

=1.05) and α, β -dipiperonylidensuccinic acid (D. S. =2.01) showed synergism with pyrethrins, but *meso*- α, β -dibenzylsuccinic-, *meso*- α, β -dipiperonylsuccinic-, *dl*- α -benzyliden- β -benzylsuccinic-, α, β -dibenzylidensuccinic-, *dl*- α -benzylsuccinic-, *dl*- α -piperonylsuccinic-, *trans*- α -benzylidensuccinic- and α -piperonylidensuccinic acid showed no synergism with the same. Whereas, all of 10

compounds showed no synergism with allethrin.

It seems that such unique synergistic activity of hibalactone with pyrethrins and allethrin is caused not only by the presence of 3,4-methylenedioxyphenyl group and double bond but by possession of γ -lactone ring or peculiar steric configuration.

Effects of Metabolic Inhibitors, Potassium Ions and DDT on Some Electrical Properties of Insect Nerve: Studies on the Mechanism of Action of Insecticides. XV. Teruo YAMASAKI and Toshio NARAHASHI* (Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Tokyo, Japan). Received Oct. 29, 1957. *Botyu-Kagaku* 22, 354-367, 1957.

60. 昆虫神経の二、三の電気的性質に及ぼす代謝阻害剤、K イオンおよび DDT の影響：
殺虫剤の作用機構に関する研究 第15報 山崎輝男・檜橋敏夫** (東京大学農学部害虫学研究室)
32. 10. 29 受理

DDT は神経の静止代謝よりもむしろ興奮代謝に働くかあるいは神経原形質膜に直接物理化学的に働いて機能変化をもたらす、という筆者らの仮説を証明するための一連の研究の一つとして、本報では昆虫神経の静止電位、活動電位、電気緊張電位、および興奮性に及ぼす二、三の代謝阻害剤や K イオンの影響を DDT と比較した。その結果仮説を支持する二、三の実験結果がえられ、ともに、代謝阻害剤の作用機構や陽極通流作用についても興味ある知見がえられた。

Resting potential of cockroach nerve has been shown to be depressed by treating with metabolic inhibitors and to be unaffected by the application of DDT. A hypothesis concerning the mode of action of DDT upon nerve has thus been presented that such changes in nerve function under the influence of DDT as an augmentation of repetitive excitability and an increase in the negative after-potential are brought about not so much by an inhibition of the "resting metabolism" of nerve as by a disturbance of the "active metabolism" of nerve or by a direct physico-chemical action on the nerve membrane causing changes in ionic permeability of the membrane¹⁵.

Excitability of nerve is known to depend partly upon the resting potential, and the latter in turn is supported by metabolic energy in the nerve, or the resting metabolism. On the other hand, maintenance of excitability is known to require in addition other source of metabolic energy, or the active metabolism¹⁵. Electrotonic potential of

nerve is known to depend on the resting potential and to have some correlation with electrical properties of the nerve membrane⁶.

In the light of such views, the present experiments were undertaken to gain further informations supporting our hypothesis, dealing with the simultaneous determinations of the changes in the resting potential, excitability, and the electrotonic potential of the nerve under the influence of metabolic inhibitors, potassium ions, or DDT, and also dealing with the effects on the DDT-induced negative after-potential of metabolic inhibitors, potassium ions, or electrotonus.

Materials and Methods

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

Nerve preparations: The isolated nerve cord, including the metathoracic ganglion and the whole abdominal ganglia, was used. It contains several giant axons, which were described in our previous paper¹⁵.

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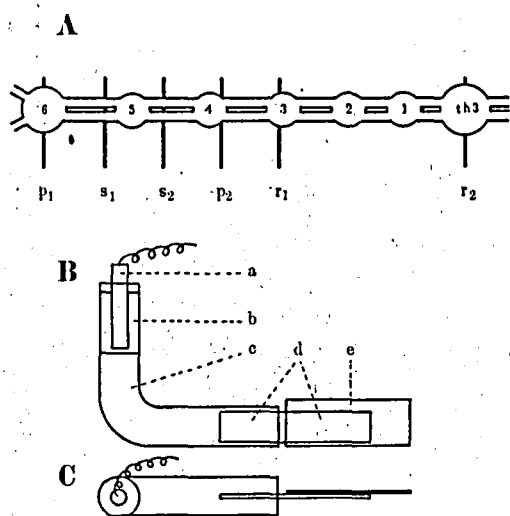


Fig. 1. Diagram of the arrangement of electrodes (A), side-view of a Zn-ZnSO₄ non-polarizable electrode (B), and its top-view (C). 1~6, the 1st~6th abdominal ganglia; th3, the 3rd thoracic ganglion; p_1 & p_2 , polarizing electrodes; s_1 & s_2 , stimulating electrodes; r_1 & r_2 , recording electrodes; a, a rod of Zn; b, ZnSO₄-saturated solution; c, Ringer-gelatin; d, a thick paper soaked with ZnSO₄-saturated solution; e, a filter paper soaked with Ringer's solution.

Stimulations and recordings: Arrangement of electrodes is illustrated by Fig. 1. Electrodes p_1 and p_2 are Zn-ZnSO₄ Ringer-gelatin type non-polarizable electrodes and were used as polarizing electrodes. Electrodes s_1 and s_2 are fine silver wires and were used as stimulating electrodes. Electrodes r_1 and r_2 are the same type as p_1 and p_2 and were used as recording electrodes. The region of the electrode p_1 and a small region between the electrodes r_1 and r_2 were crushed with forceps. The interference caused by the coupling between the stimulating electrodes s_1 and s_2 and the polarizing electrodes p_1 and p_2 could be avoided by an insertion of a resistance as high as 200 kilo-ohms in series with the secondary circuit of inductorium.

Actual structure of a Zn-ZnSO₄ non-polarizable electrode was devised so as to be applicable to the small roach nerve cord and is illustrated by Fig. 1.

A constant polarizing current with short dura-

tion of about 100 msec, usually 2~3 μ A, was applied through the electrodes p_1 and p_2 , while an electrotonic potential was recorded with the electrodes r_1 and r_2 . A brief electric shock, being a thyatron discharge intervening inductorium, was applied through the electrodes s_1 and s_2 while an action potential was recorded with the electrodes r_1 and r_2 . When the effect of electrotonus on the excitability of nerve was to be tested, electrical stimuli were delivered through the electrodes s_1 and s_2 while a polarizing current with various strength and duration, usually 2 to 10 μ A and 30 seconds to several minutes, was being applied through the electrodes p_1 and p_2 . The recording electrodes r_1 and r_2 were also used for measuring the change in the resting potential under the influence of drugs.

The distance between any two electrodes was slightly different in each experiment, but the distance between p_2 and r_1 was made as constant as possible, being within the range of 2~3 mm, because the magnitude and shape of the electrotonic potential recorded by the electrodes r_1 and r_2 on passage of a square pulse through the electrodes p_1 and p_2 depended on the distance between p_2 and r_1 as well as on the strength of the current. The interelectrode distance between p_2 and r_1 is shown in each Table or Figure.

The electrotonic potentials and the action potentials led off from the electrodes r_1 and r_2 were fed to a DC amplifier and were observed and photographed by a cathode ray oscilloscope.

Solution and drugs: The Ringer's solution used was the same as that described in our previous paper¹⁵. Metabolic inhibitors used were 5×10^{-2} M sodium monoiodoacetate, 5×10^{-2} M and 7.5×10^{-2} M sodium fluoride, 1.5×10^{-3} M, 3.1×10^{-3} M, 4.6×10^{-3} M and 1.5×10^{-2} M potassium cyanide, and 1.3×10^{-4} M rotenone. Rotenone was recently demonstrated to have a strong inhibitory action on glutamic dehydrogenase⁴. They were dissolved in Ringer's solution except for rotenone. Rotenone was applied as a suspension which had been made by an injection of the rotenone acetone solution into Ringer's solution. Potassium-rich solution was prepared by mixing Ringer's solution with

isotonic potassium solution, giving a final potassium concentration of $8.5 \times 10^{-2} \text{M}$. $1.4 \times 10^{-4} \text{M}$ *p,p'*-DDT suspended Ringer's solution was prepared by an injection of the DDT ethanol solution into Ringer's solution. Acetone or ethanol alone, being the concentration of 0.5 per cent, had no effect on the electrical properties of nerve to be examined.

Treatment with drugs: The drug-contained Ringer's solution was applied to small regions of the nerve cord with which the electrodes p_2 and r_1 were in contact and which were located between those electrodes.

These experiments were carried out at the beginning of autumn and in winter at room temperatures ranging from 24 to 26.5°C and from 14 to 18°C respectively. The temperature at any series of experiment was not varied over 0.5°C.

Results

Normal nerve: Oscillograms obtained with normal nerves are shown in each upmost row of Figs. 2, 5 and 10. The presence of a high resistance in the secondary circuit of inductorium caused a large artefact in recording the action potential; however, this artefact did not make it impossible to distinguish the action potential from the artefact and also to observe the after-potential. The negative spike action potential was followed by a positive phase, but no after-potential in either phase was observed.

A catelectrotonic potential was usually smaller in magnitude than an anelectrotonic potential. The former was superimposed with an action potential and often in addition with a series of small action potentials following the initial large action potential when the catelectrotonic current applied was stronger than the rheobase. The latter or the anelectrotonic potential sometimes showed an overshoot during or after the passage of current.

A cathodal or an anodal current with the strength of less than about $10 \mu\text{A}$, did not cause a block of conduction. The cathodal current of such strength had little effect on the shape of the action potential, while the anodal current, reversibly caused an appearance of a large negative after-potential.

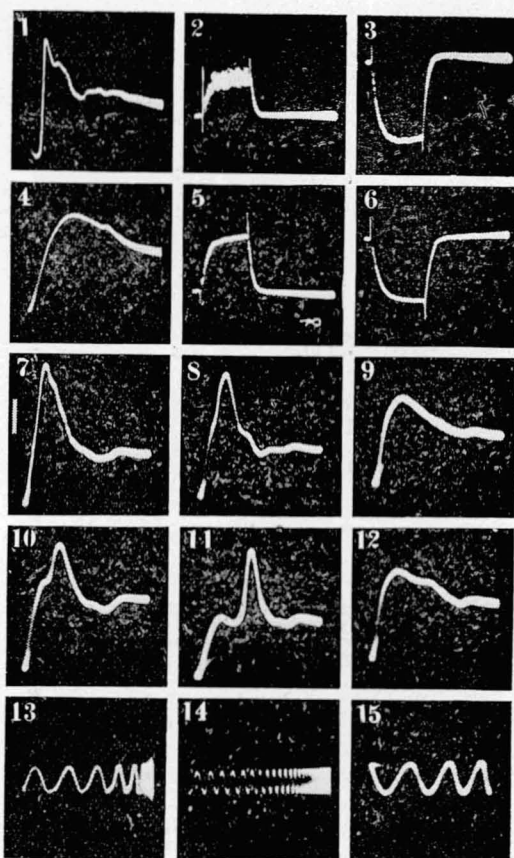


Fig. 2. Effects of $5 \times 10^{-2} \text{M}$ sodium monoiodoacetate.

1~6, conduction block and changes in electrotonic potentials, 26.5°C. 1, a normal action potential, a vertical line shows 5mV; 2 & 3, a catelectrotonic(2) and an anelectrotonic(3) potential produced at 2.5mm from the polarizing electrode by an applied short current of $3.0 \mu\text{A}$; 4, 40 minutes after application of the drug, a conduction block, only an artefact is visible; 5 & 6, a catelectrotonic(5) and an anelectrotonic(6) potential, the same time as 4.

7~12, conduction block and restoration by anodal current, the interelectrode distance $p_2 r_1$ is 1.5mm, 28.5°C. 7, a normal action potential, a vertical line shows 5 mV; 8, 31 minutes after application of the drug, a partial block; 9, 45 minutes after, a complete block; 10, 47 minutes after, immediately after applying $10 \mu\text{A}$ anodal current, a partial restoration; 11, 2 minutes after applying the anodal current, a complete restoration; 12, 15 seconds after breaking the anodal current, a reproduction of block. 13, 1000 c.p.s. for records 1 and 4; 14, 50 c.p.s for records 2, 3, 5 and 6; 15, 1000 c.p.s for records 7~12.

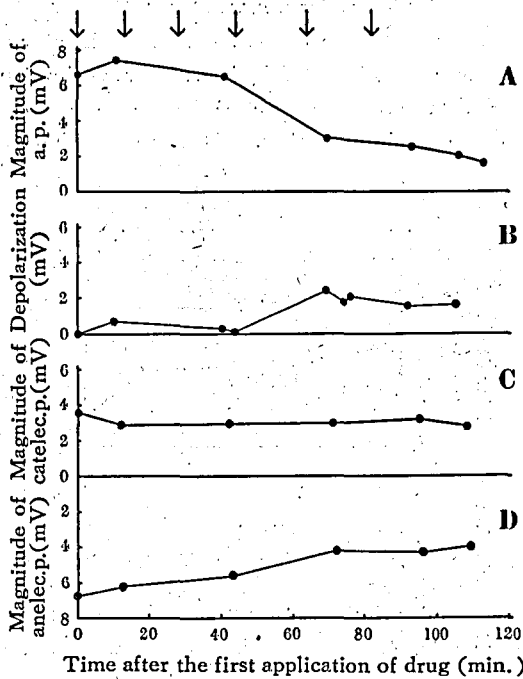


Fig. 3. Effects of $5 \times 10^{-2} \text{M}$ sodium monoiodoacetate on action potential (A), resting potential (B), catelectrotonic potential (C) and anelectrotonic potential (D). Each arrow indicates the time of drug application. The interelectrode distance p_2r_1 is 3.1mm. The strength of applied current for the production of the electrotonic potential is $2.5 \mu\text{A}$. 17.5° .

Sodium monoiodoacetate (IAA): A series of oscillograms is shown in Fig. 2, and one example of experiment is illustrated by Fig. 3. An application of $5 \times 10^{-2} \text{M}$ IAA to the nerve caused a depolarization followed by a conduction block. The depolarization started about 20 to 40 minutes after applying IAA. The action potential began to decrease in magnitude about 30 to 60 minutes after applying IAA, and completely disappeared about 1 to 2 hours after the application when the depolarization of nerve attained to 3 to 5mV. The magnitude and shape of the catelectrotonic potential were not changed during the advance of the depolarization and conduction block under the influence of IAA. On the other hand, the anelectrotonic potential was progressively depressed in magnitude by less than half of the original potential value during that period, keeping its shape unchanged. When an anelectrotonus

was applied to the depolarized and blocked region of the nerve, the action potential reappeared on an electrical stimulus. The magnitude of the action potential progressively grew up to the normal level during 30 to 60 second passage of the anodal current. This restoration was removed within several seconds upon switching off the anodal current. The latent period between the stimulus artefact and the action potential was prolonged in the restored nerve under anelectrotonus, indicating the decrease in the velocity

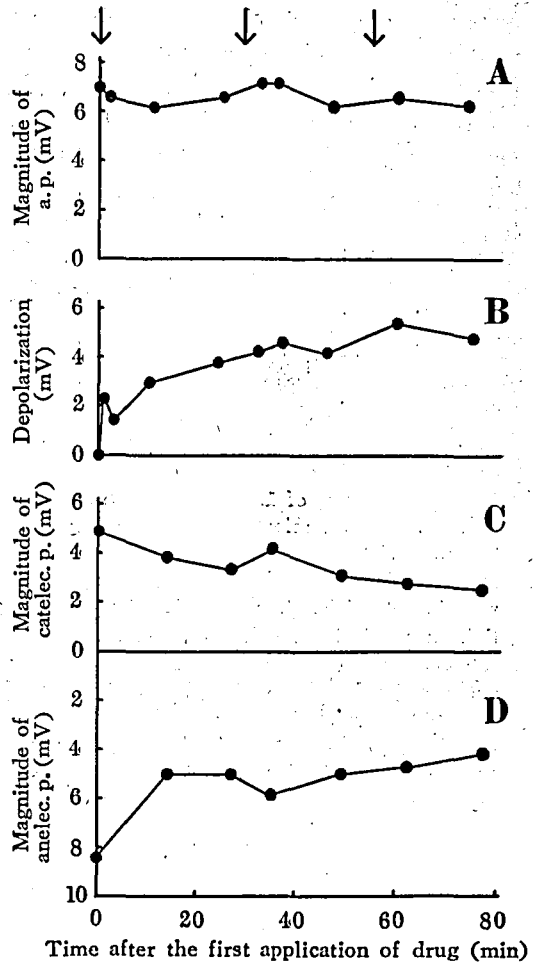


Fig. 4. Effects of $7.5 \times 10^{-2} \text{M}$ sodium fluoride on action potential (A), resting potential (B), catelectrotonic potential (C) and anelectrotonic potential (D). Each arrow indicates the time of drug application. The interelectrode distance p_2r_1 is 2.8mm. The strength of applied current for the production of the electrotonic potentials is $2.5 \mu\text{A}$. 17.5° .

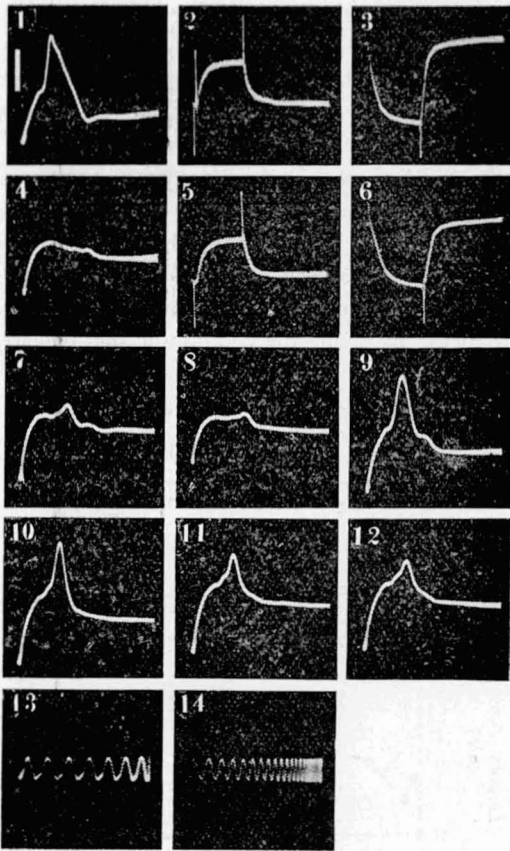


Fig. 5. Effects of 1.5×10^{-2} M potassium cyanide. The interelectrode distance $p_2 r_1$ is 3 mm. 16.5° .

1~6, conduction block and changes in electrotonic potentials. 1, a normal action potential, a vertical line shows 5mV; 2 & 3, a catelectrotonic (2) and an anelectrotonic (3) potential produced by an applied short current of $2.0 \mu A$; 4, 6 minutes after application of the drug, a conduction block, only an artefact is visible; 5 & 6, a catelectrotonic (5) and an anelectrotonic (6) potential, the same time as 4.

7~12, conduction block and restoration by anodal current. 7, 10 minutes after application of the drug, an incomplete block; 8, 30 seconds after applying $3.0 \mu A$ anodal current, no restoration; 9, 5 seconds after breaking the anodal current that continued for a period of 40 seconds, a complete restoration; 10, 10 seconds after breaking, little change; 11, 30 seconds after breaking, a reproduction of partial block; 12, 60 seconds after breaking, a further development of block.

13, 1000 c.p.s. for records 1, 4, and 7~12; 14, 50 c.p.s. for records 2, 3, 5 and 6.

of conduction under anelectrotonus,

Sodium fluoride (NaF): One example of experiment is illustrated by Fig. 4. An application of 5×10^{-2} M or 7.5×10^{-2} M NaF to the nerve caused a depolarization. It started within 10

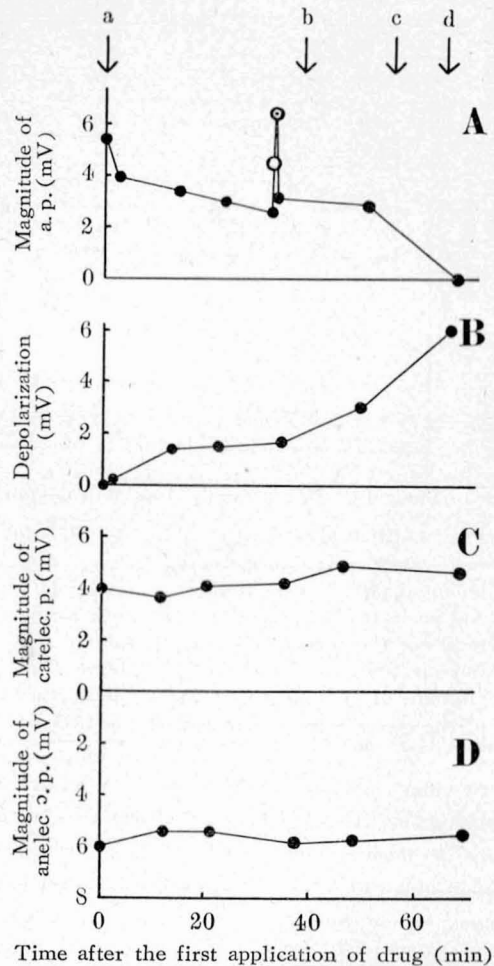
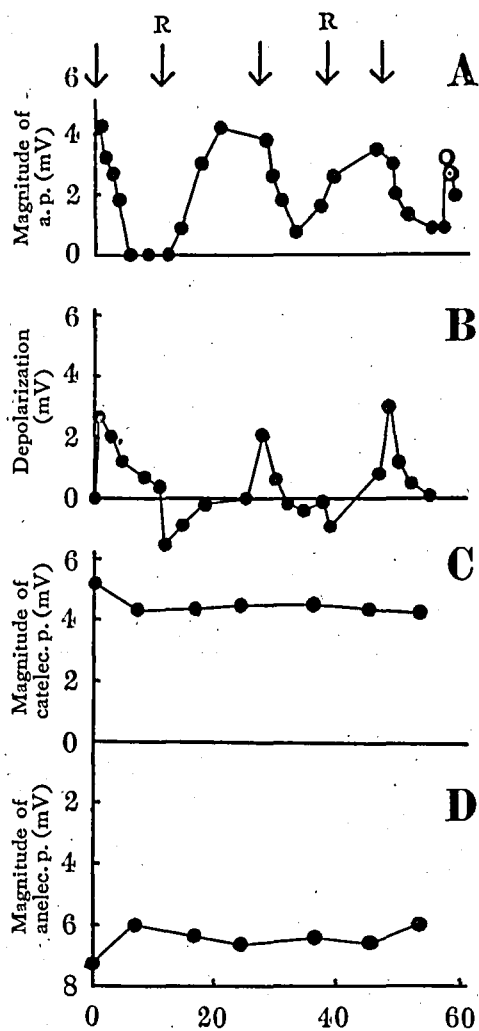


Fig. 6. Effects of low concentrations of potassium cyanide on action potential (A), resting potential (B), catelectrotonic potential (C) and anelectrotonic potential (D). The arrows a, b and c indicate the times of applying 1.5×10^{-3} M KCN, and the arrow d 3.1×10^{-3} M KCN. An open circle indicates the action potential recorded during the application of $2.0 \mu A$ anodal current, and a double circle indicates the action potential recorded immediately after breaking the anodal current. The interelectrode distance $p_2 r_1$ is 2.7 mm. The strength of applied current for the production of the electrotonic potentials is $2.0 \mu A$. 16° .



Time after the first application of drug (min)

Fig. 7. Effects of high concentration ($1.5 \times 10^{-2}M$) of potassium cyanide on action potential (A), resting potential (B), catelectrotonic potential (C) and anelectrotonic potential (D). The arrows without symbol indicate the times of KCN application, and the arrows with symbol R indicate those of the applications of normal Ringer. An open circle indicates the action potential recorded during the application of $3.0 \mu A$ anodal current, and a double circle indicates the action potential recorded immediately after breaking the anodal current. The interelectrode distance $p:r_1$ is 3.0mm. The strength of applied current for the production of the electrotonic potential is $2.0 \mu A$. 16.5° .

mimutes after applying NaF and attained to 5 to 10 mV within 1 to 2 hours, but a conduction block was never observed. The catelectrotonic potential was depressed in magnitude in most cases during the advance of the depolarization by NaF, and the anelectrotonic potential was depressed in magnitude in all cases. The depressed values of the both electrotonic potentials often reached over half of the original potential values. The shape of the both electrotonic potentials, however, remained almost unchanged.

Potassium cyanide (KCN): A series of oscillograms is shown in Fig. 5, and one example of experiment is illustrated by Figs. 6 and 7. An application of $1.5 \times 10^{-3}M$ or $3.1 \times 10^{-3}M$ KCN to the nerve caused a depolarization followed by a conduction block. The resting and the action potentials began to decrease within 10 minutes after applying KCN, and the conduction block occurred about 1 hour after the application when the depolarization attained to about 2 to 5mV. Both the catelectrotonic and the anelectrotonic potentials were not changed in both magnitude and shape in about half of the total cases, while they were slightly depressed in magnitude in another half keeping their shape unchanged. The depolarized and blocked region of the nerve could be restored by an anelectrotonus. The magnitude of the action potential progressively grew up to the normal level during 30 to 60 second passage of the anodal current, whereas in such seriously affected nerve as had been left in the blocked condition for some period of time the restoration was incomplete or even unsuccessful. This restoration was removed upon switching off the anodal current, but it took about 30 to 60 seconds to reproduce the conduction block. It was actually observed that the action potentials immediately before and after switching off the anodal current remained constant in magnitude, and that in some cases the magnitude of the action potential completely recovered immediately after switching off the anodal current although the recovery had been incomplete during the passage of current. The latent period between the stimulus artefact and the action potential was prolonged during the

passage of anodal current, indicating the decrease in the velocity of conduction under anelectrotonus.

An application of $4.6 \times 10^{-3}M$ or $1.5 \times 10^{-2}M$ KCN to the nerve produced a rapid depolarization of 2 to 5mV in most cases, but the depolarization was restored within several minutes in spite of the continuous presence of KCN. In some cases they had no effect on the resting potential. In a few cases of $4.6 \times 10^{-3}M$ KCN treatment a persistent depolarization, which was not followed by a repolarization, was actually observed, while it was not observed by the treatment with $1.5 \times 10^{-2}M$ KCN. A conduction block was usually brought about within 10 to 20 minutes after applying $4.6 \times 10^{-3}M$ or $1.5 \times 10^{-2}M$ KCN even when the resting potential had already returned to the normal level until that time. Both the catelectrotonic and the anelectrotonic potentials were either not affected or slightly depressed in magnitude keeping their shape unchanged in nearly half of the total cases. In a few cases the anelectrotonic potential was found to be slightly depressed in magnitude, the catelectrotonic potential being kept unchanged, whereas only one reverse case was encountered. The recovery from the conduction block of the nerve treated with $4.6 \times 10^{-3}M$ or $1.5 \times 10^{-2}M$ KCN by an anelectrotonus was the same as that in the case of $1.5 \times 10^{-3}M$ or $3.1 \times 10^{-3}M$ KCN which had been described in the foregoing section.

Rotenone: One example of experiment is illustrated by Fig. 8. An application of $1.3 \times 10^{-4}M$ rotenone to the nerve caused a depolarization followed by a conduction block. Both the resting and the action potentials began to decrease together within 10 to 20 minutes after applying rotenone, and the conduction block occurred within 20 to 60 minutes after the application when the depolarization attained to 4 to 9 mV. The catelectrotonic potential was not affected during the advance of the depolarization and block. The anelectrotonic potential was not affected in more than half of the total cases, and was slightly depressed in magnitude keeping its shape unchanged in the remaining cases. When an anelectrotonus was applied to the blocked

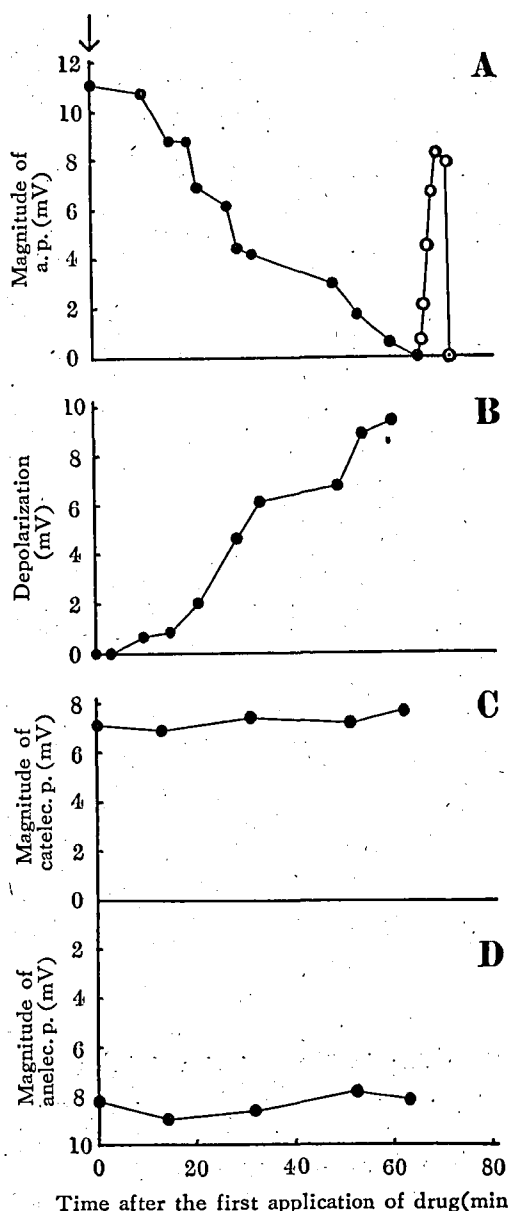
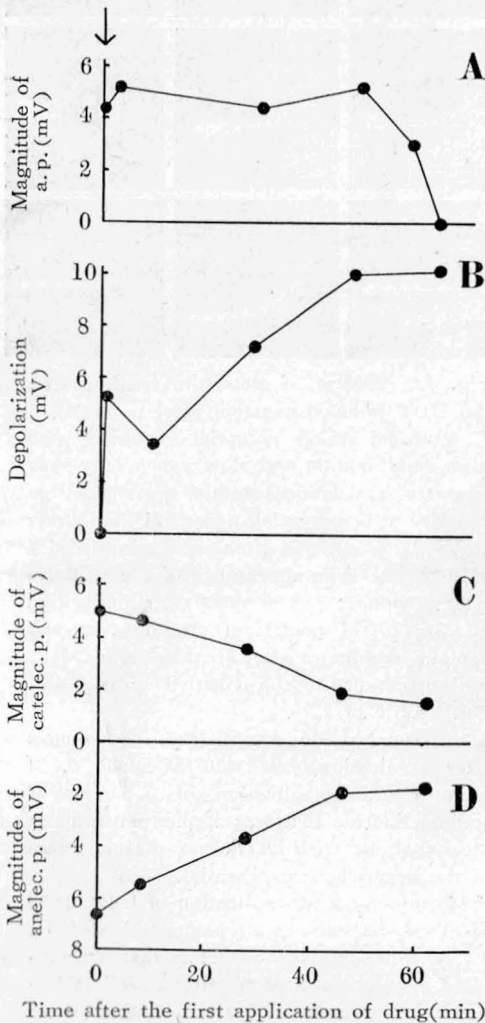


Fig. 8. Effects of $1.3 \times 10^{-4}M$ rotenone on action potential (A), resting potential (B), catelectrotonic potential (C) and anelectrotonic potential (D). The arrow indicates the time of drug application. Open circles indicate the action potentials recorded during the application of $10 \mu A$ anodal current, and a double circle indicates the action potential recorded immediately after breaking the anodal current. The interelectrode distance p_2r_1 is 3.0mm. The strength of applied current for the production of the electrotonic potentials is $2.0 \mu A$. 14.5° .

region of the nerve, the action potential appeared on stimulation within 30 to 60 seconds after the onset of the anodal current and grew progressively up to the normal level during several minute passage of the current. But the restoration was rapidly removed immediately after switching off the anodal current.

Potassium ions: One experiment is illustrated by Fig. 9. An application of $8.5 \times 10^{-2} M$ K^+ to



Time after the first application of drug(min)

Fig. 9. Effects of $8.5 \times 10^{-2} M$ potassium ions on action potential (A), resting potential (B), catelectrotonic (C) and anelectrotonic potential (D). The arrow indicates the time of drug application. The interelectrode distance $p_2 r_1$ is 3.0mm. The strength of applied current for the production of the electrotonic potentials is $3.0 \mu A$. 26.5° .

the nerve caused a rapid depolarization, and a conduction block was brought about when the depolarization advanced by over about 10mV. Both the catelectrotonic and the anelectrotonic potentials were markedly depressed in magnitude during the advance of the depolarization, and their slow phases were much more depressed than their rapid phases. The block could be restored by an anelectrotonus, which occurred progressively during 30 to 60 second passage of the anodal current and was removed within 30 seconds after switching off the current.

DDT: A series of oscillograms is shown in Fig. 10, and one example of experiment is illustrated by Fig. 11. The resting potential was not affected in most cases by an application of $1.4 \times 10^{-4} M$ DDT, and was slightly decreased in a few cases. A conduction block was never observed. The catelectrotonic potential was not depressed in magnitude, but its shape

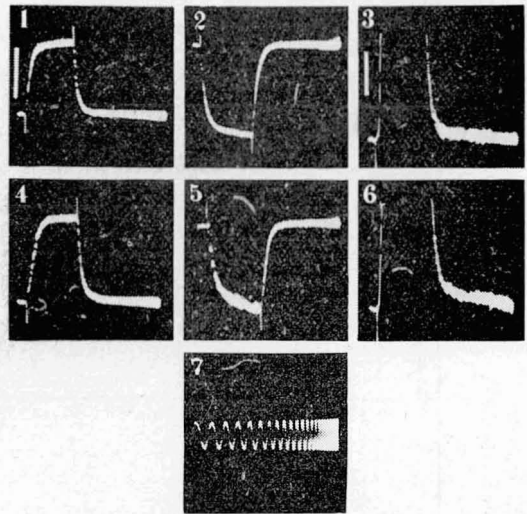


Fig. 10. Effects of $1.4 \times 10^{-4} M$ DDT on the electrotonic potentials produced at 2.0 mm from the polarizing electrode by an applied short curring of $1.5 \mu A$. $25^\circ C$. 1, a normal catelectrotonic potential, a vertical line shows 5mV; 2, a normal anelectrotonic potential; 3, a normal catelectrotonic potential recorded with high amplification, a vertical line shows 1 mV; 4 & 5, 58 minutes after application of the drug, a catelectrotonic (4) and an anelectrotonic (5) potential; 6, the same time as the records 4 and 5, a catelectrotonic potential recorded with high amplification, note a slow repolarization; 7, 50 c. p. s.

was slightly changed; its falling phase was delayed. The anelectrotonic potential was not affected in about half of the total cases, while it was slightly depressed in magnitude keeping its

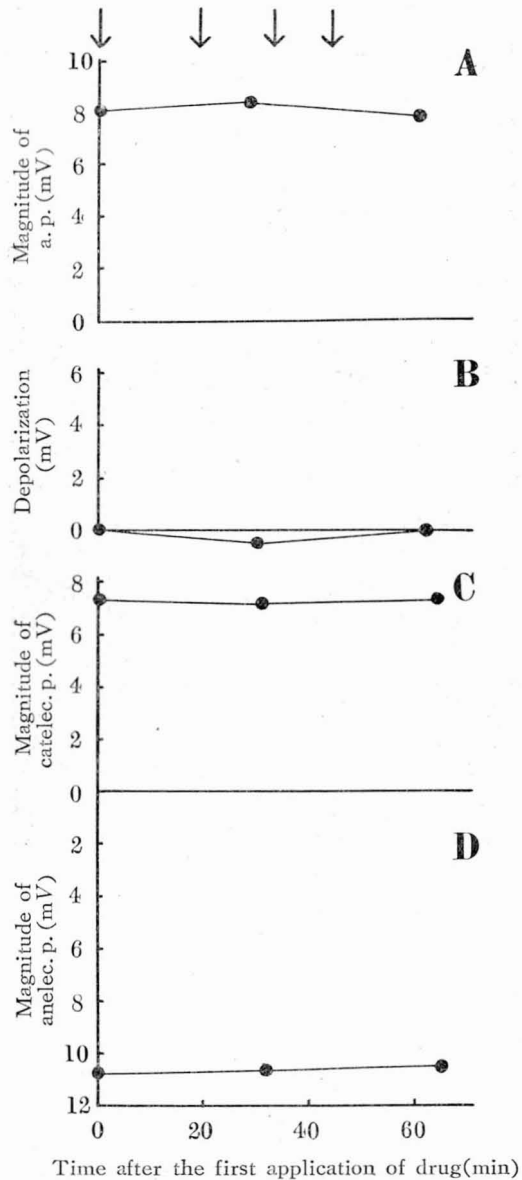


Fig. 11. Effects of 1.4×10^{-4} M DDT on action potential (A), resting potential (B), catelectrotonic potential (C) and anelectrotonic potential (D). Each arrow indicates the time of drug application. The interelectrode distance $p-r_1$ is 2.9 mm. The strength of applied current for the production of the electrotonic potentials is $2.0 \mu A$. 16.5° .

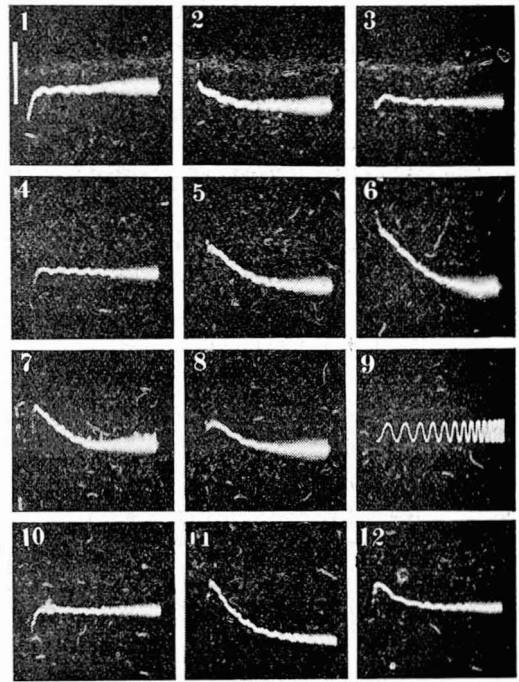


Fig. 12. Effects of metabolic inhibitors on the DDT-induced negative after-potential.

1, a normal action potential recorded with high amplification and slow sweep, only slow phases of the action potential are visible, no negative after-potential, a vertical line shows $1 mV$; 2, 56 minutes after application of 1.4×10^{-4} M DDT, an appearance of the negative after-potential; 3, 2 minutes after application of 4.6×10^{-3} M potassium cyanide that was applied 58 minutes after treating with DDT, a disappearance of the negative after-potential, 16.5° .

4, a normal action potential; 5, 60 minutes after application of 1.4×10^{-4} M DDT; 6, 24 minutes after application of 7.5×10^{-2} M sodium fluoride that was applied 68 minutes after treating with DDT, no disappearance of the negative after-potential, 16.5° .

7, 46 minutes after application of 1.4×10^{-4} M DDT; 8, 52 minutes after application of 5×10^{-2} M sodium monoiodoacetate that was applied 57 minutes after treating with DDT, a marked depression of the negative after-potential, 17° . 9, 50 c. p. s.

10, a normal action potential; 11, 67 minutes after application of 1.4×10^{-4} M DDT; 12, 33 minutes after application of 1.3×10^{-4} M rotenone that was applied 70 minutes after treating with DDT, a marked depression of the negative after-potential, 16.5° .

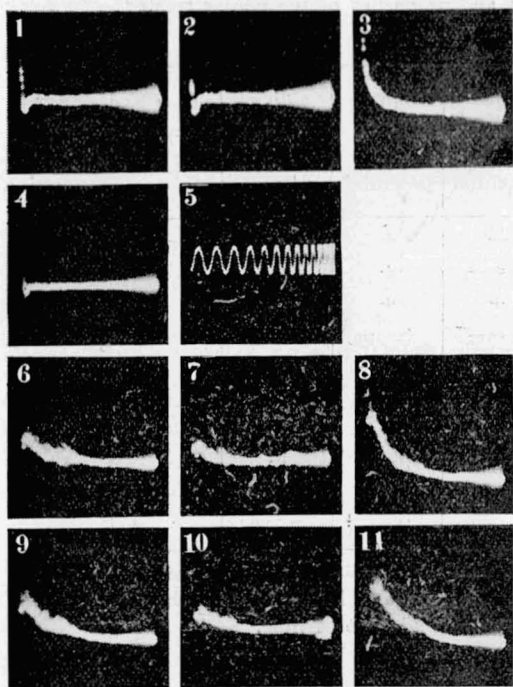


Fig. 13. Effects of electrotonus and potassium ions on negative after-potential.

1~3, effects of electrotonus on the action potential in a normal nerve, only slow phases of the action potential are visible, the interelectrode distance $p_2 r_1$ is 2.0mm, the strength of applied current is $1.5\mu A$, $25^\circ C$. 1, a normal action potential, a vertical line shows 1 mV; 2, 15 seconds after applying cathodal current, no effect; 3, 15 seconds after applying anodal current, an appearance of the negative after-potential.

4 & 6~11, effects of electrotonus and potassium ions on the DDT-induced negative after-potential, the interelectrode distance $p_2 r_1$ is 3.5mm, the strength of applied current is $2.0\mu A$, $24.5^\circ C$. 4, a normal action potential; 6, 45 minutes after application of $1.4 \times 10^{-4} M$ DDT, an appearance of the negative after-potential; 7, 15 seconds after applying cathodal current, a marked depression of the negative after-potential; 8, 15 seconds after applying anodal current, a marked augmentation; 9, 6 minutes after application of $8.5 \times 10^{-2} M$ potassium ions that was applied 59 minutes after treating with DDT, on effect; 10, 15 seconds after applying cathodal current, a marked depression; 11, 15 seconds after applying anodal current, a marked augmentation. 5, 50 c.p.s.

shape unchanged in the other half.

The effects of metabolic inhibitors, potassium ions and electrotonus on the DDT-induced negative after-potential are shown in Figs. 12 and 13. The negative after-potential which had been augmented and prolonged by the treatment with DDT was further augmented and prolonged by an anelectrotonus, the spike action potential being kept unchanged. An application of $5 \times 10^{-2} M$ IAA, $3.1 \times 10^{-3} M$ or $4.6 \times 10^{-3} M$ KCN. or $1.3 \times 10^{-4} M$ rotenone to the nerve developing the marked negative after-potential by the treatment with DDT caused a disappearance of the negative after-potential before a disappearance of the spike action potential. An application of $7.5 \times 10^{-2} M$ NaF, however, did not affect the DDT-induced negative after-potential, though NaF depolarized the nerve treated with DDT. $8.5 \times 10^{-2} M K^+$ did not selectively depress the negative after-potential, causing a simultaneous disappearance of both the spike action potential and the negative after-potential.

Discussion

The results of whole experiments are summarized in Table 1.

Depolarization of nerve caused by sodium monoiodoacetate (IAA), sodium fluoride, potassium cyanide or potassium ions is the same as that reported previously¹⁵. The results of the present experiments, however, show some important features concerning the mode of action of metabolic inhibitors upon nerve. In the first place, the block of conduction is not always dependent on the magnitude of depolarization caused by metabolic inhibitors. Since the conduction block is produced when the nerve is depolarized by over 10 mV by applying potassium ions which seem to have no inhibitory action on metabolism, the depolarization as large as 10mV seems to indicate the critical level of the resting potential to maintain excitability without suffering from metabolic disturbance. NaF does not cause the block although it depolarizes the nerve by 5 to 10 mV. This action of NaF may therefore be explained by the assumption that NaF does not

Table 1. Summary of changes in functions and properties of the nerves treated with drugs.

Drug	Concentration (M)	Depolarization	Conduction block	Depression of catelectrotonic potential	Depression of anelectrotonic potential	Depression of DDT-induced negative after-potential	Anodal restoration	After effect of anodal current on restoration
Sodium monoiodoacetate	5×10^{-2}	+	+	—	+	+	+	+
Sodium fluoride	5×10^{-2}	+	—	+ = —	+			
//	7.5×10^{-2}	+	—	+	+	—		
Potassium cyanide	1.5×10^{-3}	+	+	+ = —	+ = —		+	+
//	3.1×10^{-3}	+	+	+ = —	+ = —	+		
//	4.6×10^{-3}	+ → —	+	+ < —	+ > —	+	+	+
//	1.5×10^{-2}	+ → — > —	+	+ < —	+ > —		+	+
Rotenone	1.3×10^{-4}	+	+	—	+ < —	+	+	—
Potassium ions	8.5×10^{-2}	+	+	+	+	—	+	+
DDT	1.4×10^{-4}	+ < —	—	—	+ = —			

+ or — means presence or absence of the effect respectively, + = — means that the effect appears in about half of the total cases, + > — means that the effect appears in much more than half of the total cases, and + → — means that the effect disappears with advance of time.

affect the "active metabolism" of nerve but does affect the "resting metabolism"¹⁵⁾. On the contrary, IAA causes the block when it produces the depolarization as small as 3 to 5 mV. It seems therefore that IAA has inhibitory actions on both the resting and the active metabolisms. Rotenone may occupy an intermediate position between NaF and IAA, for the nerve is blocked when it is depolarized by 4 to 9 mV after treating with rotenone. Therefore, rotenone seems to inhibit both the resting and the active metabolisms, though its action on the active metabolism may be weaker than that of IAA. KCN seems to have dual actions on the nerve; the depolarization of 2 to 5 mV caused by lower concentrations of KCN is followed by a conduction block, while the depolarization of the same amount caused by higher concentrations of KCN is initially followed by a repolarization and then followed by a conduction block which is produced in spite of the maintenance of the normal resting potential level. These effects of KCN lead to the suggestion that KCN has inhibitory actions on both the resting and the active metabolisms and the action on the latter is stronger than that on the former.

In the second place, the differences in the mode of action of metabolic inhibitors are also

shown by the experiments of electrotonic potentials. The changes in the catelectrotonic and the anelectrotonic potentials are different in each inhibitor. The explanations for such changes in the electrotonic potentials, however, cannot be made at present.

In the third place, the conduction block caused by metabolic inhibitors or potassium ions can be restored by anodal current. This phenomenon is actually observed in other nerves^{2,6,12-14)}, and the restoration is usually considered to be due to the repolarization of nerve which was brought about by the anodal current. Though this explanation seems at first to be proper, careful observations and considerations throw some doubts. The anodal current has a restorative action not only on the depolarized and blocked nerve by the treatment with IAA, lower concentrations of KCN, rotenone or potassium ions but also on the blocked nerve with the normal resting potential by the treatment with higher concentrations of KCN. This evidence suggests the possibility of restoration due to the cause other than the repolarization. This suggestion is supported by the dramatic findings that electrical stimulations cause an increase in oxygen consumption not only of brain slices but also of mitochondria

preparation of brain^{1,3,5,7-11}). Under such an experimental condition, there is no doubt that the brain slices cannot generate the action potentials on electrical stimulations. Therefore, the electrical stimulations must affect the brain slices through their electrochemical actions upon protoplasmic constituents such as mitochondria. Under such considerations as these, it seems to be probable that anodal current restores the conduction block by causing electrochemical activation of some enzymatic constituents of axoplasm inhibited by high concentrations of KCN. Another point to be mentioned concerning the anodal restoration is the fact that the process of the anodal restoration is more or less different in each drug. The reproduction of conduction block occurs immediately after the removal of anodal current in the nerve treated with IAA or rotenone, whereas it takes about 30 to 60 seconds to reproduce the complete block in the nerve treated with KCN or potassium ions. If the anodal restoration were a simple physical event occurring at the nerve membrane, these differences would not be expected because the resting potential rapidly returns to the depolarized level upon switching off the anodal current whatever drug is applied to the nerve. These findings are also in favour of the electrochemical explanation mentioned above. This suggestion, however, is highly speculative at present, and the prevailing explanation that the anodal restoration is caused by the repolarization of nerve membrane is not implied beyond the bound of possibility.

Some of the results of experiments with DDT are to be expected from our previous reports¹⁵⁻¹⁷; the resting potential is not affected in most cases, the conduction block is never observed, and the falling phase of catelectrotonic potential is slightly delayed. These findings support our hypothesis concerning the mode of action of DDT upon nerve¹⁵ described in the preface of the present paper. The slight depression of the anelectrotonic potential observed in some of the experiments cannot be explained at the present time.

The DDT-induced negative after-potential is demonstrated to be not simply dependent upon

the resting potential. This finding supports the view that the DDT-induced negative after-potential cannot be explained in terms of a simple change in ionic flux such as a decrease in the rate of potassium efflux during the falling phase of the action potential¹⁷. Furthermore, another important point to be mentioned is presented by this experiment. The metabolic inhibitors considered in the foregoing section to inhibit the active metabolism of nerve, i.e., IAA, KCN and rotenone, have actually the depressant action on the DDT-induced negative after-potential. On the other hand, the drugs considered to have no effect on the active metabolism of nerve, i.e., NaF and potassium ions, have actually no effect on the DDT-induced negative after-potential. These results seem to indicate that the increase in the negative after-potential is caused by a stimulating action of DDT on the active metabolism of nerve. This suggestion, however, is highly speculative at present, and further experiments are necessary to draw a conclusion.

Summary

The present investigation was undertaken to gain further informations supporting our hypothesis concerning the mode of action of DDT upon nerve which had been described in the previous paper. Observations were made on the resting potential, excitability and the electrotonic potential of the cockroach nerve under the influence of metabolic inhibitors, potassium ions or DDT, and the effects of metabolic inhibitors, potassium ions or electrotonus on the DDT-induced negative after-potential were also examined.

1. The catelectrotonic and the anelectrotonic potentials were observed in the normal nerve.

2. A cathodal current, the strength of which did not cause a conduction block, had little effect on the shape of the action potential, whereas an anodal current reversibly caused an appearance of a large negative after-potential which was hardly observed in the normal nerve.

3. $5 \times 10^{-2}M$ sodium monoiodoacetate (IAA) caused a nerve depolarization followed by a conduction block. The catelectrotonic potential was not changed by IAA, while the anelectrotonic potential was depressed keeping its shape un-

changed. The conduction block was restored by an anodal current. This restoration was removed within several seconds upon switching off the anodal current.

4. $5 \times 10^{-3}M$ or $7.5 \times 10^{-2}M$ sodium fluoride caused a nerve depolarization, but a conduction block was never observed. Both the catelectrotonic and the anelectrotonic potentials were depressed in magnitude by NaF keeping their shape almost unchanged.

5. $1.5 \times 10^{-3}M$ or $3.1 \times 10^{-3}M$ potassium cyanide caused a nerve depolarization followed by a conduction block. Both the catelectrotonic and the anelectrotonic potentials were either not changed by KCN or slightly depressed in magnitude keeping their shape unchanged. The conduction block was restored by an anodal current. This restoration was removed upon switching off the anodal current, but it took about 30 to 60 seconds to reproduce the conduction block.

6. $4.6 \times 10^{-3}M$ or $1.5 \times 10^{-2}M$ potassium cyanide caused a rapid depolarization of nerve in most cases, but the depolarization was restored within several minutes in spite of the continuous presence of KCN. A conduction block was then brought about even when the resting potential had already returned to the normal level. Both the catelectrotonic and the anelectrotonic potentials were either not changed by KCN or slightly depressed in magnitude keeping their shape unchanged in nearly half of the total cases. In a few cases the anelectrotonic potential was slightly depressed in magnitude, the catelectrotonic potential being kept unchanged. The recovery from the conduction block by an anodal current was the same as that in the case of $1.5 \times 10^{-3}M$ or $3.1 \times 10^{-3}M$ KCN treatment.

7. $1.3 \times 10^{-4}M$ rotenone caused a nerve depolarization followed by a conduction block. The catelectrotonic potential was never affected by rotenone, while the anelectrotonic potential was slightly depressed in magnitude in some cases keeping its shape unchanged. The conduction block was restored by an anodal current. This restoration was rapidly removed immediately after switching off the anodal current.

8. $8.5 \times 10^{-2}M$ K^+ caused a nerve depolarization

followed by a conduction block. Both the catelectrotonic and the anelectrotonic potentials were markedly depressed in magnitude, and their slow phases were much more depressed than their rapid phases. The conduction block was restored by an anodal current. This restoration was removed within 30 seconds after switching off the anodal current.

9. The resting potential was not affected in most cases by $1.4 \times 10^{-4}M$ DDT, and was slightly decreased in a few cases. A conduction block was never observed. The catelectrotonic potential was not depressed in magnitude, but its falling phase was delayed. The anelectrotonic potential was either not changed or slightly depressed in magnitude keeping its shape unchanged.

10. The DDT-induced negative after-potential was augmented and prolonged by an anelectrotonus and completely disappeared by a catelectrotonus. It disappeared before a conduction block occurred after the application of $5 \times 10^{-2}M$ IAA, $3.1 \times 10^{-3}M$ or $4.6 \times 10^{-3}M$ KCN, or $1.3 \times 10^{-4}M$ rotenone, but $7.5 \times 10^{-2}M$ NaF or $8.5 \times 10^{-2}M$ K^+ had no effect upon it.

11. Discussions were made on the following points: the possibility that metabolic inhibitors act selectively upon either of the resting or the active metabolism of nerve, mechanism of anodal restoration from the conduction block produced by metabolic inhibitors or potassium ions, the evidence that DDT has no effect on the resting metabolism of nerve, and the speculation that DDT-induced negative after-potential is caused by a stimulating action of DDT on the active metabolism of nerve.

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On the Joint Action of Camphor Derivatives for γ -BHC and DDT. Studies on the Insecticidal Effect of Camphor Derivatives. VI. Kaoru OHTA (Laboratory of Food, Kyoto Women's University, Kyoto) and Yasunosuke IKEDA (Takamine Research Laboratory, Sankyo Co. Ltd., Yasu, Shiga Pref.). Received Oct. 31, 1957. *Botyu-Kagaku* **22**, 367, 1957 (with English résumé, 369).

61. γ -BHC, DDT との連合作用について 樟脳誘導体の殺虫効力に関する研究 第6報 太田堅 (京都女子大学 食品学教室). 池田安之助 (三共株式会社 高峰研究所) 32. 10. 31 受理

前報¹⁾の如く γ -BHC 又は DDT に樟脳誘導体特に α' -chlorocamphor を混合するときは, γ -BHC 又は DDT の殺虫効力を増大せしめるので, 薬剤相互間にある種の連合作用が存在すると思われる。よつて先に得た殺虫試験結果について, Bliss の方法により解析を試み, 更に LD₅₀ における各薬剤の協力効果を Goodwin-Bailey の degree of Synergism の単位で示した。

前報¹⁾において, γ -BHC 又は DDT に樟脳誘導体を混合してゆくと, 次第に混合物の融点は降下し, ある割合において甚だ低い融点の共融混合物を形成することが認められた。この様な共融混合物の殺虫効力について, アズキノウムシ成虫に対する室内試験を試みたところ, 樟脳誘導体特に α' -chlorocamphor を混合することによつて, γ -BHC 又は DDT の殺虫効力が増大し, 薬剤相互間に或る種の連合作用があると思われる。よつて, これら殺虫試験から得られた測

定値を基にして, 2, 3 の解析を試みたのでその結果につき報告する次第である。

I. γ -BHC に対する樟脳誘導体の連合効果

γ -BHC + 樟脳誘導体ならびに各単剤のアズキノウムシ成虫に対する殺虫試験の結果は, 前報¹⁾の第5表に示す通りであり, 薬剤間に顕著な効力差が認められる。これらの値を Bliss^{2), 3)}のプロビット単位に置きかえ, 薬量一致死率曲線 (D-M 曲線) の一次変換の計算をなし, その回帰方程式とこれに附随する 2, 3 の

Table 1. Summary of data of experiments on the relation between dosage and mortality of Azuki bean weevil, *Callosobruchus chinensis* L. in γ -BHC and its eutectic mixtures.

Code sign	Nos. of test insects	Regression equation $y=a+b(x-\bar{x})$	Degree of freedom (n)	x^2
γ -BHC	1376	$y=6.27604+0.73668(x-1.16692)$	2	0.14778
γ -BHC + α' -Cl. C.	1346	$y=6.29845+1.15867(x-1.15949)$	2	0.60979
γ -BHC + α -Br. C.	1192	$y=6.12130+0.85828(x-1.16650)$	2	0.11806
γ -BHC + C.	1166	$y=6.06539+1.04489(x-1.23684)$	2	0.93001
α' -Cl. C.	759	$y=4.35492+0.77903(x-1.88948)$	2	0.01443
α -Br. C.	795	$y=3.96662+0.85363(x-1.93236)$	2	0.07543
C.	995	$y=3.57463+0.62186(x-1.92974)$	2	0.06631