も同じで，殊に第3令をすぎてからは顕著な逆減を見せ，第2表の最後の列にしめした $b$ の逆対数値，すなわち Dyar's constant が令期の数が多くなるにしたがつて小になつていることからもうなずかれる．これは3令をすぎて後における頭幅の頻度分布は，かなり相互に重複してくるのであろうと推測されるが，事実第2図にしめした雌雄，令数別の頭幅の頻度分布曲線をみれば，3令以後その頭幅は相当程度重複し，3令までは頭幅の測定値よりする令期の決定は大体可能であるが，それ以後は不可能であると結論される．マイマイガの大きさは，4令期以後における脱皮回数ひいては発育日数の長短，摂食量の多少によつて決定される要素が大きいものと考えられる．

## 摘　　要

野辺地系マイマイガの幼虫を，温度25°，関係湿度89%の環境条件下において，ケヤキの葉をあたえて個体別に飼育し，その脱皮回数を検討，あわせて頭蓋の脱皮殻を材料にして，令期間における成長様相を考察した．

1. 野辺地系マイマイガは，その幼虫期において雌は5，6，または7回，雄は5または6回の脱皮をくりかえした．

2. 頭幅の令期間における成長様相は，第3令を境にして，ことなつたふたつの，大体直線に近い関係をしめした．

3. 頭幅による令期の決定は，第3令まではおゝむね可能であるが，それ以後は不可能である．

## 文　　献

1) Goldschmidt, R.: Wilhelm Roux' Arch. Entwicklungsmech. Organ. **116**, 136 (1929).
2) 三田村啓三：応用昆虫 **12**, 70 (1956).
3) Nagasawa, S.: Botyu - Kagaku **22**, 176 (1957).
4) ————: Jap. J. Appl. Entomol. Zool. **1**, 27 (1957).

## Résumé

Under the constant environmental condition of 25° and 89% relative humidity, the larvae of the "Noheji" race of the gypsy moth, *Lymantria dispar* L., were reared separately on leaves of the zelkova-tree, *Zelkova serrata* Makino. The females of the "Noheji" race of the gypsy moth moulted 5, 6 or 7 times in their larval stage and the males moulted 5 or 6 times. In all these cases mentioned above, the relations of log-width of exuviae of head capsule to instar number were found to be represented generally by two straight lines intersecting at a point of the 3rd instar. We shall be able to determine the instar to which a larva belongs by measuring width of exuviae of head capsule in the larvae ranging from the lst to the 3rd instars, but we shall fail to tell the instar number by this method in the larvae ranging from the 4th to the last instars.

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**Effects of Oxygen Lack, Metabolic Inhibitors, and DDT on the Resting Potential of Insect Nerve.** Studies on the Mechanism of Action of Insecticides. XII. Teruo YAMASAKI and Toshio NARAHASHI* (Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Tokyo, Japan). Received May 9, 1957. *Botyu-Kagaku* **22**, 259, 1957.

**47. 昆虫神経の静止電位に及ぼす酸素欠乏，代謝阻害剤，およびDDTの影響．** 殺虫剤の作用機構に関する研究 第12報 山崎輝男・楢橋敏夫**（東京大学農学部害虫学研究室） 32. 5. 9 受理

DDT による昆虫神経の機能変化がどのような機構でもたらされるかを究明するために，ゴキブリ神経の静止電位に及ぼす DDT の影響を，酸素欠乏あるいは代謝阻害剤の影響と比較研究した．その結果 DDT は代謝阻害剤や酸素欠乏の場合のような脱分極作用を示さず，種々な原因によつて脱分極された神経の再分極を抑制する働きがあることが判明した．既存の多くのデータや神経代謝および神経興奮の機構と結びつけて考察した結果，DDT は神経の静止代謝には影響せず，興奮代謝への生化学的影響か，あるいは神経原形質膜への直接の物理化学的作用によつて，そのイオン透過性を変え，機能変化をもたらすものと推論されるに至つた．

The mode of toxic action of DDT has been studied by many investigators along either of the following lines, i. e., the effects of DDT on various physiological functions in insects and the

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effects on the metabolism of insects. It has been clarified that DDT augments the repetitive excitability of the nerve resulting in hyper-excitation of the nerve and muscle, that it strongly stimulates the respiration of insects, that it induces the exhaustion of metabolites in insects, that it does not affect the cholinesterase activity either *in vivo* or *in vitro*, that it may affect the activities of some oxidases *in vitro*, and so on. It has been concluded in our previous papers that an augmentation of the repetitive excitability of the nerve, including both the soma and the axon, is a primary action of DDT. But the knowledge obtained up to date either by biochemical studies or by other ones cannot be used for the explanation of the cause of such a functional change in the nerve. In a series of experiments we attempted to find the cause of the functional changes in the DDT poisoned nerve. As the first step in these investigations, the effects of DDT on the resting potential of the nerve fibres of the cockroach were studied in comparison with those of oxygen lack, metabolic inhibitors, and potassium ions on the resting potential, for the maintenance of the resting potential was known to depend on the metabolic energy in the nerve.

## Materials and Methods

The abdominal nerve cords of adults of both the American cockroach, *Periplaneta americana* L. and the smoky brown roach, *P. suliginosa* S. were employed. Since the values of the resting potentials and the reactions to the drugs used in this experiment were similar in both species, the data obtained will be discussed together.

The experiments were performed during August and September at room temperature.

The central nerve cord of the cockroach has six giant axons with diameters ranging from 20 to 45 microns and ten to twelve axons with diameters ranging from 5 to 20 microns per each connective[55]. The giant axons originate in the sixth abdominal ganglion and either connect synaptically with the motoneurones in the meta-, meso-, or pro-thoracic ganglion or connect directly to the brain. They have no synaptic contacts with other axons on the way from the sixth abdominal ganglion to the brain or to the thoracic ganglia. As these giant axons are very large in diameter when compared with the other axons in the nerve cord, a potential difference measured from any point on the nerve cord is largely caused by the potential differences in the giant axons.

The potential differences between a central uninjured region of the nerve cord and a cut peripheral end dipped into Ringer-isotonic KCl solution were measured. These potential differences, undoubtedly, do not indicate the absolute values of the resting potential, however, it is sufficient to compare the relative changes in the resting potential under various environmental conditions. With this easy method of resting potential measurement it is very convenient to perform many experiments, and one can measure several preparations successively.

It may be possible to measure the potential difference by another arrangement of external electrodes. When both electrodes are located in uninjured regions of a nerve, and the drug to be tested is applied to one region, the potential differences produced by the action of the drug can easily be measured. One advantage of this method is that the control value can be taken from the same nerve, but, a disadvantage is that the original resting potential cannot be measured since only potential differences are measured by this method. However since, it is necessary for the electrodes to be widely separated, the cockroach nerve is too short for this method to be utilized.

After excision of an abdominal nerve cord from a roach, adhering tissues were removed as completely as possible in order to minimize the short circuit effect in the measurement of the resting potential.

The actual method of resting potential measurement used in this study was similar to that described by Shanes & Brown (1942). Several nerve cord preparations were soaked in Ringer's solution successively, and all preparations, usually six to ten nerve cords, for one course of the

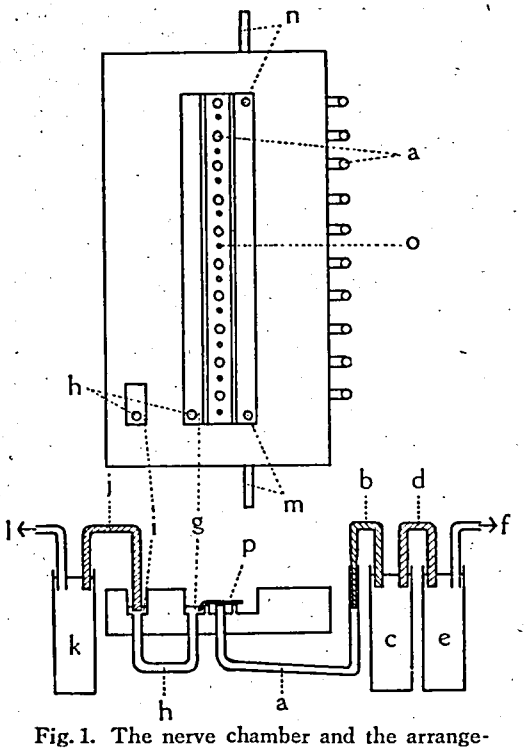


Fig. 1. The nerve chamber and the arrangement of the electrodes. Explanation see text.

experiment, were mounted at the same time on the electrodes in a bakelite "nerve" chamber (Fig. 1). The central uninjured region of each nerve was in contact with Ringer, with or without drugs, in one end of a U-shaped glass tube, a, having an inner diameter of about 3.5 mm. The other end of each glass tube lay outside the chamber and was in contact electrically with the Ringer-isotonic KCl solution, c, by means of a U-shaped glass tube, b, containing Ringer-agar. The isotonic KCl solution, c, had electrical contact with saturated KCl solution, e, by means of a U-shaped glass tube, d, containing saturated KCl-agar, and the latter solution in turn was connected to a saturated KCl type Calomel electrode, f. The cut peripheral ends of all nerves were dipped into a long common trough, g, filled with the Ringer-isotonic KCl solution, leading to a small trough, i, by a U-shaped glass tube, h. A U-shaped glass tube containing saturated KCl-agar, j, provided electrical contact between this trough, i, and the saturated KCl solution, k, which in turn was connected to the other saturated KCl type Calomel electrode, l. Successive measurements of the resting potential could be performed by successive transfers of the U-shaped glass tube, b.

The potential difference was measured with a potentiometer employing a reflection type galvanometer, having a sensitivity of $6.6 \times 10^{-9}$ A, as a null point instrument. At maximum sensitivity, the potential could be determined within 0.1mV. A Ringer-soaked cotton thread was employed for the measurement of the potential difference not derived from nerve but from electrical circuits, the value of which was subtracted from the apparent resting potentials of the nerves. An inlet, m, and an outlet, n, of gases were attached to the chamber. In order to facilitate the diffusion of gas to the nerves, the central uninjured region of each nerve was put on a Ringer-soaked filter strip, 1~2mm wide, one end of which was dipped into the end of the U-shaped glass tube, a, while the other end of which was put on a pillow made by the glass rod, o. The chamber was covered with a glass plate, in order to prevent desiccation of the nerves and to permit the admission of gases.

Two methods of drug application were employed. One was the local application of the drug containing Ringer solution to the central uninjured region of the nerve after a withdrawal of non-drug containing Ringer from the outer cut end of the U-shaped glass tube, a, the other method was the soaking of the whole nerve cord for some time in drug containing Ringer before mounting the nerve cord on the electrodes. The former method will be called the "local application method", the latter, the "soaking method".

Commercial nitrogen was led through alkali-pyrogarol solutions in order to eliminate the small amount of oxygen contained, and in turn was led through a 70cm column of water in order to saturate it with moisture. Commercial oxygen was directly led through another column of water. After being saturated with water the oxygen and nitrogen were both attached to the inlet of the chamber.

Cathodal current was applied to the nerve through silver-silver chloride non-polarizable

electrodes, one of which was dipped into the common isotonic KCl solution, c, as a cathode, and the other in the small trough, i, as an anode.

The Ringer's solution used is the same as that described in our previous paper[91], 153.9mM NaCl, 2.6mM KCl, and 1.8mM $CaCl_2$ per liter were added to 50cc of a mixture of buffer solution containing M/15 $Na_2HPO_4$ and M/15 $KH_2PO_4$, which resulted in a final pH of 7.2. The concentrations of cations were therefore as follows: 159.6mM $Na^+$, 3.1mM $K^+$, and 1.8mM $Ca^{++}$ per liter.

p,p'-DDT, methoxy analogue of DDT or methoxychlor, ethoxy analogue of DDT, and γ-BHC were tested. The suspension of insecticide made by an injection of the drug acetone solution into Ringer was applied. The control experiments proved that acetone Ringer had no effect on the resting potential of the nerve. The metabolic inhibitors tested were sodium monoiodoacetate, sodium fluoride and potassium cyanide. These were disolved in Ringer solution. The substrates which are known to be utilized in glycolysis or respiration were also tested, i. e., glucose, sucrose, sodium pyruvate and sodium lactate. These were also disolved in Ringer solution.

## Results

### Resting Potential

The resting potential of the normal nerve which was mounted after soaking in Ringer's solution for a few hours, rose gradually and attained the maximum value, this was followed by a gradual decline after maintenance of a plateau for some time (Fig. 2). The maximum resting potentials obtained are summarized in Table 1. The maximum resting potentials had a tendency to decrease from the beginning of September even at temperatures as high as 30° during the experiments. The temperatures dropped at night during September although temperatures were as high as 30° during the daytime when the experiments were performed. Hence the only possible reason for the decrease in the potential is a lowering of the rearing temperature at night.

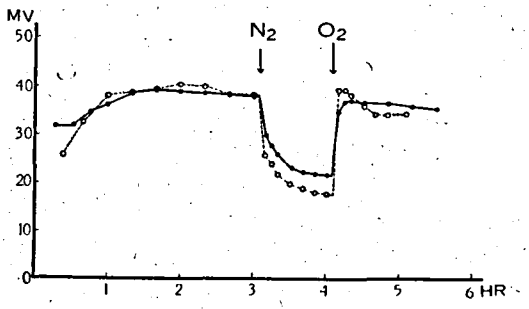


Fig. 2. Anoxic depolarization of the normal nerves. A solid line with closed circles shows no overshoot after a readmission of oxygen (Sep. 2, 28.5°), while a broken one with open circles shows an overshoot (Sep. 3, 29.5°).

### Effects of Oxygen Lack

An anoxic depolarization was induced by a continuous passage of nitrogen through the nerve chamber (Fig. 2). The depolarization was rapid during the initial phase which was followed by a slow secondary phase, and usually attained the maximum value within 30 to 60 minutes withdrawal of oxygen, amounting about 50 per cent of the resting potential (Table 1). The minimum potential remaining after the anoxic depolarization will be called "minimum resting potential". Since the resting potential declined gradually for a long period of time even with presence of oxygen, the percentages of the minimum resting potential to both the resting potential immediately before the admission of nitrogen and the resting potential determined by an extrapolation of several potential values measured at 30 to 60 minutes before the admission of nitrogen or "standard resting potential" were calculated.

A readmission of oxygen or air at any time brought about a rapid return of the resting potential to the normal level, or a repolarization, frequently showing an overshoot of the potential (Fig. 2).

When nitrogen was introduced into the chamber soon after an attaining of the maximum resting potential, the minimum resting potential during anoxia, in terms of the percentage to the standard resting potential, showed a nearly constant value of from 40 to 60 per cent in all experiments. After progression of the natural decline of the resting potential, a second admission of nitrogen

Table 1. Anoxic depolarization of the nerve fibres. Mean values (mV).

| Date (Temp.) | No. of exp. | Max.[1] pot. (1) | 30 min. after $N_2$ (I) Standard[2] pot. (2) | Min.[3] pot. (3) | (3)/(2) % | (3)/(1) % | 60 min. after $N_2$ (I) Standard[2] pot. (4) | Min.[3] pot. (5) | (5)/(4) % | (5)/(1) % | 30 min. after $N_2$ (II) Standard[2] pot. (6) | Min.[3] pot. (7) | (7)/(6) % | (7)/(1) % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8.4 (31.5°C) | 3 | 48.0 | 41.3 | 23.0 | 55.5 | 47.9 | 39.5 | 19.3 | 49.0 | 40.3 | | | | |
| 8.5 (29.5°C) | 4 | 44.8 | 41.9 | 24.2 | 57.8 | 54.0 | | | | | 30.9 | 23.8 | 81.6 | 52.8 |
| 8.6 (30.5°C) | 4 | 41.9 | 40.5 | 19.9 | 49.5 | 47.6 | 40.1 | 18.9 | 47.6 | 45.1 | 20.6 | 19.8 | 95.7 | 47.3 |
| 8.12 (28.0°C) | 3 | 38.6 | 38.7 | 19.8 | 50.8 | 51.1 | | | | | | | | |
| 8.25 (32.0°C) | 2 | 44.0 | 44.9 | 20.2 | 44.7 | 45.4 | | | | | 33.4 | 21.0 | 62.3 | 47.6 |
| 8.26 (32.5°C) | 2 | 43.8 | 39.8 | 17.9 | 45.1 | 42.0 | | | | | 22.5 | 16.7 | 74.4 | 39.0 |
| 8.27 (33.0°C) | 2 | 34.4 | 36.7 | 21.2 | 57.0 | 62.5 | | | | | 27.6 | 20.9 | 73.3 | 61.6 |
| 8.28 (33.0°C) | 2 | 48.5 | 45.5 | 27.8 | 61.2 | 57.5 | | | | | 38.1 | 29.0 | 75.3 | 59.7 |
| 9.2 (28.5°C) | 3 | 41.1 | 38.4 | 24.7 | 64.4 | 60.4 | 38.0 | 23.4 | 61.8 | 57.3 | | | | |
| 9.3 (29.5°C) | 3 | 36.7 | 33.5 | 20.7 | 61.8 | 56.2 | 32.7 | 19.1 | 59.0 | 52.0 | | | | |
| 9.4 (31.0°C) | 2 | 36.8 | 37.9 | 24.2 | 63.8 | 65.6 | 38.7 | 16.9 | 43.8 | 45.9 | | | | |
| 9.7 (27.0°C) | 6 | 37.2 | 34.8 | 22.8 | 32.4 | 61.4 | 34.4 | 20.9 | 61.1 | 56.3 | | | | |
| 9.16 (23.0°C) | 3 | 40.1 | 36.5 | 22.3 | 61.6 | 55.9 | 35.2 | 19.9 | 57.1 | 49.6 | | | | |
| 9.19 (30.0°C) | 3 | 35.3 | 26.2 | 15.0 | 58.6 | 42.7 | 24.9 | 14.7 | 60.6 | 41.4 | 20.0 | 16.4 | 84.6 | 46.7 |

[1] The maximum resting potential obtained in oxygen or air after mounting the nerve on the electrodes.

[2] The resting potential determined by an extrapolation of several potential values measured 30 to 60 minutes before the onset of depolarization.

[3] The minimum resting potential remaining after depolarization.

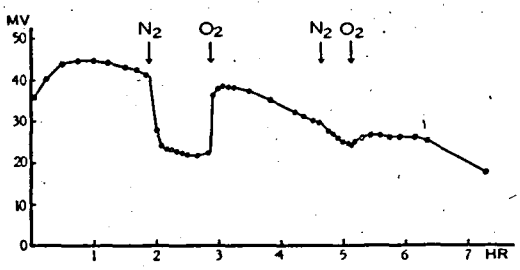


Fig. 3. Anoxic depolarizations of the normal nerve, showing a decreased degree of depolarization after a natural decline of the resting potential. Aug. 6, 30.5°.

brought about a smaller depolarization than did the first, maintaining a constant non-depolarized potential. As a result of this the percentage of the ratio between the minimum resting potential during the second anoxia and the standard resting potential was higher than that of the first anoxia (Fig. 3, Table 1). These results indicate that the natural decline of the resting potential occurring in oxygen or air is largely due to the depression of an anoxia sensitive fraction of the resting potential.

The anoxia sensitive fraction of the resting potential will be called "aerobic resting potential", and the other or anoxic insensitive fraction "anaerobic resting potential". The aerobic and the anaerobic resting potentials each composed about half of the total resting potential.

### Effects of Substrates

Since it has been clarified by the above mentioned experiments that metabolic energy is required for the maintenance of the resting potential, several different substrates were applied to the nerve. After soaking the nerves in either 0.1 M glucose, 0.1 M sucrose, 0.02M sodium pyruvate, or 0.02M sodium lactate for 60 or 90 minutes, the resting potentials and its reactions

Table 2. Effects of substrates on the resting potential. Mean values (mV). For explanations see Table 1.

| Date (Temp.) | Substrate (Conc.) | No. of exp. | Max. pot. (1) | 30 min. after $N_2$ | | | |
|---|---|---|---|---|---|---|---|
| | | | | Standard pot. (2) | Min. pot. (3) | (3)/(2) % | (3)/(1) % |
| 8.12 (28°C) | Nil | 3 | 38.6 | 38.7 | 19.8 | 50.8 | 51.1 |
| | Pyruvate (0.02M) | 3 | 44.6 | 42.5 | 21.0 | 49.6 | 47.2 |
| | Lactate (0.02M) | 3 | 40.7 | 39.9 | 20.5 | 51.1 | 50.1 |
| 8.7 (28°C) | Glucose (0.1M) | 3 | 42.7 | 37.6 | 24.6 | 65.3 | 57.4 |
| | Sucrose (0.1M) | 3 | 40.7 | 39.0 | 21.3 | 54.8 | 52.2 |

to anoxia were examined. These substrates did not affect the resting potential or the anoxic depolarization (Table 2).

Effects of Metabolic Inhibitors

Sodium monoiodoacetate (IAA): The resting potential of the nerve was measured after soaking it for one hour in 0.0001M, 0.001M, or 0.005M IAA Ringer. 0.0001M IAA had little or no effect; the resting potential increased to the maximum value which was followed by a gradual decline as in the case of the control nerve. 0.001M IAA had a slight depressant action; the nerve treated with 0.001M IAA showed much less increase in potential after mounting it on the electrodes and more rapid decline of the potential after the maximum value was reached. 0.005M IAA had a much stronger depressant action than 0.001M. The resting potential values of the nerves treated with IAA for five hours are shown in Table 3.

The local application of IAA also brought about depolarization of the nerve with long latent periods (Fig. 4).

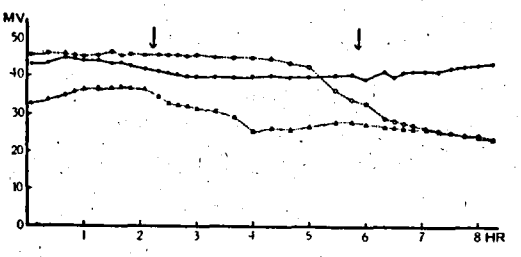


Fig. 4. Effects of monoiodoacetate (IAA) and NaF on the resting potential. Local application method. A solid line with closed circles shows the control nerve, a broken one with open circles the nerve treated with 0.001M IAA at the first arrow, and a dotted one with triangles the nerve treated with 0.002M NaF at the first arrow and then with 0.003M NaF at the second arrow Sep. 27, 22.5°.

Table 3. Effects of monoiodoacetate on the resting potential. Soaking method. Mean values (mV). Aug. 14, 29°. For explanations see Table 1.

| Conc. (M) | No. of exp. | Max. pot. (1) | 5 hrs. after treatment | |
|---|---|---|---|---|
| | | | Pot. (2) | (2)/(1) % |
| 0 | 2 | 45.3 | 40.3 | 89.5 |
| 0.0001 | 2 | 43.0 | 37.8 | 87.9 |
| 0.001 | 2 | 43.2 | 25.0 | 57.8 |
| 0.005 | 2 | 34.1 | 0.6 | 1.8 |

The anoxic depolarization of the nerves soaked for one hour in IAA Ringer was measured. One such experiment is illustrated by Fig. 5, and results are indicated in Table 4. The anoxic depolarization became much less when the decrease of the resting potential was brought about by a prolonged action of IAA, but the anaerobic resting potential remained constant unless the total resting potential declined markedly as in the case of the higher concentration of IAA, 0.002M. These results indicate that the lower concentrations of IAA, 0.0001M and 0.001M, largely depress the aerobic resting potential, while the higher concentration of IAA, 0.002M, completely

Table 4. Anoxic depolarization of the nerve treated with monoiodoacetate (IAA). Soaking method. Mean values (mV). For explanations see Table 1.

| Date (Temp.) | IAA (M) | Substrate (M) | No. of exp. | Max. pot. (1) | 30 min. after $N_2$(I) | | | | 30 min. after $N_2$ (II) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Standard pot. (2) | Min. pot. (3) | (3)/(2) % | (3)/(1) % | Standard pot. (4) | Min. pot. (5) | (5)/(4) % | (5)/(1) % |
| 8.25 (32°C) | 0 | Nil | 2 | 44.0 | 44.9 | 20.2 | 44.7 | 45.4 | 33.6 | 21.0 | 62.3 | 47.6 |
| | 0.0001 | Nil | 2 | 45.1 | 41.8 | 19.5 | 46.7 | 43.2 | 40.0 | 24.7 | 61.7 | 54.6 |
| | 0.001 | Nil | 2 | 32.5 | 27.4 | 18.9 | 69.0 | 57.8 | 22.0 | 19.6 | 88.6 | 60.0 |
| | 0.002 | Nil | 2 | 25.1 | 20.9 | 13.2 | 63.5 | 52.7 | 2.5 | 2.0 | | 8.0 |
| 8.26 (32.5°C) | 0 | Nil | 2 | 43.7 | 39.8 | 17.9 | 45.1 | 42.0 | 22.5 | 16.7 | 74.4 | 39.0 |
| | 0.001 | Nil | 2 | 37.1 | 32.1 | 21.4 | 66.5 | 57.6 | 25.1 | 20.2 | 80.5 | 54.3 |
| | 0.001 | Pyruvate (0.02) | 2 | 39.0 | 32.0 | 21.9 | 68.4 | 56.0 | 27.0 | 22.1 | 81.6 | 56.5 |
| | 0.001 | Lactate (0.02) | 2 | 41.8 | 29.4 | 21.5 | 73.2 | 52.4 | 21.8 | 17.7 | 81.2 | 43.1 |
| 9.12 (29°C) | 0 | Nil | 3 | 38.4 | 33.3* | 20.2* | 60.5* | 52.7* | | | | |
| | 0.001 | Nil | 3 | 22.3 | 16.0* | 14.3* | 89.3* | 65.8* | | | | |

* 60 min. after $N_2$.

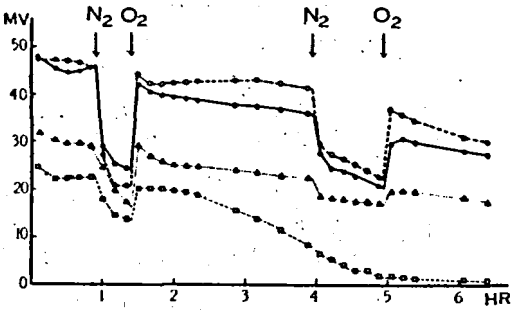


Fig. 5. Anoxic depolarization of the nerves treated with IAA. Soaking method. A solid line with closed circles shows the control nerve, a broken one with open circles the nerve soaked for 1 hour in 0.0001M IAA, a dotted one with triangles the nerve soaked in 0.001M IAA, and a broken one with squares the nerve soaked in 0.002M IAA. Aug. 25, 32°.

depresses the aerobic resting potential and partly depresses the anaerobic one.

The presence of 0.02M sodium pyruvate or 0.02M sodium lactate in Ringer did not alter the depressant effect of IAA (Table 4).

Sodium fluoride (NaF): The resting potential of nerve was measured after soaking it for one hour in 0.01M, 0.02M, or 0.03M NaF Ringer. The maximum resting potential was hardly affected by 0.01M NaF, was slightly depressed by 0.02M NaF, and was depressed to about 50 per cent by 0.03M NaF (Table 5). The local application of 0.02M NaF also brought about the depolarization of the nerve. But the mechanism of NaF depolarization must be different from that of IAA, because no latent period was observed with NaF (Fig. 4).

The anoxic depolarization of the nerve soaked

Table 5. Anoxic depolarization of the nerve treated with sodium fluoride. Soaking method. Mean values (mV). Aug. 28, 33°. For explanations see Table 1.

| NaF (M) | No. of exp. | Max. pot. (1) | 30min. after $N_2$ (1) | | | | 30 min. after $N_2$ (II) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Standard pot. (2) | Min. pot. (3) | (3)/(2) % | (3)/(1) % | Standard pot. (4) | Min. pot. (5) | (5)/(4) % | (5)/(1) % |
| 0 | 2 | 48.5 | 45.5 | 27.8 | 61.2 | 57.5 | 38.5 | 29.0 | 75.3 | 59.7 |
| 0.01 | 2 | 42.9 | 40.2 | 22.1 | 56.1 | 51.8 | 36.9 | 25.9 | 70.4 | 61.8 |
| 0.02 | 2 | 34.3 | 34.1 | 17.5 | 52.2 | 51.6 | 29.2 | 20.2 | 68.8 | 59.1 |
| 0.03 | 2 | 20.8 | 20.7 | 12.1 | 58.0 | 58.3 | 12.8 | 10.5 | 82.0 | 50.4 |

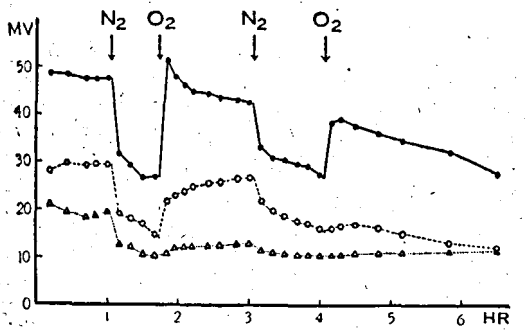


Fig. 6. Anoxic depolarization of the nerves treated with NaF. Soaking method. A solid line with closed circles shows the control nerve, a broken one with open circles, the nerve soaked for 1 hour in 0.02M NaF, and a dotted one with triangles, the nerve soaked in 0.03M NaF. Aug. 28, 33°.

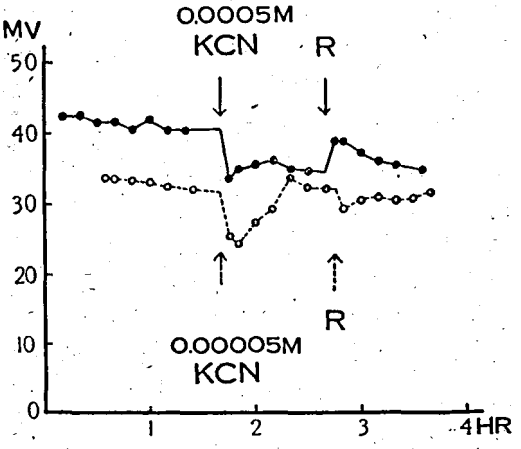


Fig. 7. Effect of KCN on the resting potential. Local application method. A solid line with closed circles shows the nerve treated with 0.0005M KCN and then returned to Ringer, and a broken one with open circles the nerve treated with 0.00005M KCN and then returned to Ringer. Sep. 20, 22°.

for one hour in NaF Ringer was different from that of the nerve treated with IAA Ringer (Fig. 6, Table 5). This could easily be observed in the much more depolarized nerve soaked in the high concentration of NaF, 0.03M. In consequence of this the percentage of the ratio of the minimum resting potential during anoxia to the standard resting potential showed about the same value as that of the control. Both the aerobic and the anaerobic resting potentials must be depolarized equally by the action of NaF. With the higher concentration of NaF, 0.03M, the repolarization after the readmission of oxygen was incomplete.

Potassium cyanide (KCN): Only the local application method was employed. A rapid depolarization was produced by an application of 0.00005M, 0.0005M, or 0.0025M KCN Ringer, but there was a tendency to recover in spite of the presence of KCN (Fig. 7). An overshoot of the resting potential beyond the normal level was often observed as is shown in Fig. 7 In this case a decline of the potential was brought about after an attainment of the maximum value. Like NaF, KCN showed no latent period. The percentage of the ratio of the minimum resting potential to the standard resting potential showed a relatively high value, about 80 per cent (Table 6).

Effects of Insecticides

p, p'-DDT: The local application of $5\times10^{-5}$M

Table 6. Effects of potassium cyanide on the resting potential (mV). Sep. 20, 22°. For explanations see Table 1.

| Nerve | KCN treatment | KCN (M) | Max. pot. (1) | After treatment | | |
|---|---|---|---|---|---|---|
| | | | | Standard pot. (2) | Min. pot. (3) | (3)/(2) % |
| A | I | 0.0005 | 28.7 | 22.0 | 18.0 | 81.8 |
| | II | 0.0005 | | 27.3 | 22.6 | 82.7 |
| | III | 0.0005 | | 24.7 | 21.0 | 85.0 |
| B | I | 0.0005 | 42.5 | 39.7 | 33.5 | 84.3 |
| | II | 0.0005 | | 32.0 | 37.7 | 117.8 |
| | III | 0.0005 | | 32.5 | 31.0 | 95.3 |
| C | I | 0.0005 | 29.8 | 30.0 | 23.0 | 76.6 |
| | II | 0.0005 | | 32.5 | 27.1 | 83.3 |
| | III | 0.0005 | | 31.2 | 28.1 | 90.0 |
| D | I | 0.00005 | 34.7 | 22.0 | 17.5 | 79.5 |
| | II | 0.0025 | | 19.0 | 16.7 | 87.8 |
| | III | 0.0025 | | 18.7 | 16.6 | 88.7 |
| E | I | 0.00005 | 48.4 | 35.7 | 38.8 | 108.6 |
| | II | 0.0005 | | 30.8 | 24.1 | 78.2 |
| | III | 0.0025 | | 31.8 | 26.3 | 82.7 |
| F | I | 0.00005 | 27.8 | 27.4 | 21.9 | 79.9 |
| | II | 0.0005 | | 21.8 | 16.4 | 75.2 |
| | III | 0.0025 | | 14.8 | 13.0 | 87.8 |

DDT Ringer for several hours had no effect on the resting potential or on the anoxic depolarization (Fig. 8, Table 7).

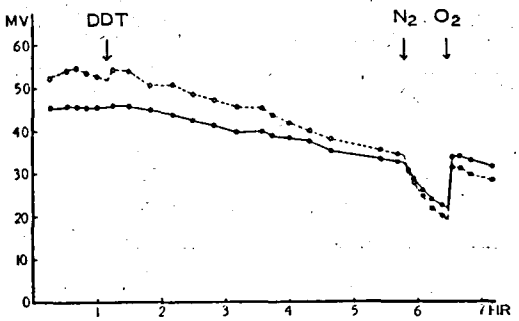


Fig. 8. Effect of p,p'-DDT on the resting potential and the anoxic depolarization. Local application method. A solid line with closed circles shows the control nerve, and a broken one with open circles the nerve treated with $5\times10^{-5}$M DDT. Sep. 1, 28°.

The effects of soaking the nerve for 3 to 4 hours in $5\times10^{-5}$M DDT Ringer on the resting potential and the anoxic depolarization were examimed. The resting potential of the nerve just mounted on the electrodes after soaking it in DDT Ringer was smaller than that of the control. The rate of increase in the resting potential and the maximum resting potential reached were also smaller. The percentage of the ratio of the minimum resting potential during anoxia to the standard resting potential was about the same value as that of the control (Fig. 9, Table 8). These results indicate that both the aerobic and the anaerobic resting potentials are equally depolarized by the soaking in DDT Ringer.

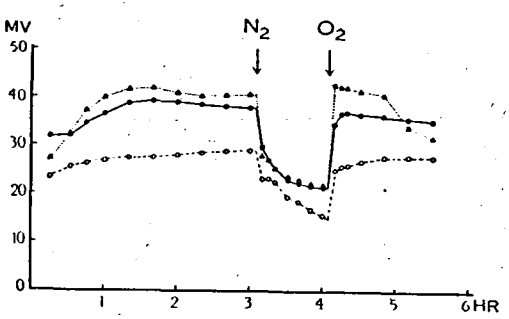


Fig. 9. Anoxic depolarization of the nerves treated with p,p'-DDT and γ-BHC. Soaking method. A solid line with closed circles shows the control nerve, a broken one with open circles the nerve soaked for 3 hours in $5\times10^{-5}$M DDT, and a dotted one with triangles the nerve soaked in $5\times10^{-5}$M BHC. Sep. 2, 28.5°.

In order to examine by another way whether or not DDT acts upon the depolarization and the repolarization processes, a cathodal current was applied to the nerve treated with DDT Ringer (Fig. 10, Table 9). Both the magnitude

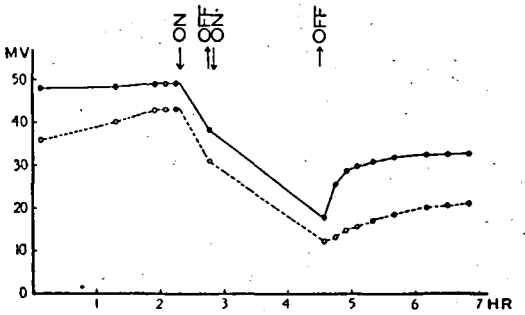


Fig. 10. Effect of a cathodal current on the resting potential. A solid line with closed circles shows the control nerve, and a broken one with open circles the nerve locally treated with $5\times10^{-5}$M p,p'-DDT. Sep. 24, 24°.

and rate of the depolarization caused by the cathodal current and the maximum repolarization potential after removal of the current were not altered in the presence of DDT. However, the rate of repolarization after the removal of the current was much decreased under the influence of DDT.

DDT analogues: The effects of $5\times10^{-5}$M methoxychlor and ethoxy analogue of p,p'-DDT on the resting potential were the same as those of DDT (Table 8).

γ-BHC: Soaking the nerve for 3 to 4 hours in $5\times10^{-5}$M γ-BHC Ringer had no effect on the resting potential of the nerve, on the rate of increase in the resting potential after mounting, on the maximum resting potential attained, or on the anoxic depolarization (Fig. 9, Table 8).

### Effects of Potassium Ions

It has long been known that potassium ions induce a depolarization of nerve and muscle[10,45,63]. Potassium depolarization is not caused by metabolic disturbances in the nerve as in the case

Table 7. Anoxic depolarization of the nerve treated with DDT. Local application method. Mean values (mV). For explanations see Table 1.

| Date (Temp.) | DDT (M) | No. of exp. | Max. pot. (1) | Pot. immediately before $N_2$ | 30 min. after $N_2$ | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Standard pot. (2) | Min. pot. (3) | (3)/(2) % | (3)/(1) % |
| 9.1 (28°C) | 0 | 5 | 40.9 | 29.6 | 28.1 | 20.2 | 71.7 | 49.3 |
| | $5\times10^{-5}$ | 5 | 43.6 | 29.6 | 28.0 | 19.4 | 69.8 | 42.7 |
| 9.10 (25°C) | 0 | 3 | 47.3 | 36.5 | 32.8 | 25.6 | 77.9 | 54.2 |
| | $5\times10^{-5}$ | 4 | 47.3 | 39.3 | 35.9 | 27.7 | 76.7 | 58.3 |

Table 8. Anoxic depolarization of the nerve treated with DDT, DDT analogues, and BHC. Soaking method. Mean values (mV). For explanations see Table 1.

| Date (Temp.) | Insecticide (M) | No. of exp. | Pot. immediately after mounting | Max. pot. (1) | 30 min. after $N_2$ | | | | 60 min. after $N_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Standard pot. (2) | Min. pot. (3) | (3)/(2) % | (3)/(1) % | Standard pot. (4) | Min. pot. (5) | (5)/(4) % | (5)/(1) % |
| 8.27 (33°C) | Nil | 2 | 30.7 | 34.4 | 36.7 | 21.2 | 57.0 | 62.5 | | | | |
| | DDT $(5\times10^{-5})$ | 2 | 19.9 | 25.5 | 22.0 | 10.8 | 50.0 | 42.4 | | | | |
| | $CH_3O$-DDT $(5\times10^{-5})$ | 2 | 20.1 | 25.6 | 23.9 | 12.3 | 50.8 | 48.2 | | | | |
| | $C_2H_5O$-DDT $(5\times10^{-5})$ | 2 | 22.8 | 24.2 | 23.6 | 11.3 | 49.6 | 46.4 | | | | |
| | BHC $(5\times10^{-5})$ | 2 | 31.2 | 33.7 | 30.5 | 19.7 | 64.7 | 58.4 | | | | |
| 9.2 (28.5°C) | Nil | 3 | 33.0 | 41.1 | 38.4 | 24.7 | 64.4 | 60.4 | 38.0 | 23.4 | 61.8 | 57.3 |
| | DDT $(5\times10^{-5})$ | 4 | 21.6 | 26.5 | 25.8 | 15.8 | 61.1 | 58.8 | 26.2 | 12.0 | 44.5 | 43.7 |
| | BHC $(5\times10^{-5})$ | 3 | 27.4 | 41.5 | 40.1 | 24.5 | 60.9 | 59.2 | 40.9 | 22.3 | 54.7 | 54.0 |
| 9.3 (29.5°C) | Nil | 3 | 25.9 | 36.7 | 33.5 | 20.7 | 61.8 | 56.2 | 32.7 | 19.1 | 59.0 | 52.0 |
| | DDT $(5\times10^{-5})$ | 4 | 14.2 | 23.1 | 19.1 | 10.9 | 58.1 | 46.0 | 18.7 | 9.7 | 54.3 | 41.2 |
| | BHC $(5\times10^{-5})$ | 3 | 19.8 | 31.8 | 31.6 | 23.0 | 72.8 | 72.3 | 32.0 | 21.1 | 66.0 | 66.6 |

Table 9. Depolarization by a cathodal current of the nerve treated with DDT. Local application method. Mean values (mV). For explanations see Table 1.

| Date (Temp.) | DDT (M) | No. of exp. | Max. pot. (1) | Pot. immediately before current (2) | During current | | | After current | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Min. pot. (3) | (3)/(1) % | (3)/(2) % | Max. repol. pot. (4) | (4)/(1) % | (4)/(2) % | (4)/(3) % | Recovery time (min.) |
| 9.24 (24°C) | 0 | 5 | 38.0 | 36.8 | 18.1 | 48.6 | 50.6 | 25.8 | 68.3 | 71.0 | 141.8 | 26 |
| | $5\times10^{-5}$ | 5 | 43.7 | 41.5 | 20.1 | 45.2 | 49.4 | 28.6 | 63.9 | 64.7 | 148.2 | 49 |
| 9.29 (22.5°C) | 0 | 5 | 42.8 | 41.2 | 28.9 | 68.4 | 70.6 | 33.7 | 80.4 | 82.9 | 117.9 | 18 |
| | $5\times10^{-5}$ | 5 | 46.4 | 43.7 | 28.7 | 61.6 | 65.0 | 33.2 | 71.8 | 75.7 | 116.9 | 37 |

Table 10. Anoxic depolarization of the nerve treated with K-rich Ringer. Soaking method. Mean values(mV). Sep. 4, 31°. For explanations see Table 1.

| $K^+$ conc. (mM) | No. of exp. | Max pot. (1) | 30 min. after $N_2$ | | | | 60 min. after $N_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Standard pot. (2) | Min. pot. (3) | (3)/(2) % | (3)/(1) % | Standard pot. (4) | Min. pot. (5) | (5)/(4) % | (5)/(1) % |
| 3.1 (Normal Ringer) | 2 | 36.8 | 37.9 | 24.2 | 63.8 | 65.6 | 38.7 | 16.9 | 43.8 | 45.9 |
| 15.0 | 3 | 25.6 | 24.5 | 14·4 | 59.0 | 56.6 | 24.4 | 11.6 | 47.2 | 45.3 |

of IAA and NaF but is caused by a change in the concentration gradient outside and inside of the nerve fibres. The resting potential of the nerve soaked for 3 hours in potassium-rich Ringer (15mM $K^+$) was depressed (Fig. 11, Table 10), but the percentage of the ratio of

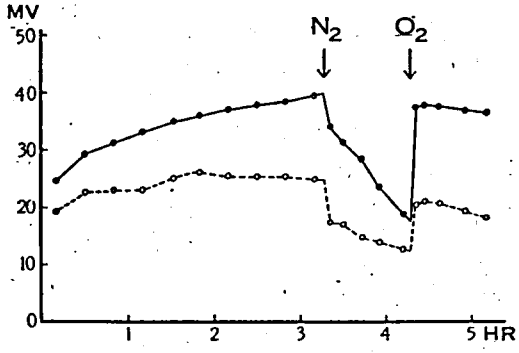


Fig. 11. Anoxic depolarization of the nerve treated with K-rich Ringer. Soaking method. A solid line with closed circles shows the nerve soaked for 3 hours in normal Ringer (3.1mM K), and a broken one with open circles the nerve soaked in 15mM K Ringer. Sep. 4, 31°.

the minimum resting potential during anoxia to the standard resting potential was about equal to that of the control nerve. These results indicate that potassium ions equally depress the aerobic and anaerobic fractions of the resting potential.

## Discussion

The mode of production of bioelectric potentials in nerve and muscle has long been studied by many investigators since an ionic hypothesis was first proposed by Bernstein (1902). At present, the sodium theory proposed by Hodgkin (1951) is the most valuable one and is widely accepted. The resting potential can be explained by this sodium theory. The resting membrane is readily permeable to both potassium and chloride ions, but is sparingly permeable to sodium ions. Since an active sodium pump mechanism removes sodium ions from the interior as fast as they diffuse in, the concentration of sodium ions inside the nerve fibre is much lower than that of sodium ions outside the nerve fibre. As a consequence of this sodium distribution and of a high internal concentration of impermeable anions, such as are provided by glutamic and aspartic acids, the potassium and chloride ions are distributed very unequally under the condition of Donnan equilibrium. The internal potassium content is much greater than the external, while the chloride ion content is greater outside the nerve than inside. The resting potential, E, being the potential obtaining in a Donnan equilibrium, is related to the relative concentration of potassium or chloride ions by the equation:

$$E = \frac{RT}{F} \log_e \frac{[K]_i}{[K]_o} = \frac{RT}{F} \log_e \frac{[Cl]_o}{[Cl]_i}$$

$[K]_i$ and $[K]_o$ being internal and external potassium concentration respectively, $[Cl]_i$ and $[Cl]_o$ internal and external chloride concentration respectively, R, the gas constant, T, absolute temperature, and F, Faraday. Because the excised nerves are no longer in a steady state, the above mentioned equations cannot be applied to them. Hodgkin & Katz (1949) proposed a slightly modified equation for nerves which are in a non-steady state, and their observed resting potentials were in good agreement with that of their calculated values.

Thus, resting potentials can be explained definitely in terms of ionic concentration gradients. Furthermore, many studies proved the

importance of metabolic energy in the maintenance of the resting potential. For example, nerve and muscle can be depolarized by anoxia and metabolic inhibitors such as cyanide, monoiodoacetate, sodium fluoride, or sodium azide [16,17,23,39,42,45,70,72]. Depolarization can also be caused in insect nerve by lack of oxygen and metabolic inhibitors as is shown in the present experiments. But as to how metabolism relates to the maintenance of the resting potential is not clear. The sodium pump mechanism and the potassium retention force, which retains the potassium ions inside the nerve fibre against a concentration gradient, both may operate with metabolic energy [29,67,69,71].

The relationship between the resting potential and the metabolism in the nerve will first be discussed in the light of the present experiments. The mean value of the resting potentials of the roach nerves is 40.8mV (range, 29.7～51.4mV) as is shown in Table 1. Although this value undoubtedly does not show an absolute value of the resting potential, this may be relatively close to the absolute value, because the absolute resting potentials of the nerves determined in other animals show the following values: the giant axon of squid (*Loligo*), 61mV [10] or 62mV [28], that of another squid (*Sepia*), 62mV [84], that of crab (*Homarus*), 62mV [27], and the myelinated nerve fibre of frog (*Rana*) 71mV [31,32]. The reason that relatively high values of the resting potential could be obtained in the roach by external electrodes may be as follows: the giant axons are much larger than the other axons in the nerve cord, and the larger the diameter of the axon or the smaller the short circuit, the larger the potential which can be recorded.

The resting potential of the roach nerve cord declines rapidly when nitrogen is introduced into the nerve chamber, and attains a minimum value within 30 to 60 minutes. This decline of the potential in the cockroach is more rapid than that in the frog nerve [45,72]. The difference in the diameter of the nerve trunk, or of the thickness and property of the nerve sheath may determine differences in diffusion rates of gases through the nerve membrane.

The resting potential can be divided into two fractions: an aerobic resting potential which is depolarized by anoxia declines gradually for a long period of time in oxygen or air, and an anaerobic resting potential which is not depolarized by anoxia is sustained for a long period of time in the same conditions. Each composes about half the total resting potential.

The reason for the ineffectiveness of several substrates on both the resting potential and the anoxic depolarization remains unknown. Two alternative explanations may be applied: either the nerve membrane are impermeable to the substrates or the substrates cannot be utilized as sources of energy.

The mode of the depolarizing action of IAA and NaF may be different in some aspects. The lower concentrations of IAA, 0.0001M and 0.001M, largely depolarize the aerobic resting potential, while the higher concentration, 0.002M, completely depolarizes the aerobic and partly depolarizes the anaerobic fraction of the resting potential. The threshold concentration of NaF that produces depolarization is higher than that of IAA, showing a value of 0.02M. Furthermore, NaF depolarizes both the aerobic and the anaerobic resting potentials equally even at the threshold concentration. These differences can be expected as the mode of inhibition of enzymes by these two inhibitors is different. However, further explanations of these phenomena cannot be proposed at the present time.

Depolarization by the KCN treatment occurs very rapidly, but to a lesser degree than that caused by oxygen lack. Furthermore, a partial recovery of the resting potential is frequently observed in spite of the presence of KCN in Ringer, showing a difference from either the IAA and NaF induced depolarization of the insect nerve or the depolarization of the nerve of other animals produced by KCN [45,87]. These actions of KCN cannot be attributed to the effect of $K^+$, because the concentrations of KCN used are very low.

As is expected from the former reports on other animals [4,9,10,14,32,43,45,63,73,74], the nerve of the cockroach is also depolarized by the

potasssium-rich Ringer. This depolarization may not be due to a metabolic disturbance but may be due to the change in the potassium ion concentration gradient inside and outside the nerve fibres. Therefore, an equal depression of the resting potential in both the aerobic and the anaerobic fractions is brought about.

Thus it has been clarified that the nerve fibres of the cockroach require metabolic energy for the maintenance of the resting potential. In the following several paragraphs we will review the mode of toxic action of DDT, and then will discuss the cause of the functional changes in the nerve under the influence of DDT.

The effects of DDT on the various functions of nerve have been studied by many investigators and it was clarified that an augmentation of the repetitive excitability of the nerve is a most important primary action of DDT [13,18,56,57,86,88–93].

The effects of DDT on some metabolic cycles have also been studied, especially on several oxidases and dehydrogenases. But the results obtained are conflicting; some investigators reported the inhibitory action of DDT on the activity of succinoxidase or cytochrome oxidase [1, 35, 47, 51, 53, 60,61], some reported the accelerating action of DDT upon oxidase[81], and others reported that DDT had no effect on succinoxidase, cytochrome oxidase, or dehydrogenases [6,15,36,37, 40,48,75]. Careful consideration of the differences in the experimental conditions described in the above mentioned reports suggests that DDT may affect the oxidase activity under some *in vitro* conditions, i. e., with higher concentrations of DDT or with suitable solvents of DDT, while DDT cannot affect the oxidase activity *in vivo* except that during the later stage of DDT poisoning the oxidase may be depressed. In the later stage of poisoning, not only oxidases but also other enzymes may be secondarily depressed. In spite of many accumulated data concerned with the enzymatic actions of DDT on the muscle or on the whole insect body, no enzymatic experiments to explain the functional changes in the nerve caused by DDT have been undertaken to date upon the insect nerve.

The effects of DDT on the carbonic anhydrase activity [80] and on the phosphate metabolism [59,78, 79] were also studied, but no conclusions could be drawn about the cause of the functional changes in the nerve. Since cholinesterase activity is not affected by DDT both *in vivo* and *in vitro* [54 62.77,82,83], the hyperexcitability of the nerve brought about by the action of DDT must be quite different in nature from that brought about by the organophosphates, parathion and TEPP.

Both the increase in oxygen consumption of the DDT poisoned insect [7,8,24,44,46,52] and the marked metabolic exhaustion in it [11, 46,50,58] must have resulted from violent muscular activity and must be a manifestation of the secondary action of DDT.

The above mentioned reports do not propose any explanations of the cause of functional changes in the nerve poisoned with DDT. Any enzymatic actions of DDT cannot be related to the changes in the functions of the nerve. The present experiments indicate that the mode of action of DDT on the nerve is quite different in nature from that of both metabolic inhibitors and potassium ions.

The local application of DDT Ringer on the roach nerve cord affects neither the resting potential nor the anoxic depolarization. These results are in good agreement with those of the crab (*Libinia*) nerve [66], indicating that DDT does not affect the metabolism sustaining the resting potential. The soaking of the nerves for several hours in DDT Ringer, however, shows some effects: an increase in the rate of the depolarization occurs during the soaking period, and a slowing in the rate of the repolarization is brought about after mounting the nerve on the electrodes. The depolarization of the normal cockroach nerve brought about by soaking the nerve in Ringer may be caused by either the lack of oxygen or the cathodal current provided by the cut ends of the nerve; when an injury current flows inwardly across the cut end of the nerve and outwardly across the intact region of the nerve, the intact region of the nerve must be slowly depolarized. The possibility that the cause of depolarization is the lack of oxygen

was excluded by the experiments in which the nerves were kept on Ringer-soaked filter paper for several hours; they were depolarized to the same degree as the controls soaked in Ringer.

The repolarization of the nerve after mounting must be brought about by a removal or a marked depression of the cathodal current caused by a decrease in the short circuit outside the nerve. Whatever the proper explanation may be, it can be postulated that DDT also affects the rate of depolarization or repolarization brought about by other ways, i. e., artificially applied cathodal current. As was described in the foregoing section, DDT slows the rate of the repolarization after a removal of the cathodal current, but it has no effect on the rate of the depolarization by the cathodal current.

These observations are very interesting for they have some similarity to an increase and prolongation of the negative after-potential caused by DDT and reported earlier[33,34]. In order to discuss the possible events occurring under the influence of DDT, a widely accepted excitation theory, the sodium theory[25], will be cited here briefly to explain the action potential. The nerve membrane becomes highly and selectively permeable to sodium ions when a stimulus of any kind is applied to the nerve. Therefore, sodium ions enter into the nerve according to the concentration gradient, establishing an equilibrium potential,

$$E_{Na} = \frac{RT}{F} \log_e \frac{[Na]_i}{[Na]_o}$$

This equilibrium potential brings about a depolarization and a reversal of the membrane potential. Since an increase in the membrane permeability to potassium ions occurs soon after, the reversed membrane potential is repolarized rapidly to the normal level. These processes can be recorded as an action potential. The sodium ions entered into the nerve fibre during the excitation are pumped out by an energy driven sodium pump mechanism, and the potassium ions flowed out are absorbed by an active metabolic force[25,29,69,71].

Based upon this theory, the increase and prolongation of the negative after-potential may be explained in terms of the changes in the rate of ionic movement across the nerve membrane. The slowing of the repolarization after a removal of the cathodal current under the influence of DDT may be explained in terms of a depression of potassium absorption, because the depolarization produced by the cathodal current must be caused by a leakage of the potassium ions from inside the nerve fibre. These changes in the rate of ionic movement must be caused either by the physico-chemical actions of DDT upon the nerve membrane bringing about the changes in the ionic permeability, or by the actions of DDT on the metabolism relating strictly to the excitation process, for example, the sodium pump or the potassium retention mechanism. These relations can also be discussed from another point of view. Nerve metabolism can be divided into two categories: an active metabolism acting directly and strictly on the excitation process, and a resting metabolism acting on the maintenance of the resting potential. This idea is supported by many experiments which indicate different or specific actions of various metabolic inhibitors and narcotics on each type of metabolism. For example, sodium azide, which does not block conduction in lower concentrations, has no effect on the rate of oxygen consumption of the resting nerve, but it depresses the increase in the rate of oxygen consumption during activity of the nerve. Methyl fluoroacetate has an opposite effect, depressing the rate of oxygen consumption of the resting nerve, while keeping that of the active state unchanged[12]. Chloretone inhibits the rate of oxygen uptake in both the resting and active nerves, but the inhibition is greater in the active nerve[5]. Several narcotics, at lower concentrations do not affect the rate of respiration of brain slices, but do inhibit the rate of respiration in brain slices which were electrically tetanized[49]. From the above mentioned results and discussions, it can be concluded that DDT does not affect the resting metabolism of the nerve. The energy for the sodium pump and the potassium retention mechanism may be supplied by the active matabolism.

Since narcotics, which inhibit the active meta-

bolism of nerve as described above, decrease the ionic permeability of the nerve membrane [2,19,20,22,30,64,65,68], there may be a correlation between the increase in the ionic permeability which occurs during excitation and the active metabolism of the nerve. Based upon these points of view, it can be supposed that DDT acts on the active metabolism of the nerve, and it affects either the ionic permeability in active state or the sodium pump and the potassium retention mechanism.

The other possible effect of DDT is a direct physico-chemical action on the nerve membrane as mentioned earlier. The changes in the ionic permeability of the membrane must be necessarily induced by such a direct action of DDT. This idea is supported by the experiments already reported [92] which clarified that DDT acted more strongly on the insect nerve at lower temperature than at higher temperature. If DDT had acted on the enzymatic reactions, its effects would be stronger at the higher temperature than at the lower one. Furthermore, if an adsorption of DDT on the special structure of the nerve membrane were a primary and important event, DDT would be effective at the lower temperature than at the higher one.

The various theories of the action of DDT and its relation with metabolism and function of the nerve is illustrated schematically in Fig. 12.

Based on this hypothesis it can be claimed that DDT acts on the nervous function by eventually changing the mechanism of the ionic transfer across the membrane. It remains to be solved whether DDT disturbs the active metabolism or it affects the membrane physico-chemically. Direct determination of the ionic permeability of nerve membrane poisoned with DDT has not been made. Tobias (1948) only reported that the sodium content of the roach nerve cord poisoned with DDT increased. This result can clearly be explained by the present hypothesis.

This hypothesis can be demonstrated by the application of several methods, for example, the direct measurements of the resting and the action potentials of the nerve fibre [26,41], or the direct measurements of the ionic fluxes across the membrane by the tracers, $Na^{24}$ and $K^{42}$ [38]. Experiments using the former method are being conducted in our laboratory.

## Summary

The effects of p,p′-DDT, its ethoxy analogue, methoxychlor, and γ-BHC on the resting potential of the cockroach nerve cord have been studied and compared with those of oxygen lack, metabolic inhibitors, and potassium ions.

1. The resting potential, a potential difference between an uninjured central region of the nerve and a cut peripheral end soaked in Ringer-isotonic KCl solution, attains the mean value of 40.8mV.

2. The resting potential has a tendency to decline when the rearing temperature of the roaches is lowered.

3. The resting potential declines rapidly when nitrogen is introduced into the nerve chamber, attaining the maximum value of 50 per cent of the resting potential within 30 to 60 minutes. The potential returns to the normal level very rapidly on a readmission of oxygen or air, and often shows an overshoot of the potential. An anoxia-sensitive fraction of the resting potential was named "aerobic resting potential", while an anoxia-insensitive fraction, "anaerobic resting potential".

4. The aerobic resting potential declines gradually in oxygen or air over a long period of time, but the anaerobic one is maintained at a constant level.

5. Sodium pyruvate, sodium lactate, glucose and sucrose have no effect on the resting potential and the anoxic depolarization.

6. The nerve is depolarized whether the nerve is soaked in monoiodoacetate solution or the nerve is locally treated with it. Lower concentrations of monoiodoacetate depolarize largely the aerobic resting potential, while higher concentration completely depolarizes the aerobic resting potential and partly depolarizes the anaerobic resting potential. The presence of sodium pyruvate or sodium lactate in the solution does not alter the effect produced by monoiodoacetate.

7. Sodium fluoride also depolarizes the nerve

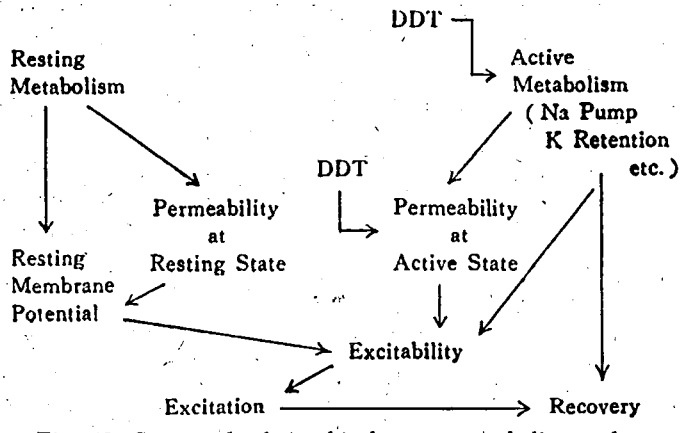


Fig. 12. Suggested relationship between metabolism and function in the nerve, and the possible points of action of DDT.

whether the soaking or the local application method is applied. Above threshold concentrations, it equally depolarizes both the aerobic and the anaerobic resting potentials.

8. A local application of KCN brings about a depolarization, which is followed frequently by a partial repolarization.

9. p,p′-DDT has no effect on the resting potential and the anoxic depolarization when locally applied.

10. p,p′-DDT shows some effects when the nerve is soaked for several hours in DDT Ringer: the nerve is much more depolarized in the presence of DDT while it is soaking in Ringer, and the rate of the repolarization brought about after mounting it on the electrodes decreases. Such a depolarization in the presence of DDT is brought about by an equal depression of both the aerobic and the anaerobic resting potentials.

11. p,p′-DDT has no effect on the depolarization produced by a cathodal current, but slows the rate of the repolarization after removal of the current.

12. Ethoxy analogue of p,p′-DDT and methoxychlor have the same effect as that of DDT on the resting potential and the anoxic depolarization. γ-BHC has no effect upon them.

13. 15mM K+ brings about a depolarization. It depolarizes both the aerobic and the anaerobic resting potentials equally.

14. These data were discussed in the light of the knowledge of metabolism and function of the nerve and the effects of DDT on the nervous function and metabolism in insects obtained to date. A hypothesis was proposed which is shown in Fig. 12. It was suggested that DDT affects the nerve function by changing the ionic permeability of the nerve membrane, and that such a change in permeability is caused by either the action of DDT on the active metabolism of the nerve or the direct physico-chemical action of DDT on the nerve membrane.

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## References

1) Anderson, A. D., R. B. March & R. L. Metcalf: Ann. Ent. Soc. Amer. **47**, 595(1954).
2) Bennett, A. L. & K. G. Chinburg: J. Pharmacol. Exper. Therap. **88**, 72 (1946).
3) Bernstein, J.: Pflüg. Arch. Ges. Physiol. **92**, 521 (1902).
4) Bishop, G. H.: J. Cell Comp. Physiol. **1**, 177 (1932).
5) Bronk, D. W. & F. Brink: Feder. Proc. **10**, 19 (1951).
6) Brown, B. E. & A. W. A. Brown: J. Econ. Ent. **49**, 675 (1956).
7) Buck, J. B. & M. L. Keister: Biol. Bull. **97**, 64 (1949).
8) Buck, J. B., M. L. Keister & I. Posner: Ann. Ent. Soc. Amer. **45**, 369 (1952).
9) Cowan, S. L.: Proc. Roy. Soc. B **115**, 216 (1934).
10) Curtis, H. J. & K. S. Cole: J. Cell. Comp. Physiol. **19**, 135 (1942).
11) Dahm, P. A. & C. W. Kearns: Ann. Ent.

Soc. Amer. **44**, 573 (1951).
12) Doty, R.W. & R.W. Gerard: Amer. J. Physiol. **162**, 458 (1950).
13) Dresden, D.: Physiological Investigations into the Action of DDT. Drukkerij en Uitgeverij G. W. van der Wiel & Co., Arnhem (1949).
14) Feng, T.P. & Y.M. Liu: J. Cell. Comp. Physiol. **34**, 33 (1949).
15) Fukami, J., M. Suko & C. Kusano: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1952).
16) Furusawa, K.: J. Physiol. **67**, 325(1929).
17) Gerard, R.W.: Amer. J. Physiol. **92**, 498 (1930).
18) Gordon, H. T. & J. H. Welsh: J. Cell. Comp. Physiol. **31**, 395 (1948).
19) Goto, K. & S. Sato: J. Jap. Physiol. Soc. **14**, 364 (1952a).
20) ——, ——: ibid. **14**, 427 (1952b).
21) Guttman, R.: J. Gen. Physiol. **22**, 567 (1939).
22) Hardt, A. & A. Fleckenstein: Arch. Exp. Pathol. Pharmak. **207**, 39 (1949).
23) van Harreveld, A.: Amer. J. Physiol. **147**, 669 (1946).
24) Harvey, G.T. & A.W.A. Brown: Can. J. Zool. **29**, 42 (1951).
25) Hodgkin, A. L.: Biol. Rev. **26**. 339 (1951).
26) Hodgkin. A. L & A. F. Huxley: Nature **144**, 710 (1939).
27) ——, ——: J. Physiol. **104**, 176 (1945).
28) Hodgkin, A.L. & B. Katz: ibid. **108**, 37 (1949).
29) Hodgkin, A. L. & R. D. Keynes: ibid. **128**, 28 (1955).
30) Holland, W. C.: J. Pharmacol. Exper. Therap. **111**, 1 (1954).
31) Huxley, A.F. & R. Stämpfli: J. Physiol. **112**, 476 (1951a).
32) ——, ——: ibid. **112**, 496 (1951b).
33) Ishii, T. & T. Yamasaki: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1953).
34) ——, ——: ibid. (1956).
35) Johnston, C.D.: Arch. Biochem. & Biophys. **31**, 375 (1951).
36) Judah, J.D.: Brit. J. Pharmacol. **4**, 120 (1949).
37) Kanehisa, K.: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1953).
38) Keynes, R. D.: J. Physiol. **114**, 119 (1951).
39) Koch, E.: Pflüger's Arch. **216**, 100 (1927).
40) Koshihara, T. & J. Fukami: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1953).
41) Ling, G. & R.W. Gerard: J. Cell. Comp. Physiol. **34**, 383 (1949a).
42) ——, ——: ibid. **34**, 413 (1949b).
43) ——, ——: Nature **165**, 113 (1950).
44) Lord, K.A.: Ann. Appl. Biol. **36**, 113 (1949).
45) Lorente de Nó, R.: A Study of Nerve Physiology. The Studies from the Rockefeller Institute for Medical Reseach **131** and **132**. New York (1947).
46) Ludwig, D.: Ann. Ent. Soc. Amer. **39**, 496 (1946).
47) Ludwig, D., M. C. Barsa & C. T. Cali: ibid. **48**, 165 (1955).
48) Matsumoto, Y, & M. Hayakawa: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1952).
49) McIlwain, H.: Biochem. J. **53**, 403 (1953).
50) Merrill, R. S., J. Savit & J. M. Tobias: J. Cell. Comp. Physiol. **28**, 465 (1946).
51) Morrison, P. E. & A. W. A. Brown: J. Econ. Ent. **47**, 723 (1954).
52) Otsuka, M.: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1951).
53) Perry, A.S. & B. Sactor: Ann. Ent. Soc. Amer. **48**, 329 (1955).
54) Richards, A. G. & L. K. Cutkomp: J. Cell. Comp. Physiol. **26**, 57 (1945).
55) Roeder, K.D.: J. Exp. Zool. **108**, 243 (1948).
56) Roeder, K. D. & E. A. Weiant: J. Cell.

Comp. Physiol. **32**, 175 (1948).
57) ——, ——: Ann. Ent. Soc. Amer. **44**, 372 (1951).
58) Rosedale, J.: Ent. Soc. S. Africa **11**, 34 (1948).
59) Sacklin, J. A., L. C. Terriere & L. F. Remmert: Science **122**, 377 (1955).
60) Sacktor, B.: Ph. D. Thesis, Rutgers Univ., New Brunswick, N. J. (1949).
61) ——: J. Econ. Ent. **43**, 832 (1950).
62) Sakai, M.: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1955).
63) Shanes, A. M.: J. Cell. Comp. Physiol. **23**, 193 (1944).
64) ——: Science **107**, 679 (1948a).
65) ——: Biol. Bull. **95**, 245 (1948b).
66) ——: J. Gen. Physiol. **33**, 75 (1949).
67) ——: ibid. **33**, 643 (1950).
68) ——: J. Cell. Comp. Physiol. **38**, 17 (1951a).
69) ——: J. Gen. Physiol. **34**, 795 (1951b).
70) ——: Feder. Proc. **10**, 611 (1951c).
71) ——: Ann. N. Y. Acad. Sci. **55**, 1 (1952).
72) Shanes, A. M. & D. E. S. Brown: J. Cell. Comp. Physiol. **19**, 1(1942).
73) Shanes, A. M. & Hopkins: J. Neurophysiol. **11**, 331 (1948).
74) Steinbach, H. B.: J. Cell. Comp. Physiol. **15**, 373 (1940).
75) Suzuki, R., T. Yamasaki & T. Ishii: Abstracts of Meeting of the Society for Applied Zoology and Applied Entomology, Tokyo (1951).
76) Tobias, J. M.: J. Cell. Comp. Physiol. **31**, 125 (1948).
77) Tobias, J. M., J. J. Kollros & J. Savit: ibid. **28**, 159 (1946).
78) Tomizawa, C. & J. Fukami: Ōyō-Kontyū **12**, 1 (1956a).
79) ——, ——: Botyu-Kagaku **21**, 133(1956b).
80) Torda, C. & H. G. Wolff: J. Pharmacol. Exper. Terap. **95**, 444 (1949).
81) ——, ——: Proc. Soc. Exp. Biol. Med. **74**, 744 (1950).
82) Truhaut, R. & D. Vincent: Ann. Pharm. Francaises **5**, 159 (1947).
83) ——, ——: Bull. Soc. Chim. Biol. **30**, 694 (1948).
84) Weidmann, S.: J. Physiol. **114**, 372(1951).
85) ——: ibid. **129**, 568 (1955).
86) Welsh, J. H. & H. T. Gordon: J. Cell. Comp. Physiol. **30**, 147 (1947).
87) Wright, E. B.: Amer. J. Physiol. **148**, 174 (1947).
88) Yamasaki, T. & T. Ishii: Ōyō-Kontyū **7**, 157 (1952a).
89) ——, ——: ibid. **8**, 111 (1952b).
90) ——, ——: ibid. **9**, 87 (1953).
91) ——, ——: Botyu-Kagaku **19**, 1 (1954a).
92) ——, ——: ibid. **19**, 39 (1954b).
93) ——, ——: Jap. J. Appl. Zool. **19**, 16 (1954c).

## 正 誤 表

防虫科学 第 22 巻 I 春川忠吉博士古稀祝賀記念号

| 頁 | 行 | 誤 | 正 |
|---|---|---|---|
| 112 | 右下 6 | 対して第1化期増殖率 | 対して第2化期増殖率 |
| 148 | 左上 22 | 4.5±0.5分 | 4.5±0.5日 |
| 〃 | 右下 12 | 棲息密度 | 棲息密度 |
| 149 | 右下 2 | numbers | number |
| 208 | 下 5 | 燻蒸倉庫ガス | 燻蒸倉庫のガス |
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| 210 | 右上 22 | ガス濃度に比較して | ガス濃度に比例して |
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