Interaction of DDT with the Components of Lobster Nerve Membrane Conductance

TOSHIO NARAHASHI and HANS G. HAAS

From the Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27706

ABSTRACT The falling phase of action potential of lobster giant axons is markedly prolonged by treatment with DDT, and a plateau phase appears as in cardiac action potentials. Repetitive afterdischarge is very often superimposed on the plateau. Voltage-clamp experiments with the axons treated with DDT and with DDT plus tetrodotoxin or saxitoxin have revealed the following: DDT markedly slows the turning-off process of peak transient current and suppresses the steady-state current. The falling phase of the peak transient current in the DDT-poisoned axon is no longer expressed by a single exponential function as in normal axons, but by two or more exponential functions with much longer time constants. The maximum peak transient conductance is not significantly affected by DDT. DDT did not induce a shift of the curve relating the peak transient conductance to membrane potential along the potential axis. The time to peak transient current and the time for the steady-state current to reach its half-maximum are prolonged by DDT to a small extent. The finding that, under the influence of DDT, the steady-state current starts flowing while the peak transient current is partially maintained supports the hypothesis of two operationally separate ion channels in the nerve membrane.

The insecticide DDT is known to exert two major effects on nerve, namely the initiation of repetitive afterdischarge and the prolongation of an action potential. The repetitive discharge has long been known to occur in sensory, central, and motor nervous systems in DDT-poisoned insects, and is directly responsible for the symptoms of DDT poisoning; i.e. hyperexcitability, ataxia, and convulsion (Roeder and Weiant, 1948; Yamasaki and Ishii, 1952 *a*; 1954 *a*, *b*; Narahashi and Yamasaki, 1960 *a*; Welsh and Gordon, 1947; Lalonde and Brown, 1954). The prolongation of the falling phase of the action potential was first observed by Shanes (1949) using external electrodes on crab nerve, and was confirmed with cockroach nerve (Yamasaki and Ishii, 1952 *b*; Yamasaki and Narahashi, 1957 *a*, *b*). Extended experiments by means of intracellular microelectrodes on cockroach giant axons have revealed that the increased negative afterpotential in DDT is further

augmented and prolonged by removal of potassium from the bathing medium forming a plateau phase like cardiac action potential (Narahashi and Yamasaki, 1960 *a*, *b*). From the observations of delayed rectification and the measurements of membrane conductance during the plateau, it was suggested that the increase in potassium conductance of the nerve membrane or the sodium inactivation, or both, are partially inhibited thereby causing the prolongation of action potential.

The present study is concerned with the mechanism whereby the action potential is prolonged by treatment with DDT. The major part of the experiments was performed by means of voltage-clamp technique in an attempt to interpret the DDT action in terms of ionic conductances of the nerve membrane. The results to be described here definitely demonstrate the validity of the previous hypothesis, and suggest certain important features of membrane conductance changes. It turns out that DDT may become one of the most interesting chemicals as a tool in neurophysiology.

A preliminary report of this study has been published (Narahashi and Haas, 1967).

METHODS

Material Giant axons in the circumoesophageal connectives of the lobster, *Homarus americanus*, were used in the present experiments.

Current Clamp The resting and action potentials under current-clamp conditions were measured by means of intracellular capillary microelectrodes filled with 3 M KCl. The circumoesophageal connective was desheathed and split in half longitudinally. Recording was made from the giant axons which were still partially embedded in the half of the connective. The action potentials were produced by external stimuli applied to one end of the connective, or by transmembrane currents applied through the current microelectrode inserted near (about 100 μ) the recording microelectrode. After observing control responses, the bathing medium was replaced by the one containing DDT. The experiments were done at room temperature (22°C).

Voltage Clamp The method of sucrose-gap voltage-clamp with the completely isolated single axons was essentially the same as that described previously for squid axons (Moore, Blaustein, Anderson, and Narahashi, 1967) except that the nerve chamber was modified to adapt to the lobster axon. The method of correcting for leakage currents was also the same in principle as that described previously (Moore et al., 1967). This was based on the observation that the leakage current, which immediately followed the capacitative current, declined exponentially and reached a steady-state magnitude, and that the leakage current was symmetrical in its time course and magnitude both for hyperpolarizing and for depolarizing pulses (unpublished observation). The membrane currents associated with step hyperpolarizations of 40, 60, and 80 mv were recorded together with a family of membrane currents associated with step depolarizations. The magnitudes of the hyperpolarizing mem-

brane currents were then plotted against the membrane potentials at every 0.2 msec after the beginning of the pulse and at the steady state. This resulted in a family of straight lines for various moments in the time course of current, and was used to correct the depolarizing membrane currents for their leakage components which were assumed to be the same as and opposite to the hyperpolarizing leakage currents.

Unlike the current-clamp experiments mentioned above, it was necessary to carry out separate voltage-clamp experiments with normal axons and with axons soaked in DDT solution for a period of 40 min (with two exceptions in which the soaking period was 30 min). This was because the "artificial node" established by two sucrose streams under sucrose-gap conditions survived only up to 20–30 min in most cases while it took more than 20 min for DDT to exert its full effect. Thus the experiments were actually carried out in the following way: an isolated axon was soaked in DDT solution for 40 min, and then transferred to the sucrose-gap chamber which contained no DDT. The effect of DDT was irreversible after washing with normal, DDT-free medium. Since it took another 10–30 min for the measurements of membrane currents to be started after mounting the preparation in the chamber, the measurements were actually made 50–70 min after isolation of the axon preparation. The separate measurements of the control axon preparations that had not been exposed to DDT solution were made 15–145 min after the isolation of axons. There was no statistically significant correlation between the time and the peak transient and steady-state conductances ($P > 0.05$). Therefore, it was assumed that no deterioration occurred in this period of time, and all the control measurements were combined regardless of the time after isolation.

A few additional experiments were done in the same artificial node starting with normal solution and applying DDT solution in an attempt to trace the time course of the change in membrane current produced by DDT. All the voltage-clamp experiments were performed at 7–10°C. Since it took only less than 1 min for a family of membrane currents to be recorded, the change in temperature during this period of time was negligible.

Solutions The following artificial seawater was used as the bathing medium (mM): Na^+468, K^+10, Ca^{2+}25, Mg^{2+}8, Cl^-533, SO_4^{2-}4, HCO_3^-2.5, pH 7.9.

DDT Purified *p,p'*-DDT (dichlorodiphenyltrichloroethane) was supplied by Geigy Agricultural Chemicals (Ardsley, N.Y.). It was dissolved in ethanol to make up stock solution, which was in turn injected into a bathing medium to give a DDT suspension. The final concentration of ethanol in the test solution was a maximum of 1% (by volume), which was found to have no effect on the electrical excitability of nerve by itself (see also Moore, Ulbricht, and Takata, 1964).

Tetrodotoxin and Saxitoxin Tetrodotoxin was supplied by Sankyo Company (Tokyo, Japan). It was dissolved in deionized water to make up stock solution, which was kept in a refrigerator. The stock solution was diluted with bathing media daily. The stock solution of saxitoxin was supplied by Dr. E. J. Schantz, Department of the Army, Fort Detrick, Frederick, Maryland. It was also diluted daily. A concentration of 3×10^{-7} M was used for both toxins throughout the experiments.

RESULTS

Current Clamp

In the normal lobster axons, the falling phase of the action potential was followed by a slow repolarization or negative afterpotential (Fig. 1, A1). The negative afterpotential started increasing some 20 min after introduction of DDT at a concentration of 5×10^{-4} M (Fig. 1, A2). This latent time varied very much among preparations, and probably depends on the degree of

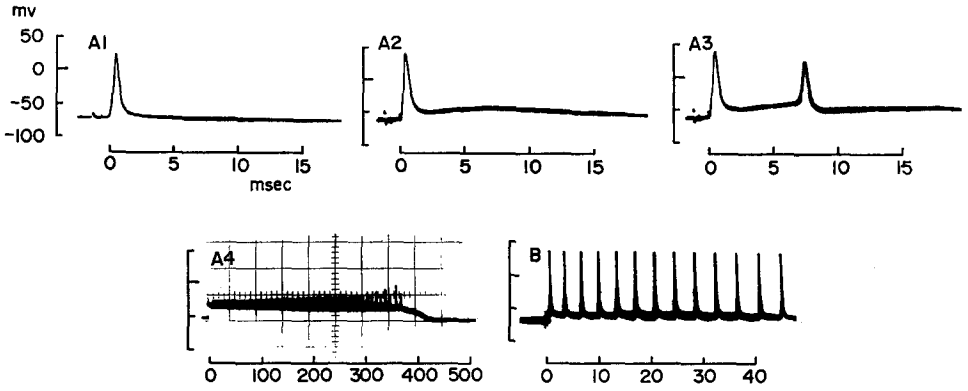

FIGURE 1. Action potentials before and after application of 5×10^{-4} M DDT. A1, control record before DDT; A2, 36 min in DDT, increase in negative afterpotential; A3, 42 min in DDT, afterdischarge superimposed on the increased negative afterpotential; A4, 78 min in DDT, repetitive afterdischarge superimposed on a prolonged, plateau action potential. The initial spike is too faint to be reproduced. B, another axon, repetitive afterdischarge superimposed on a slightly increased negative afterpotential, 9 min in DDT. Preparations, series A, 1-13-67-A; B, 1-5-67.

exposure of the axon to the external medium. In most preparations, a repetitive afterdischarge appeared during the augmented and prolonged negative afterpotential (Fig. 1, A3 and B). The magnitude of the negative afterpotential and the number of spikes in the afterdischarge grew progressively with time. Finally, the negative afterpotential increased so markedly that a plateau phase was formed similar to that seen in a cardiac action potential. Repetitive discharges were superimposed on the plateau in some cases (Fig. 1, A4), but in other cases they were lacking (Fig. 2). Despite the drastic change in the falling phase of the action potential, its magnitude and rising phase remained almost unchanged. The resting potential underwent little or no change from treatment with DDT.

The magnitude and duration of the plateau depended partly on the membrane potential. To study the effect of change of membrane potential on the plateau, the membrane was first hyperpolarized by passing an inward current through the current microelectrode for more than 5 sec, and an action po-

tential was elicited by a brief outward current while the membrane was still being hyperpolarized. The level of the plateau remained almost constant in the face of hyperpolarization (Fig. 2). The duration of the plateau was prolonged by moderate hyperpolarization, but there was a critical level of membrane potential beyond which the duration started shortening (Fig. 2). A similar change in the plateau was observed in a K-free seawater. The hyper-

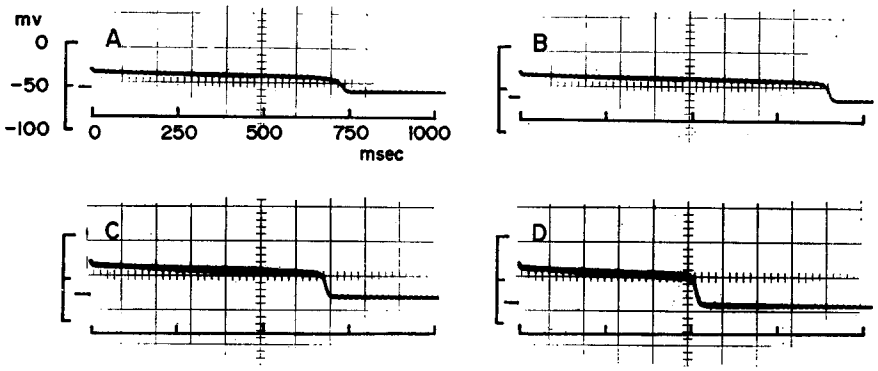

FIGURE 2. Effect of hyperpolarization on a prolonged plateau action potential in an axon exposed to 5×10^{-4} M DDT for 110 min. Each anodal hyperpolarization started some 5 sec before producing action potentials by a brief cathodal pulse. A, no polarizing current; B-D, three different magnitudes of hyperpolarization. With increasing hyperpolarization, the duration of the plateau is first prolonged and then shortened, while the initial level of the plateau remains almost unchanged. The initial spikes are too faint to be reproduced. Record A1 of Fig. 1 serves as the control before applying DDT. Preparation 1-13-67-A.

polarization due to the removal of potassium caused the change in the plateau similar to that produced by anodal hyperpolarization, and further changes in membrane potential by polarizing currents in K-free seawater produced the change in the plateau similar to that in seawater. Thus it can be said that the plateau formation in K-free DDT solution observed with cockroach giant axons (Narahashi and Yamasaki, 1960 *a*, *b*) was due to hyperpolarization.

The duration of the plateau depended also on the frequency of stimulation. Beyond a certain limit, the higher the frequency the shorter the plateau. Such a long latent period for the plateau duration agrees with the observations with DDT-poisoned cockroach axons (Narahashi and Yamasaki, 1960 *b*).

Voltage Clamp

As described in the Methods section, most of the voltage-clamp experiments were carried out separately with normal axons and with axons soaked in

DDT solution for 40 min because of the rather short survival times of the artificial node. Since isolated single axons were used, the 40 min treatment with DDT at a concentration of 5×10^{-4} M was enough to produce a prolonged plateau.

Membrane Current The families of membrane currents associated with step depolarizations in a normal and in a DDT-poisoned axon are illustrated in Fig. 3. By comparison of the family of currents of the DDT-poisoned axon

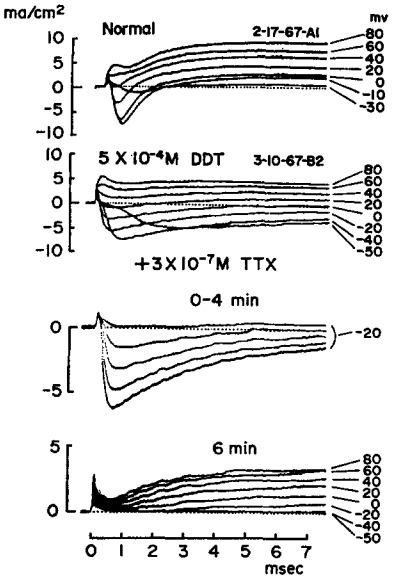

FIGURE 3. Families of membrane currents associated with step depolarizations from the holding membrane potential of −100 mv to membrane potentials indicated in a normal axon and in an axon treated with DDT and with DDT plus tetrodotoxin (TTX). The third set of records shows changes in the current during the course of TTX blockage; the largest current was recorded immediately before introducing TTX, and the smallest current 4 min after the introduction of TTX. The dotted lines refer to the zero base lines.

(second set) with that of the normal axon (top set), the following two changes by DDT immediately become apparent:

1. The peak transient currents decay much more slowly in the DDT-poisoned axon, leaving an *inward* steady-state current with small depolarizations.
2. The magnitudes of the steady-state currents are smaller in the DDT-poisoned axon than in the normal one.

Since the concentration of potassium in the external media was kept constant, and since the concentration of potassium in the axoplasm did not seem to have changed remarkably, as the resting potential remained constant, the inward steady-state currents seen in DDT cannot be due to inward flow of potassium ions. It is most likely that they represent the residual component of the peak transient currents. In order to test this idea, 3×10^{-7} M tetrodotoxin (TTX) was applied to the DDT-poisoned axon. TTX is known to selectively block the peak transient component of the membrane current without affecting the steady-state component at all (Narahashi, Moore, and

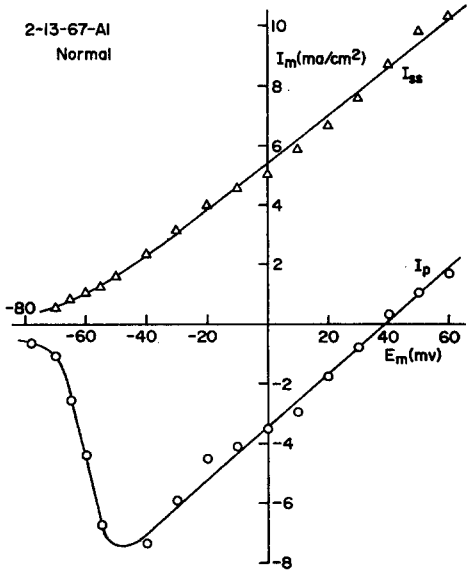

FIGURE 4 A

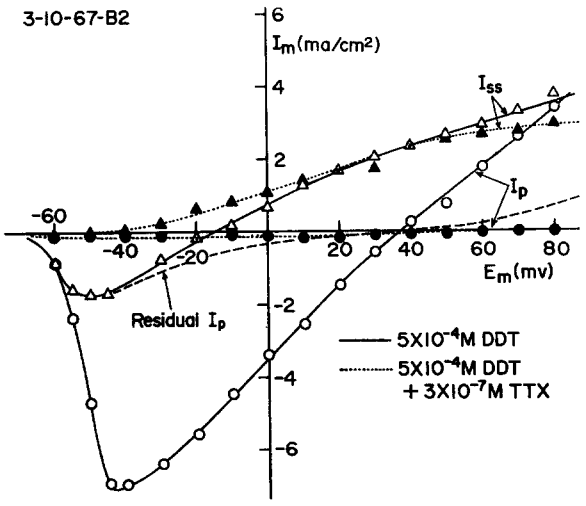

FIGURE 4 B

FIGURE 4. Current voltage relations for peak transient current (I_p) and for steady-state current (I_{ss}) in a normal axon (A) and in an axon treated with DDT and with DDT plus tetrodotoxin (TTX) (B). The residual component of peak transient current was obtained by subtracting I_{ss} in DDT plus TTX from that in DDT, and is shown by a broken line.

Scott, 1964; Nakamura, Nakajima, and Grundfest, 1965; Takata, Moore, Kao, and Fuhrman, 1966; Moore et al., 1967). The third set of records of Fig. 3 shows the change in membrane current at −20 mv membrane potential during the course of TTX blockage. It is clearly seen that the *inward* steady-state current has now been converted into an *outward* steady-state current as the peak transient current is blocked. This clearly indicates that the inward steady-state current is due to the residual component of the peak transient current which decays very slowly in DDT. The family of membrane currents associated with various step depolarizations is shown in the bottom

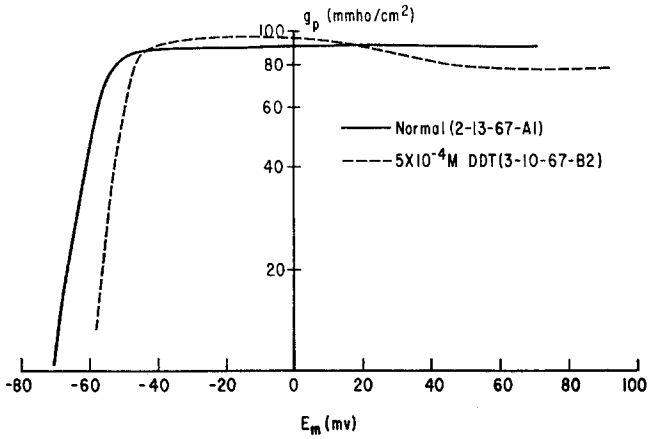

FIGURE 5. The curves relating the maximum peak transient conductance, calculated by equation (1), to membrane potential in a normal and in a DDT-treated axon.

set of Fig. 3. It is clear that the steady-state currents are much smaller in magnitude than those from the normal axon.

Current-Voltage Relations The current-voltage relations both for the peak transient and for the steady-state currents are plotted in Figs. 4 A and B. The peak transient current in the DDT-poisoned axon (B) does not differ greatly from that in the normal axon (A). The steady-state current in DDT is inward in direction at membrane potentials ranging from −60 to −15 mv, but is converted into outward in direction by the treatment with TTX over the entire range of membrane potential. It is noteworthy that the curve for the steady-state current in DDT and that in DDT plus TTX intersect each other at the membrane potential where the peak transient current in DDT reverses its polarity; i.e. at the equilibrium potential for the peak transient current. The residual component of the peak transient current is calculated by subtracting the steady-state current in DDT plus TTX from that in DDT, and is drawn with a broken line in Fig. 4 B. It can be seen clearly that the residual current has the same equilibrium potential as the

TABLE I

Maximum values for peak transient conductance (g_p) and for steady-state conductance (g_{ss}), and membrane potential where g_p attains its half-maximum ($E_{m/2}$) in normal, in DDT-treated, and in DDT- and tetrodotoxin (TTX)-treated axons. The concentrations of DDT and TTX are 5×10^{-4} M and 3×10^{-7} M, respectively.

Normal axons

Preparation	Time in DDT	Time after dissection	g_p*	g_{ss}	$E_{m/2}$*
	min	*min*	*mmho/cm²*	*mmho/cm²*	*mv*
2-8-67-B1	0	85	100	100	—
2-13-67-A1	0	15	92	80	−60
2-13-67-A2	0	30	88	150	—
2-13-67-A3	0	50	130	129	—
2-15-67-A2	0	40	139	141	—
2-15-67-B1	0	95	78	71	−49
2-15-67-B2	0	105	52	56	−64
2-15-67-B3	0	130	63	65	−49
2-17-67-A1	0	15	138	74	−21
2-17-67-A2	0	30	43	64	—
2-20-67-A2	0	40	78	58	—
2-20-67-A3	0	60	117	80	−26
3-27-67-A2	0	25	137	86	−32.5
3-27-67-A3	0	35	100	56	−44.5
3-27-67-B1	0	120	98	66	−29
3-27-67-B3	0	145	107	62	−30
3-29-67-A1	0	120	43	43	—
3-31-67-A1	0	45	63	39	−57
Mean±SE			93±7.3	79±3.8	−42±4.5

DDT-treated and DDT + TTX-treated axons

Preparation	Time in DDT	Time after dissection	g_p*		g_{ss}		$E_{m/2}$*
			DDT	DDT+TTX	DDT	DDT+TTX	DDT
3-2-67-A1	40	50	92	—	44	—	—
3-2-67-A2	40	60	34	—	21	—	−51
3-2-67-A3	40	65	73	—	39	—	−48
3-2-67-A4	40	70	58	—	42	—	−41.5
3-8-67-A1	30	60	33	8	37	32	−34.5
3-8-67-B1	30	50	53	—	68	—	−25
3-10-67-B1	40	50	71	—	50	—	—
3-10-67-B2	40	60	100	2	51	30	−51
3-13-67-A1	40	50	110	1	48	21	−28.5
3-15-67-A1	40	70	—	—	69	37	—
3-15-67-B2	40	65	114	—	50	—	—
3-24-67-A1	40	60	—	—	49	26	—
3-24-67-B1	40	50	97	—	60	—	—
Mean±SE			76±8.7	3.7±2.1	48±3.5	29±2.7	−40±4.1

* The difference between the mean values from normal and DDT-treated axons is statistically insignificant ($P > 0.05$).

peak transient current. This supports the idea that the inward steady-state current observed in the DDT-poisoned axon is due to the residual component of the peak transient current.

The magnitude of the true steady-state current in the DDT- and TTX-treated axon is much smaller than that in the normal axon (Figs. 4 A and B). Because the peak transient current decays rather quickly to very small values in normal axons in seawater, it is not necessary to block the current by TTX for the purpose of obtaining the true steady-state current of normal untreated axons.

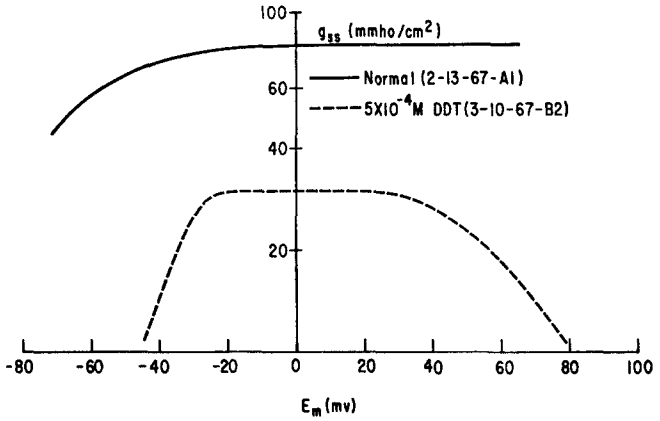

FIGURE 6. The curves relating the maximum steady-state slope conductance, calculated by equation (2), to membrane potential in a normal and in a DDT-treated axon.

Time Course of Change in Membrane Current after Application of DDT In a few preliminary experiments, continuous observations were made on the membrane current before and after application of DDT. The steady-state current had started decreasing within about 10 min after introducing DDT before the slowing of the falling phase of the peak transient current became apparent.

Membrane Conductances The membrane conductance (g_p) at the peak transient current (I_p) was calculated by the following equation:

$$g_p = \frac{I_p}{E - E_p} \qquad (1)$$

where E is the membrane potential and E_p is the membrane potential where I_p reverses its polarity. The peak transient conductances in a normal and in a DDT-poisoned axon are plotted against membrane potential on a semi-logarithmic scale in Fig. 5. Both conductance curves show a similar pattern.

Numerical data on the maximum value for the peak transient conductance

are given in Table I. The average value is 76 mmho/cm² for DDT-poisoned axons as against 93 mmho/cm² for fresh untreated axons. The difference is not statistically significant ($P > 0.05$).

Numerical data on the membrane potential where g_p attains its half-maximum ($E_{m/2}$) are also given in Table I. It is apparent from the mean values that there is no significant shift ($P > 0.05$) of the conductance curve along the potential axis.

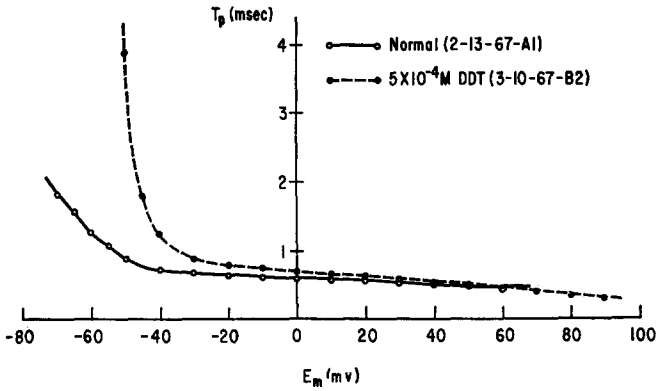

FIGURE 7. The time to peak transient current plotted as a function of membrane potential in a normal and in a DDT-treated axon.

Because of difficulty in estimating the equilibrium potential for the steady-state current, the slope steady-state conductance (g_{ss}) was calculated by the equation:

$$g_{ss} = \frac{dI_{ss}}{dE} \qquad (2)$$

where I_{ss} refers to the steady-state current. The steady-state conductances in a normal and in a DDT- and TTX-treated axon are plotted on a semilogarithmic scale in Fig. 6. In this particular DDT- and TTX-poisoned axon, the maximum value for the steady-state conductance is much smaller than that of the normal axon, and the conductance decreases with stronger depolarizations resulting in a bell-shaped curve.

Numerical data on the maximum steady-state conductance in normal, DDT-treated, and DDT- and TTX-treated axons are given in Table I. The conductances in DDT plus TTX represent the true steady-state conductances corrected for the residual component of the peak transient current under the influence of DDT, and give the mean value of 29 mmho/cm² as against the control mean value of 79 mmho/cm². Thus the maximum steady-state conductance is suppressed to 37% normal value by poisoning with DDT.

TABLE II

Time to peak transient current (T_p) associated with step depolarizations from the holding potentials (E_{hold}) to the membrane potentials indicated in normal axons and in axons treated with 5×10^{-4} M DDT.

Preparation	E_{hold}	T_p*		
		At − 40 mv	At − 20 mv	At 0 mv
	mv	*msec*	*msec*	*msec*
		Normal axons		
2-8-67-A1	−140	0.65	0.63	0.60
2-13-67-A1	−130	0.75	0.65	0.60
2-13-67-A2	−130	0.95	0.74	0.63
2-13-67-A3	−130	1.00	0.70	0.60
2-15-67-A2	−110	0.85	0.60	0.49
2-15-67-B1	−110	0.72	0.55	0.49
2-15-67-B2	−110	0.65	0.58	0.52
2-15-67-B3	−110	0.85	0.62	0.52
2-17-67-A1	−100	—	1.07	0.72
2-17-67-A2	−100	1.80	1.25	0.85
2-20-67-A2	−100	1.40	0.87	0.61
2-20-67-A3	−100	1.70	1.08	0.56
3-27-67-A2	−100	0.85	0.70	0.44
3-27-67-A3	−100	0.85	0.58	0.50
3-27-67-B1	−100	1.25	0.81	0.60
3-27-67-B3	−100	1.80	1.09	0.75
3-29-67-A1	−100	—	0.60	0.50
3-31-67-A1	−100	0.85	0.70	0.63
Mean±SE		1.06±0.101	0.77±0.051	0.59±0.022
		DDT-treated axons		
3-2-67-A1	−100	2.00	1.38	1.04
3-2-67-A2	−100	2.60	1.20	1.06
3-2-67-A3	−100	2.10	1.02	0.90
3-2-67-A4	−100	2.40	1.10	0.86
3-8-67-A1	−100	3.20	1.04	0.80
3-8-67-B1	−100	2.60	1.22	0.74
3-10-67-B1	−100	0.85	0.66	0.64
3-10-67-B2	−100	1.25	0.85	0.80
3-13-67-A1	−100	—	1.20	0.76
3-15-67-A1	−100	1.50	0.84	0.76
3-15-67-B2	−100	—	0.80	0.64
3-24-67-A1	−100	—	—	0.64
3-24-67-B1	−100	—	0.54	0.44
Mean±SE		2.06±0.248	0.99±0.085	0.78±0.053

* The difference between the mean values from normal and DDT-treated axons at each membrane potential is statistically significant ($P < 0.01$).

Time to Peak Transient Current The time to peak transient current from normal and DDT-poisoned axons is taken as a measure of the rate of conductance increase and is plotted as a function of membrane potential in Fig. 7. Although the time to peak transient current in the DDT-poisoned axon is in the same order of magnitude as that in the normal axon with stronger depolarizations, it deviates from the normal curve and is prolonged markedly with weaker depolarizations; i.e., at −40 mv or inside more negative membrane potentials.

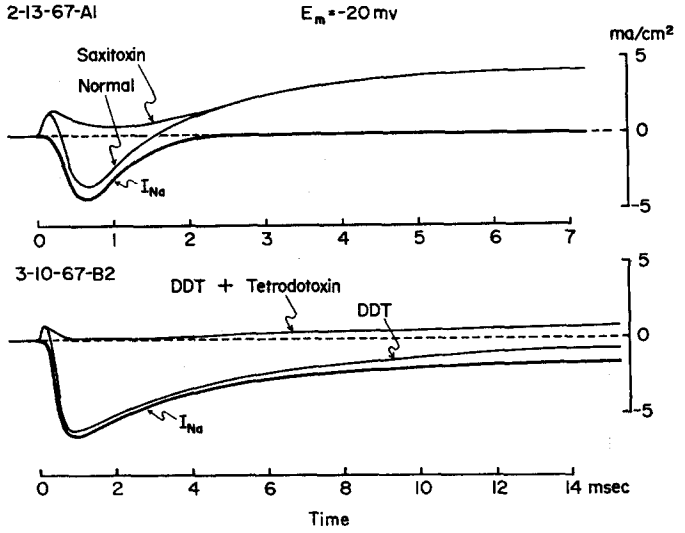

FIGURE 8. Separation of membrane current into peak transient (sodium) current and steady-state (potassium) current by the use of saxitoxin or tetrodotoxin at a concentration of 3×10^{-7} M in a normal and in a DDT-treated axon. The membrane current in saxitoxin and that in DDT plus tetrodotoxin show the steady-state (potassium) currents. The peak transient (sodium) current (I_{Na}) was obtained by subtraction of the steady-state (potassium) current from the total current.

Numerical data on the time to peak transient current are given in Table II, in which measurements are made at three membrane potentials; i.e., at −40, −20, and 0 mv. On an average, the time to peak transient current is prolonged by DDT 1.94-fold at −40 mv, 1.28-fold at −20 mv, and 1.32-fold at 0 mv. These values for the prolongation are statistically significant at each membrane potential ($P < 0.01$).

Time Course of Decline of Peak Transient Current In order to measure the time course of the falling phase of the peak transient current, the membrane current must be separated into two components. Fig. 8 shows this process. In normal axons, saxitoxin (STX) was applied at a concentration of 3×10^{-7} M to block the peak transient component. STX behaves in exactly the

same way as TTX in its highly selective inhibition of the peak transient current of lobster nerve (Narahashi, Haas, and Therrien, 1967). By subtraction of the membrane current in STX from the normal membrane current, the true peak transient current, which is carried mostly by sodium ions under normal conditions, can be obtained. The membrane current in STX is carried mostly by potassium ions. The peak transient component decays in

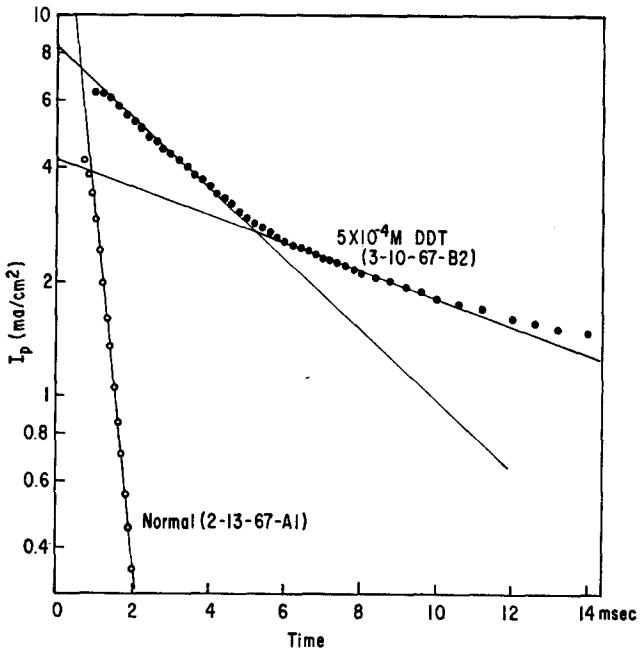

FIGURE 9. Semilogarithmic plot of the time course of the falling phase of the peak transient (sodium) current at −20 mv in a normal and in a DDT-treated axon after correction for the steady-state (potassium) current in the same way as in Fig. 8. The straight lines were drawn by eye. The time constants of the falling phase are estimated as 0.47 msec for the normal axon, and 4.7 msec and 12.0 msec for the initial and second phases of the DDT-poisoned axon, respectively.

about 2 msec from the beginning of the current. The lower set of Fig. 8 illustrates the similar procedure of subtraction in a DDT-poisoned axon by the use of TTX. The membrane current in DDT plus TTX is carried mostly by potassium ions. It is clear that the true peak transient current decays much more slowly than in the normal axon, and is still flowing almost steadily 14 msec after the onset of current.

The falling phases of the peak transient (sodium) currents corrected for the steady-state (potassium) currents in the manner shown in Fig. 8 are plotted against time on a semilogarithmic scale in Fig. 9. The measurements from the normal axon fall on a straight line having a time constant of 0.47

msec. On the contrary, the measurements from the DDT-poisoned axon fall on two or possibly more straight lines with time constants of 4.7 msec for the initial phase and 12 msec for the second phase.

Measurements of the time constant (τ_h) of the falling phase of peak transient current at the membrane potential of −20 mv are listed in Table III.

TABLE III

Time constant (τ_h) of the falling phase of peak transient (sodium) current corrected for steady-state (potassium) current by the use of saxitoxin and tetrodotoxin in normal and in DDT-treated axons, respectively. The concentration of DDT is 5×10^{-4} M, and that of saxitoxin and tetrodotoxin is 3×10^{-7} M. The measurements are made on the currents associated with step depolarizations from the holding potentials (E_{hold}) to the membrane potential of −20 mv.

Preparation	E_{hold}	τ_h	
		1st phase	2nd phase
	mv	*msec*	*msec*
		Normal axons	
2-13-67-A1	−130	0.47	—
2-13-67-A2	−130	0.70	—
2-13-67-A3	−130	0.74	—
2-15-67-A2	−110	0.48	—
2-15-67-B1	−110	0.56	—
2-20-67-A2	−100	0.67	—
2-20-67-A3	−100	0.96	—
Mean±SE		0.65±0.06	
		DDT-treated axons	
3-8-67-A1	−100	2.32	12.7
3-10-67-B2	−100	4.70	12.0
3-13-67-A1	−100	4.50	30.0
3-15-67-A1	−100	3.45	7.9
3-24-67-A1	−100	1.30	6.35
3-24-67-B1	−100	1.35	2.5
Mean±SE		2.93±0.62	11.9±3.94

The mean value for the initial phase in DDT-poisoned axons is 2.93 msec as against the normal mean value of 0.65 msec, indicating a 4.5-fold slowing. When the mean value for the second phase in DDT-poisoned axons is compared with the normal mean value, the slowing is as much as 18.3-fold.

Time Course of the Turning-On Process of Steady-State Current In order to compare the time course of the turning-on process of the steady-state current, measurements were made on the time for the corrected steady-state current to reach its half-maximum. Normal and DDT-poisoned axons were treated

by STX and TTX respectively at a concentration of 3×10^{-7} M, and the steady-state (potassium) current obtained in the manner shown in Fig. 8 was measured. Fig. 10 shows the relationship between the time and the membrane potential in a normal and in a DDT-poisoned axon. The time is considerably prolonged by DDT over the entire range of membrane potential. The measurements in Table IV were made at the membrane potentials

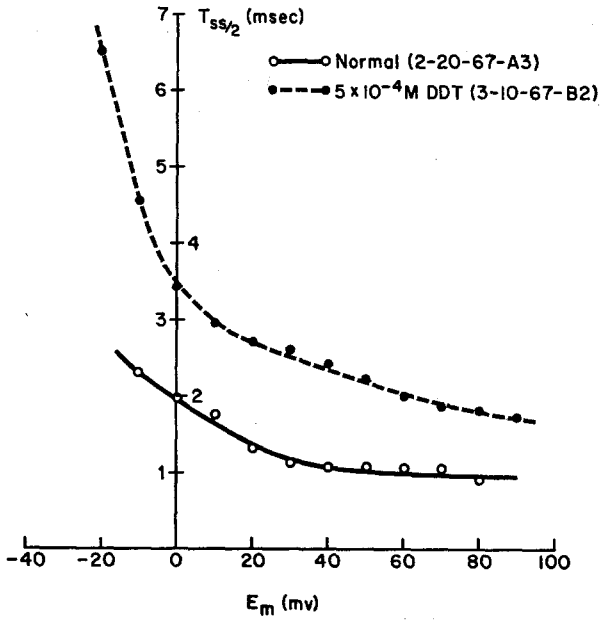

FIGURE 10. The time for steady-state current to reach its half-maximum plotted as a function of membrane potential in a normal and in a DDT-treated axon. The measurements were made on the steady-state (potassium) current after blocking the peak transient current by saxitoxin (3×10^{-7} M) in the normal axon and by tetrodotoxin (3×10^{-7} M) in the DDT-treated axon in the manner shown in Fig. 8. The holding membrane potential was −100 mv for both axons.

of 0 and +20 mv. The average values show that the time is slowed by the treatment with DDT from the normal value of 2.19 msec to 3.51 msec at the 0 mv membrane potential (1.6-fold), and from the normal value of 1.87 msec to 2.61 msec at the +20 mv membrane potential (1.4-fold). Since the time course of the turning-on process of the steady-state current changes with the holding membrane potential (Frankenhaeuser and Hodgkin, 1957; Cole and Moore, 1960), the mean values are also calculated for the measurements made at the holding potential of −100 mv as shown in parentheses in Table IV. The calculations give the same amount of slowing by DDT of the time for the steady-state current to reach its half-maximum; i.e. 1.6-fold slowing at 0 mv and 1.4-fold slowing at +20 mv.

DISCUSSION

The present experiments have definitely demonstrated that DDT slows down the turning-off process of the peak transient current and inhibits the steady-state current. This supports the previous hypothesis of DDT action on nerve (Narahashi and Yamasaki, 1960 *a*, *b*).

TABLE IV

Time for steady-state current to reach its half-maximum ($T_{ss/2}$) in normal and in DDT-treated axons. The steady-state current is obtained after correction for the peak transient current by the use of saxitoxin in the normal axons and by the use of tetrodotoxin in the DDT-treated axons. The concentration of DDT is 5×10^{-4} M, and that of saxitoxin and tetrodotoxin is 3×10^{-7} M. The measurements are made on the currents associated with step depolarizations from the holding potentials (E_{hold}) to the membrane potentials indicated.

Preparation	E_{hold}	$T_{ss/2}$	
		At 0 mv	At + 20 mv
	mv	*msec*	*msec*
		Normal axons	
2-13-67-A1	−130	2.60	2.20
2-13-67-A2	−130	2.35	2.10
2-13-67-A3	−130	3.20	2.80
2-15-67-A2	−110	1.05	0.95
2-15-67-B1	−110	1.45	1.35
2-17-67-A1	−100	2.70	2.25
2-20-67-A3	−100	1.95	1.45
Mean±SE		2.19±0.75 (2.33)*	1.87±0.25 (1.85)*
		DDT-treated axons	
3-8-67-A1	−100	4.25	3.30
3-10-67-B2	−100	3.40	2.70
3-13-67-A1	−100	2.90	1.85
Mean±SE		3.51±0.42	2.61±0.45

* Mean values at $E_{hold} = -100$ mv.

Comparison with Frog Nerve Fibers

Hille[1] also observed a similar, prolonged tail of the peak transient current in DDT-poisoned frog nodes of Ranvier. There were two components of the peak transient current under the influence of DDT, namely the initial transient component which decays as quickly as in normal axons and the tail

[1] Hille, B. 1967. Personal communication.

component which decays very slowly. The ineffectiveness of DDT on the initial falling phase in nodes of Ranvier is in contrast with the 4.5-fold drastic slowing by DDT in lobster axons.

Comparison with Veratrine Alkaloids

The slowing of the off process of the peak transient current caused by DDT is to some extent comparable to that caused by veratrine alkaloids. Ulbricht (1965) observed by voltage-clamp technique with frog nodes of Ranvier that the initial sodium current kept flowing for several seconds. It is not known how long the residual peak transient current flows under the influence of DDT, because the measurements were not made beyond some 15 msec in the present experiments. However, judging from the fact that the duration of action potential in DDT-poisoned axons is of the order of several hundred msec to 1 sec in most cases, the residual current probably does not flow beyond 1 sec.

Sites of Action of DDT in the Nerve Membrane

With regard to the sites of action of DDT in the nerve membrane, separate consideration should be given to the delay in the turning-off process of peak transient current and to the inhibition of the steady-state current. The term channel used here does not necessarily mean an anatomical structure but simply refers to a conceptual pathway through which ions flow. However, in order to make further considerations easier, the channel is assumed to be a tunnel having a diameter of a few angstroms. It is not necessary to assume a uniform diameter through the entire length of the channel.

1. THE SLOWING OF THE TURNING-OFF PROCESS OF PEAK TRANSIENT CURRENT The peak transient channel can be visualized as having a gate at its external opening, because TTX, which is not lipid-soluble, can block the channel only from outside the nerve membrane (Narahashi, Anderson, and Moore, 1966, 1967). Since the turning-on process of the peak transient current is not impaired by DDT, the mechanism of gate opening must be kept intact. The gate may be closed by the mechanism located at the gate itself, or by the mechanism located rather near the internal opening of the channel. The reason for assuming the gate closing mechanism at or near the internal membrane surface lies in the observation that the turning-off process of the peak transient current is highly sensitive to changes in composition of internal solutions or to drugs applied internally. For example, the turning-off process is delayed by internal perfusion of cesium (Adelman and Senft, 1966), potassium-free sodium fluoride solution (Chandler and Meves, 1966), low ionic strength media (Adelman, Dyro, and Senft, 1965 *a*, *b*), and the insecticide allethrin (Narahashi and Anderson, 1967). Since DDT is lipid-soluble, it can

reach the internal surface where the mechanism of the peak transient turning-off process could be located.

2. THE INHIBITION OF THE STEADY-STATE CURRENT Unlike the peak transient channel, the gate of the steady-state channel may be located on the internal surface of the nerve membrane. This is based on the observation that tetraethylammonium and cesium, both of which do not permeate the nerve membrane, can block the steady-state current only from inside the membrane as far as squid giant axons are concerned (Tasaki and Hagiwara, 1957; Armstrong and Binstock, 1965; Chandler and Meves, 1965; Pickard, Lettvin, Moore, Takata, Pooler, and Bernstein, 1964; Adelman and Senft, 1966). In view of such an asymmetric structure, it is unlikely that DDT has an equal affinity for both openings of the channel. Thus there are at least two possible ways in which the steady-state current is blocked by DDT: (*a*) DDT plugs or interferes with the gate at the internal surface of the membrane. (*b*) DDT penetrates into the membrane at the interchannel regions thereby exerting the channel-blocking effect by intermolecular forces. We presently have no data to exclude either of them.

Two Operationally Separate Channel Hypothesis

The data of Table IV show that the turning-on process of the steady-state current is delayed 1.6-fold (at 0 mv) or 1.4-fold (at +20 mv) by treatment with DDT. On the other hand, the turning-off process of the peak transient current is delayed as much as 4.5-fold for the initial phase and 18.3-fold for the second phase (Table III and Fig. 8). This indicates that the steady-state (potassium) current starts flowing while the residual (sodium) current continues to flow. Much less overlap is seen in normal axons (Fig. 8). A corollary of these results is that the peak transient and steady-state channels function independently. This supports our previous hypothesis of two operationally separate channels. The experimental evidence supporting this idea was obtained by the use of TTX (Narahashi et al., 1964; Takata et al., 1966; Moore et al., 1967).

Charge-Transfer Complexes

Nothing is known about the molecular structure of the site of action of DDT on the nerve membrane. It has recently been found that DDT forms charge-transfer complexes with nerve components (Matsumura and O'Brien, 1966 *a*, *b*; O'Brien and Matsumura, 1964). It remains to be studied whether these complexes reflect any drug-receptor complexes responsible for the conductance changes observed in the present study. However, there is the possibility that certain protein components of the nerve membrane are the target site of DDT.

We are indebted to Dr. J. W. Moore for helpful discussion during the course of this study and to Dr. E. J. Schantz for his kind supply of the saxitoxin sample. Thanks are also due to Mr. E. M. Harris for construction and maintenance of much of the electronic equipment, to Mr. R. Solomon for construction of the nerve chamber, and to Miss J. C. Topping for analyses of the data.

Figs. 3, 4, and 9 are reprinted from Narahashi and Haas (1967) (copyright 1967 by the American Association for the Advancement of Science).

This study was supported by the National Institutes of Health grant NB06855 and the Duke Endowment grant 84-0954.

Dr. Haas is a recipient of the Senior Foreign Scientist Fellowship of the National Science Foundation.

Received for publication 23 June 1967.

REFERENCES

ADELMAN, W. J., JR., F. M. DYRO, and J. SENFT. 1965 *a*. Long duration responses obtained from internally perfused axons. *J. Gen. Physiol.* **48** (5, Pt. 2):1.

ADELMAN, W. J., JR., F. M. DYRO, and J. SENFT. 1965 *b*. Prolonged sodium currents from voltage clamped internally perfused squid axons. *J. Cellular Comp. Physiol.* **66** (Suppl. 2):55.

ADELMAN, W. J., Jr., and J. P. SENFT. 1966. Voltage clamp studies on the effect of internal cesium ion on sodium and potassium currents in the squid giant axon. *J. Gen. Physiol.* **50**:279.

ARMSTRONG, C. M., and L. BINSTOCK. 1965. Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. Gen. Physiol.* **48**:859.

CHANDLER, W. K., and H. MEVES. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol., (London).* **180**:788.

CHANDLER, W. K., and H. MEVES. 1966. Incomplete sodium inactivation in internally perfused giant axons from *Loligo forbesi*. *J. Physiol., (London).* **186**:121P.

COLE, K. S., and J. W. MOORE. 1960. Potassium ion current in the squid giant axon: Dynamic characteristic. *Biophys. J.* **1**:1.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol., (London).* **137**:217.

LALONDE, D. I. V., and A. W. A. BROWN. 1954. The effect of insecticides on the action potentials of insect nerve. *Can. J. Zool.* **32**:74.

MATSUMARA, F., and R. D. O'BRIEN. 1966 *a*. Absorption and binding of DDT by the central nervous system of the American cockroach. *J. Agr. Food Chem.* **14**:36.

MATSUMURA, F., and R. D. O'BRIEN. 1966 *b*. Interactions of DDT with components of American cockroach nerve. *J. Agr. Food Chem.* **14**:39.

MOORE, J. W., M. P. BLAUSTEIN, N. C. ANDERSON, and T. NARAHASHI. 1967. Basis of tetrodotoxin's selectivity in blockage of squid axons. *J. Gen. Physiol.* **50**:1401.

MOORE, J. W., W. ULBRICHT, and M. TAKATA. 1964. Effect of ethanol on the sodium and potassium conductances of the squid axon membrane. *J. Gen. Physiol.* **48**:279.

NAKAMURA, Y., S. NAKAJIMA, and H. GRUNDFEST. 1965. The action of tetrodotoxin on electrogenic components of squid giant axons. *J. Gen. Physiol.* **48**:985.

NARAHASHI, T., and N. C. ANDERSON. 1967. Mechanism of excitation block by the insecticide allethrin applied externally and internally to squid giant axons. *Toxicol. Appl. Pharmacol.* **10**:529.

NARAHASHI, T., N. C. ANDERSON, and J. W. MOORE. 1966. Tetrodotoxin does not block excitation from inside the nerve membrane. *Science* **153**:765.

NARAHASHI, T., N. C. ANDERSON, and J. W. MOORE. 1967. Comparison of tetrodotoxin and procaine in internally perfused squid giant axons. *J. Gen. Physiol.* **50**:1413.

NARAHASHI, T., and H. G. HAAS. 1967. DDT: Interaction with nerve membrane conductance changes. *Science.* **157**:1438.

NARAHASHI, T., H. G. HAAS, and E. F. THERRIEN. 1967. Saxitoxin and tetrodotoxin: Comparison of nerve blocking mechanism. *Science.* **157**:1441.

NARAHASHI, T., J. W. MOORE, and W. R. SCOTT. 1964. Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. Gen. Physiol.* **47**:965.

NARAHASHI, T., and T. YAMASAKI. 1960 *a*. Mechanism of increase in negative after-potential by dicophanum (DDT) in the giant axons of the cockroach. *J. Physiol., (London).* **152**:122.

NARAHASHI, T., and T. YAMASAKI. 1960 b. Behaviors of membrane potential in the cockroach giant axons poisoned by DDT. *J. Cellular Comp. Physiol.* **55**:131.

O'BRIEN, R. D., and F. MATSUMURA. 1964. DDT: A new hypothesis of its mode of action. *Science.* **146**:657.

PICKARD, W. F., J. Y. LETTVIN, J. W. MOORE, M. TAKATA, J. POOLER, and T. BERNSTEIN. 1964. Caesium ions do not pass the membrane of the giant axon. *Proc. Natl. Acad. Sci. U.S.* **52**:1177.

ROEDER, K. D., and E. A. WEIANT. 1948. The effect of DDT on sensory and motor structures in the cockroach leg. *J. Cellular Comp. Physiol.* **32**:175.

SHANES, A. M. 1949. Electrical phenomena in nerve. II. Crab nerve. *J. Gen. Physiol.* **33**:75.

TAKATA, M., J. W. MOORE, C. Y. KAO, and F. A. FUHRMAN. 1966. Blockage of sodium conductance increase in lobster giant axon by tarichatoxin (tetrodotoxin). *J. Gen. Physiol.* **49**:977.

TASAKI, I., and S. HAGIWARA. 1957. Demonstration of two stable potential states in the squid giant axon under tetraethylammonium chloride. *J. Gen. Physiol.* **40**:859.

ULBRICHT, W. 1965. Voltage clamp studies of veratrinized frog nodes. *J. Cellular Comp. Physiol.* **66** (Suppl. 2):91.

WELSH, J. H., and H. T. GORDON. 1947. The mode of action of certain insecticides on the anthropod nerve axon. *J. Cellular Comp. Physiol.* **30**:147.

YAMASAKI, T., and T. ISHII.[2] 1952 *a*. Studies on the mechanism of action of insecticides. IV. The effects of insecticides on the nerve conduction of insect. *Oyo Kontyu.* **7**:157.

YAMASAKI, T., and T. ISHII.[2] 1952 *b*. Studies on the mechanism of action of insecticides. V. The effects of DDT on the synaptic transmission in the cockroach. *Oyo Kontyu.* **8**:111.

YAMASAKI, T., and T. ISHII.[2] 1954 *a*. Studies on the mechanism of action of insecticides. VII. Activity of neuron soma as a factor of development of DDT symptoms in the cockroach. *Botyu-Kagaku* (Scientific Insect Control). **19**:1.

YAMASAKI, T., and T. ISHII.[2] 1954 *b*. Studies on the mechanism of action of insecticides. IX. Repetitive excitation of the insect neuron soma by direct current stimulation and effects of DDT. *Oyo Dobutsugaku Zasshi.* **19**:16.

[2] Former name of T. Narahashi.

YAMASAKI, T., and T. NARAHASHI. 1957 *a*. Increase in the negative after-potential of insect nerve by DDT. Studies on the mechanism of action of insecticides. XIII. *Botyu-Kagaku* (Scientific Insect Control). **22**:296.

YAMASAKI, T., and T. NARAHASHI. 1957 *b*. Intracellular microelectrode recordings of resting and action potentials from the insect axon and the effects of DDT on the action potential. Studies on the mechanism of action of insecticides. XIV. *Botyu-Kagaku* (Scientific Insect Control). **22**:305.