost straightness. Although the prostration by this dosage of nereistoxin continued over 10 hours, the insect recovered from the poisoning¹⁾ taking an

approximately reversed sequence of the poisoning symptoms.

In the observation with lower and higher dosages, the same symptoms appeared in about the same sequence as described above, excepting that the first restless locomotion and the twitches of legs in the prostrated insect scarcely manifested in the test with a lower dosage.

Different symptoms were evoked by the injection of nereistoxin. Immediately after the treatment with the dosage of $50 \mu\text{g/g}$, the insect was paralysed promptly. After about 2 min. the insect was completely paralysed. The sequence of the symptoms resembled to that in the injected house fly as described later.

Entomograms of German cockroach In the experiments of leg-jerk entomography of the cockroach, the characteristic waves were recorded from the insect poisoned by nereistoxin. A typical record is shown in Fig. 1. Immediately after

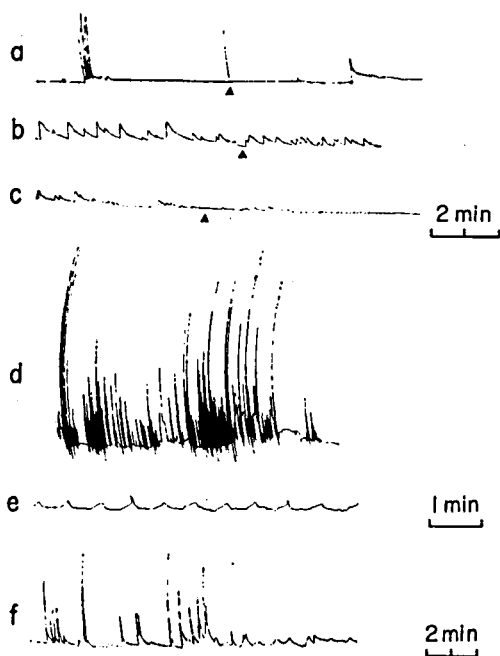


Fig. 1. Leg-jerk entomograms of the poisoned adult German cockroaches, *Blattella germanica* L. a ~ c: nereistoxin $125 \mu\text{g/g}$; a: 0~10 min. after the treatment; b: 11~19 min.; c: 23~34 min.; d, e: DDVP $200 \mu\text{g/g}$; d: 0~5 min.; e: 30~36 min.; f: lindane $50 \mu\text{g/g}$, 58~72 min.

▲: mechanical stimulation.

the application of nereistoxin, quickly repetitive jerks appeared (Fig. 1 a). These jerks are considered apparently not to be an artifact because the other insecticides tested in the experiments or acetone alone did not evoke the jerks of this kind. That the jerk appeared as a result of mechanical stimulus indicated that the insect was not completely knocked down or prostrated.

The recorded waves that followed were intermittent repetitive waves of quick flexion and slow extension (Fig. 1 b). The frequency and the amplitude were rather small and the locomotion continued for a fairly long period. The jerk of leg resulted from the reaction to a mechanical stimulus already disappeared in this period. The locomotion gradually decreased in its amplitude followed by the complete immobilization (Fig. 1 c).

The entomograms recorded from the cockroaches poisoned by DDVP and lindane were quite different from those of nereistoxin. The followings are the typical records from the insects poisoned by these insecticides.

DDVP: Twitches of high frequency with superimposed flexion and extension set in immediately after the treatment (Fig. 1 d). Frequently the succession of twitches altered with the slowly and irregularly repetitive locomotion with low amplitude and frequency. Following occurred jerk was repetitive with low amplitude and its flexion and extension were slow (Fig. 1 e). In the early period of this stage a single twitch was superimposed on each of the slow jerks. The waves continued with fairly regular frequency until the insect was completely prostrated.

Lindane: In the early period of the poisoning preceded by the latent period, several twitches were frequently superimposed on a single flexion of low amplitude (Fig. 1 f). The twitches gradually lost their intensity and only weak tremors remained before the complete prostration.

Symptoms of the poisoned house fly The posture and the locomotion of legs were mainly observed on the house fly fixed on a tooth-pick. The succession of symptoms of intoxication by nereistoxin appeared in the main three stages as described below.

1) Primary prostration stage: Almost immediately after the injection, the fly was narcotized

becoming flaccid and loosing leg prehensivity. Sometimes legs were half contracted. The insect was passive and only reactive to a strong mechanical stimulus.

2) Leg-tremoring stage: In the second stage, a fine tremor or a vibration of legs appeared. The tremoring legs almost extended. The tremor was high in frequency and successive. The reaction to a mechanical stimulus disappeared completely. The abdominal part extended. Sometimes defecation was observed. The degree of tremor was low at the beginning in the stage and gradually increased, then decreased reaching to the following stage. The flection of slightly tremoring legs appeared at the end of this stage.

3) Complete prostration stage: The tremor disappeared, then the fly was prostrated completely. The legs were in a contracted posture. The abdominal part still extended.

The sequence of symptoms of the house fly injected with various dosages is shown in Fig. 2. Although the three stages apparently appeared in turn in the flies treated with a high dosage (50 $\mu\text{g/g}$) of nereistoxin, the order was considerably disarranged in the test with low dosages (6 and 20 $\mu\text{g/g}$) in which some insects recovered from the poisoning. In some cases of the low

dosage tests, the tremor continued more than a day, but in the others it was interrupted by the prostration.

In the poisoning of house fly by DDVP and lindane which were topically applied and injected, the sequence of the symptoms were quite different from that of nereistoxin. The most distinct difference of nereistoxin was the manifestation of the "primary prostration" which did not appear in the poisoning by the other insecticides.

Effects on the nervous discharges In the house fly nereistoxin caused an increase in the frequency of motor discharges. The development of the burst of discharges evolved in the order described below. A typical oscillographical record is shown in Fig. 3.

Stage 1: After a very short latent period, small spikes manifested in a fairly high frequency.

Stage 2: The spikes of somewhat higher potentials were superimposed on the spike train of lower potentials. The superimposed spikes were forming a train gradually (Fig. 3 b).

Stage 3: The violent outburst of spike train was established with the increase in the frequency and the amplitude. Besides, the characteristic projection of enormous spikes occurred increasing the frequency in degrees. In this stage the

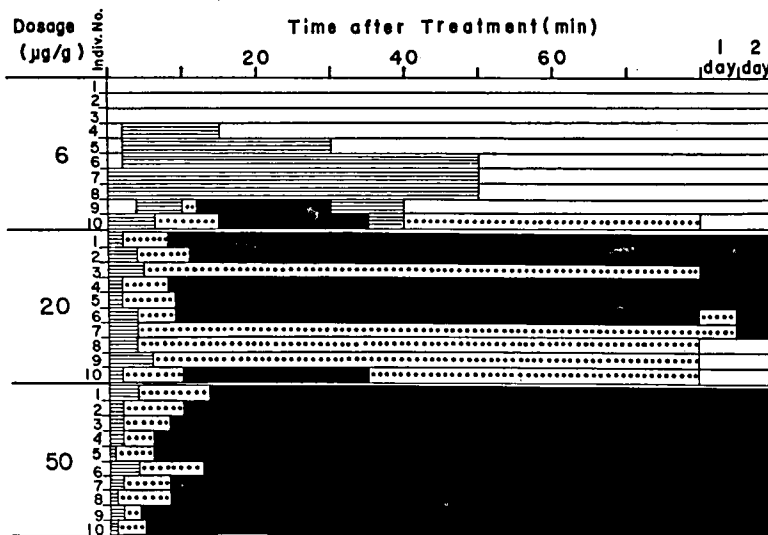


Fig. 2. The time sequence of the toxic symptoms in the female house flies, *Musca domestica* L. injected with nereistoxin. blank: normal, lined: primary prostration, dotted: leg-tremoring, dark: complete prostration. The detailed explanation on the symptoms is in the text.

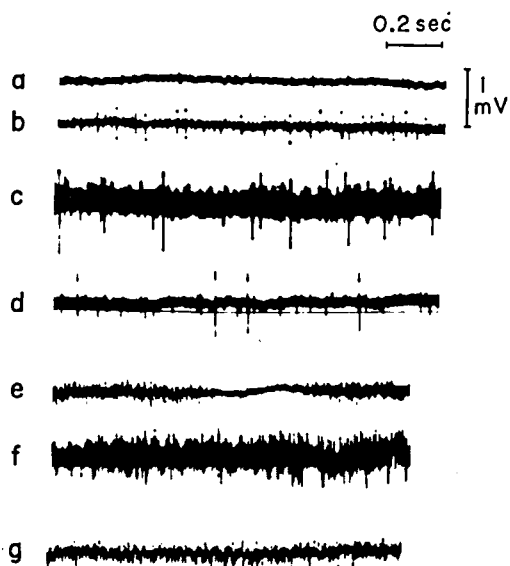


Fig. 3. The motor discharges led off from the hind legs of the ganglion-exposed female house flies, *Musca domestica* L. a : normal (before treatment), b ~ d : nereistoxin 10^{-4} M; b : 5min. after the treatment; c : 14 min., d : 21min., e, f : lindane 5×10^{-5} M; e. : 13 min.; f : 17 min.; g: DDVP 10^{-4} M, 2 min.

burst of discharges provoked the tremor of legs (Fig. 3c).

Stage 4 : The frequency of enormous spike gradually diminished, the continuous spike train still remaining. Then the amplitude of the trained spikes gradually decreased.

Stage 5 : The intermittent spikes occurred protruding on the continuous train of a low amplitude (Fig. 3d).

Stage 6 : The superimposed spikes in Stage 5 did not appear any more. Finally the discharges diminished gradually. The further application of nereistoxin did not manifest any changes in the discharges, even if the concentration of the compound was higher than that in the prior treatment.

Although it was difficult to make demarcation of the different stages for the gradual changes that followed one another, the approximate times of the initiation of the stages could be determined by the continuous observation on the oscillograms. The sequence of the abnormal discharges evoked by nereistoxin is summarized in Table 1.

When the ganglion of the house fly was exposed to the perfusate of nereistoxin, the higher concentration shortened the period of each stage. When treated with the high concentrations (10^{-3} and 10^{-4} M), each of the stages proceeded orderly. In preparations treated with lower concentration, however, the stages appeared irregularly. In the five preparations treated with 10^{-4} M, one of them manifested the extraordinary sequence of the stages, and the lowest concentration (5×10^{-5} M) evoked the repetitive appearance of the different stages of the discharges for a long period.

The relationship between the nervous discharges and the symptoms was observable from the result obtained with the flies injected with nereistoxin (Table 1). The house flies in the "primary prostration" stage evoked the discharges of Stages 1 and 2. As observed on the flies of which ganglion was exposed, it was evident that also in the injected flies the tremor of legs due to the violent burst occurred in Stage 3. Since the injection of low dosages of nereistoxin scarcely evoked the leg-tremor and the complete prostration as observed in the experiments on the symptoms (Fig. 2), the injection of $6 \mu\text{g/g}$ caused only Stage 1 and Stage 2 in most cases.

The action potentials led off from the house flies treated with lindane and DDVP did not bear any resemblance in their aspects to those from the insect treated with nereistoxin. The treatment with lindane caused intermittent spike trains (Fig. 3e) which were followed by the spike trains of higher potentials (Fig. 3b). DDVP caused the burst of discharges, but the potentials were comparatively low (Fig. 3g).

The treatment of the German cockroach with nereistoxin initiated a stimulatory effects in the motor discharges led off from the legs (Fig. 4). The spike discharges evoked by the compound were rather weaker than in the house flies.

Effects on the respiratory rate The amount of oxygen uptake was increased to a great extent in the cockroaches treated with nereistoxin. Yet, the increase was promptly restored to the normal extent. No detectable changes were observed compared with the normal insect after the restoration to the normal extent (Fig. 5).

The respiratory activity of the isolated muscle

Table 1. Sequence of motor discharges in the female house flies, *Musca domestica* L. treated with nereistoxin.*

Concentration or dosage of nereistoxin	Indiv. no.	Stage of nervous discharges					
		1	2	3	4	5	6
<i>Perfusate</i>							
10 ⁻³ M	1	0.5	1	1.5	3.5	4.5	7.5
	2	0.5	1.5	2	4	6	9
2×10 ⁻⁴ M	1	1	3	5	7	10	10
	2	2	5	6	7	9	14
	3	1	4	7	9	10	15
10 ⁻⁴ M	1	2	3	5	7	8	10
	2	1.5	3	6	8	9	12.5
	3	2	6	12.5	16	17	19
	4	2	3.5	12.5	24	48	60
5×10 ⁻⁵ M	5**	{	3	5.5	13		
				15	16.5		
				18	21		
	1**	{	2	11	19		
				19.5	20		
				21	22		
24				25			
56							
<i>Injection</i>							
20 μg/g	{	2 pp	3 pp	10 lt	30 lt	15 cp	20 cp
		2 pp	4 pp	10 lt		15 lt	30 lt
		4 pp		15 lt		20 cp	
				15 lt			
6 μg/g	{	10 pp	10 pp	50 lt***			
		20 pp	20 pp				

* The explanation on the stages of the nervous discharges is in the text. In the test with the perfusate, the numerals indicate the approximate times (min.) of the initial appearance of the specified stages after the treatments. The numerals in the test of injection indicate the times (min.) when the injected flies were settled for the oscillography. The abbreviations are the stages of the toxic symptoms; thus, pp: primary prostration, lt: leg-tremoring, cp: complete prostration. The detailed explanations of each stage are in the text.

** The stages appeared irregularly.

*** The complete prostration preceded.

of the American cockroach was almost unaffected by 5×10^{-4} M of nereistoxin. Thus, the amount of oxygen consumed were 288 μ l/h/g before the exposure and 301 μ l/h/g after the exposure.

Discussion

As it was revealed from the results of present experiments, there were distinct differences in the symptoms of the poisoning by nereistoxin from other common insecticides. The effects of nereistoxin on the locomotion and the nervous discharges indicated that nereistoxin is a nervous excitant. Although many common insecticides including those tested for the comparisons in the present experiments are well-known for the ab-

normal nervous excitation in insects, the results of entomography and oscillography indicated that the mode of action of nereistoxin is characteristically different from those of the other insecticides.

In previous paper published by some workers, only simple descriptions are given on the symptoms of the insect poisoned by nereistoxin; the poisoned insects were likely to be anaesthetized followed by the revival from the poisoning^{6,7)}. On the vertebrate the report on the symptoms of nereistoxin was early published by Nitta²⁾. According to his report, when nereistoxin hydrogen oxalate was injected, the general sequence

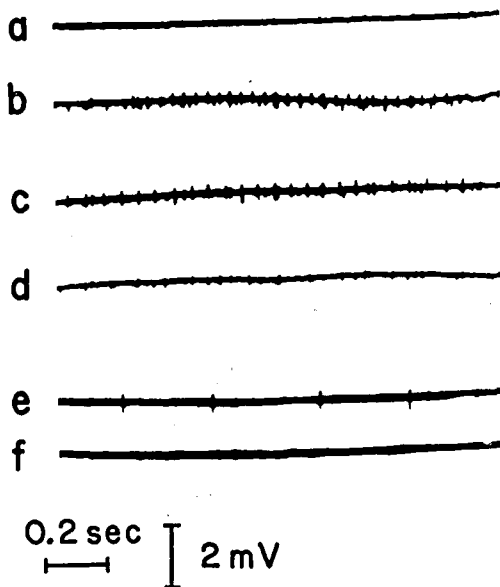


Fig. 4. The motor discharges led off from the hind leg of the adult German cockroach, *Blattella germanica* L. of which central nerve cord was exposed. Nereistoxin 10^{-4} M. a: before treatment, b: 2 min. after the treatment, c: 4 min., d: 7 min., e: 24 min., f: 27 min.

of symptoms was: firstly the sluggish posture appeared, then excitation tetanic contraction, convulsion or tremor and the test animal finally passed into the prostration. The symptoms in the insect as described in the present report, rather resembled those in the vertebrate. That

is, when the house fly or the cockroach was injected with nereistoxin, the symptom appeared at first was a kind of prostration which was followed by tremor of legs or muscle. Then the complete prostration set in continuing until death. However the German cockroach which was treated topically on the cuticle manifested neither the primary prostration nor the tremor of legs. Obvious tetanic movements in the cockroach were only the weak twitches of legs which were also recorded in the entomograms. Thus, in nereistoxin-poisoned insects, the more resemblance to the vertebrate injected with nereistoxin was apparent in the case of injected insects than in those treated on their cuticle.

Although two different treatments, the injection and the topical application, apparently yielded the different sequence in the poisoning symptoms of German cockroach, the symptoms in the topical application resembled those in the injection of low dosages in many cases. In the house fly, unfortunately, the topical application of the compound has no effect¹⁾, but many individuals which were injected with low dosages did not manifest the tremors (Table 1 and Fig. 2). These facts suggest that if the dosages which were able to evoke the tremors with the injection were applied topically on the cuticle, the actual concentration in the insect body must have been low on account of slow penetration to the

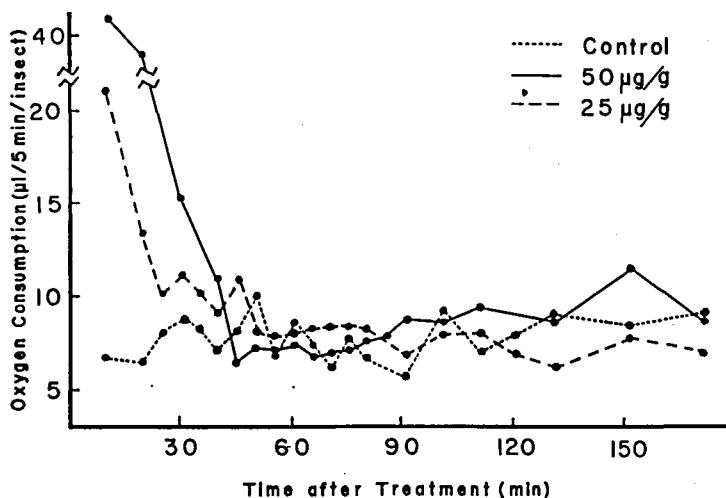


Fig. 5. The respiratory rate of the adult German cockroaches, *Blattella germanica* L. treated with nereistoxin topically on the cuticle.

cuticle or the degradation in the insect body.

Since the knock-down or the "primary prostration" was induced by the low dosages in the house fly (Fig. 2), although the insects revived from the prostration, the primary action of nereistoxin is conceivable not to relate to the manifestation of the tremor or legs which can be seen with the naked eye.

From the results of electro-physiological experiments, it was obvious that nereistoxin has an activity to stimulate the nervous discharges. The compound increased the motor discharges in the *in-situ* preparations of the house fly and the German cockroach. It is presumed that in these experiments nereistoxin behaved in the same way as it was injected into the insect body, since the compound must have reached to the nervous tissue directly. Therefore, as shown in Table 1, the appearance of the effect on the nervous discharges was quite similar between the preparations in the perfusate experiment and those which were injected.

In the house fly injected with nereistoxin and being in the "primary prostration", the discharges promptly increased in their amplitude and frequency (Table 1). The increase was also observed in the early period in the preparations of which nervous tissue was exposed (Table 1 and Fig. 3). However, the discharges were less violent in these periods (Stages 1 and 2) in the degree of burst than those appeared in the following period corresponding to Stage 3. In addition some of the insects which were injected by low dosages manifested neither the tremors nor the violent burst of discharges (Table 1). Considering that the violent burst associated with the tremor of legs, these results proved the knock-down of the insect or the manifestation of the "primary prostration" is related to the evocation of the spike discharges in a low level (Stage 1 and 2) which were observed in the early period after the treatment.

However it is still uncertain that the tremor of legs and the violent burst of discharges were not the direct results of the primary action of nereistoxin, but probably a nervous deterioration.

There was no evidence to indicate the direct effects of nereistoxin on the tissue respiration of

the cockroach muscle. Hence the increase of the respiratory rate in the nereistoxin-poisoned cockroach is considered to be due to the excitatory effects on the nervous discharges.

Although the possible mechanism of the insecticidal action of nereistoxin is obscure at the present time, it is noticeable that the effects of the compound on certain organs in the vertebrate coincide with the stimulatory effect on the insect. That is, in the vertebrate certain dosages stimulate the reflex arc of spine, the heart pulsation and the muscle contraction of the intestine, the uterus and the pupil. However, higher dosages provoke the tetanus of the muscle⁶⁾.

On the other hand, the roles of atropine and eserine on the action of nereistoxin in the vertebrate⁶⁾ would be worthwhile to pay attention to study the possible function of cholinergic system as a site of action of nereistoxin. In fact the recent study by the author showed nereistoxin antagonize acetylcholine in the contraction of rectus abdominis muscle of frog.^{7),8)}

Summary

The symptoms of the poisoning by nereistoxin in the German cockroach and in the house fly were investigated.

In the cockroach the topical application of the compound provoked the excitation, that is, the hyper-activity in a low level at the early period of the intoxication and the intermittent twitches of legs which were followed by the prostration in the late period (Fig. 1). The tremor of the leg of house fly injected with nereistoxin appeared to be preceded by the "primary prostration" and followed by the "complete prostration".

The motor discharges led off from the legs of the house fly and the cockroach increased in the frequency and the amplitude by perfusing nereistoxin onto the exposed ganglions or by the injection into the body (Fig. 3 and 4).

In the house fly the manifestation of the spike trains in the nervous potentials was associated with the symptoms of the poisoned insect (Table 1). That the "primary prostration" associated with the manifestation of the low amplitude spike train (Stages 1 and 2 in the sequence of the abnormal discharges by nereistoxin) indicates that the primary action of the compound is excitation.

The sequence of symptoms and that of the burst of discharges in the insects treated with nereistoxin were quite different from those treated with common insecticides (Fig. 1 and 3).

The increase of the respiratory rate in the nereistoxin-poisoned cockroach (Fig. 5) is considered to be due to the nervous excitation, since the compound has no detectable effects on the tissue respiration of the muscle.

It is concluded from the results of the present experiments that nereistoxin acts as a characteristic nervous excitant of which mechanism of action is different from those of common insecticides.

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also acknowledges Dr. S. Asahina, the National Institute of Health, Mr. R. Hatta and Dr. T. Kobayashi in the author's laboratory for their suggestion to continue the work.

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Studies on the Insecticidal Action of Nereistoxin, 4-N,N-dimethylamino-1,2-dithiolane.
III. Antagonism to Acetylcholine in the Contraction of Rectus Abdominis Muscle of Frog.
Michihiko SAKAI (Kyoto Herbal Garden, Research and Development Division, Takeda Chemical Industries Ltd. Ichijōji, Sakyō-ku, Kyoto) Received February 23, 1966. *Botyu-Kagaku*, 31 61. 1966

9. イソメ毒 (Nereistoxin, 4-N,N-dimethylamino-1,2-dithiolane) の殺虫作用に関する研究 III. Acetylcholine によるカエル腹直筋収縮に対する拮抗作用 坂井道彦 (武田薬品工業株式会社 研究開発本部・京都試験農園) 41. 2. 23 受理

従来報告されているイソメ毒の脊椎動物における薬理作用から、イソメ毒がコリン作動性の器官の一部、特にアセチルコリン (Ach) 受容体に作用することが予想される。

カエル腹直筋を用いた実験の結果、本薬物はかなりの低濃度で筋の Ach による収縮を抑制するが、筋自体の収縮能は高濃度でも抑制されないことを確めた。イソメ毒のみの施用は筋に収縮反応を起さなかった。従って、イソメ毒はカエル腹直筋においては Ach 受容体を Ach に対して拮抗的に閉塞するものであって、受容体の depolarizant ではなく、また筋自体の収縮能を抑制するものでもないことが認められる。このことから、おそらく昆虫においてもコリン作動性器官の Ach 受容体がイソメ毒の作用点であることが想像される。

イソメ毒濃度と筋の Ach による収縮を抑制する効果とは一定の関係があったので、これを利用してイソメ毒の topical application で中毒したチャバネゴキブリ体内のイソメ毒を定量したところ、イソメ毒は速やかに表皮を透過して体内に侵入すること、および中毒からの回復は体内のイソメ毒の量の減少に伴って起ることを認めた。

In the previous report of the author, it was pointed out that the insecticidal action of nereistoxin^{1,2)} is due to its excitant effect on the

nervous discharges³⁾. However, the mechanism of its action is still obscure at the present time. To investigate the mechanism in insects, it is