were transferred again at 30°. Pupation does not take place among the larvae kept at 30° continuously, but occurs among those kept more than two months at 20° and more than $3^1/_3$ months at 25°. Most of the adults, however, were in a state of metathetely at their emergence. On the fish meal, the higher percentage of pupation as well as the fewer number of moulting was observed as compared with that on the dried pupae under the same condition of temperature (Table 10).

About half of the larvae have completed diapause in the begining of February under narural temperature condition, and emerged in the begining of April. Several per cent of larvae undergo two year larval stage even if they are bred under favourable condition. The incidence of the larvae of two or three year cycle is prevalent on the unfavourable diets. (Table 2, 3).

Resistance of House Flies to Insecticides and the Susceptibility of Nerve to Insecticides. Studies on the Mechanism of Action of Insecticides. XVII. Teruo YAMASAKI & Toshio NARAHASHI (Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Japan). Received July 25, 1958. *Botyu-Kagaku* **23**, 146, 1958.

27. イエバエの殺虫剤抵抗性と神経感受性

殺虫剤の作用機構に関する研究 第17報 山崎輝男・楢橋敏夫（東京大学　農学部　害虫学研究室）33. 7. 25 受理

昆虫の殺虫剤抵抗性の機構の研究はとくにイエバエについて広く行われてきている。DDT 抵抗性イエバエでは体内における DDT 解毒力が正常系統に比べて著しく強いことが一般に認められており，さらに神経の DDT 感受性が正常系統に比べて低いといわれている。しかしわが国の抵抗性イエバエについてはこの種の研究は少ない。よって二，三の薬剤に対する抵抗性イエバエを材料として抵抗性の機構を究明すべく，まず神経の薬剤感受性の系統による違いを調べた。その結果 BHC およびディールドリンに比較的強い系統では，その神経感受性が低いことが証明され，この因子が抵抗性発現の原因の一つであることが明らかにされた。

The mechanism involved in the resistance of insects to insecticides has so far been studied by many investigators. Among a number of insecticides, DDT resistance was most extensively studied because of its practical importance and also because of the accumulated data concerning the mode of action of DDT.

Since DDT, having entered an insect body through the cuticle or the mouth, is detoxified or accumulated in some tissues during the course of reaching a site of action or the nervous tissues, the DDT resistance must be due to lower penetrability of DDT through the cuticle, higher capacity of detoxication, larger amounts of accumulation in the tissues other than the site of action or lower susceptibility of nerves to DDT[67,69]. More than one of these factors may play a major role in the resistance phenomenon. The penetrability of DDT through the cuticle of resistant house flies was found to be not lower than that of susceptible house flies[20,33,47,59]. It was demonstrated that stronger capacity to convert DDT to its nontoxic derivative, DDE, in DDT-resistant house flies plays a majar role in resistance[1,23,24,26,31,33-35,39,45,47-50,58-59]. However, thereshould be one or more additional defense mechanisms since much greater amounts of undetoxified DDT remain in the survived resistant flies than in the dead susceptible flies[1,33,47,50].

In the light of such views, it would be expected that the nerves of resistant strains of house flies would be less sensitive to DDT than those of susceptible strains, because the nervous tissue is demonstrated to be an important site of action of DDT[2,5-7,11,19,42,43,51,55,60-65,70-73,78]. In fact several experiments have been carried out demonstrating that this is indeed the case in some resistant strains of house flies[20,38,44,51,56]. Though the possibility that such a lower sensitivity of nerve to DDT in resistant strains is due to the high capacity of detoxication in the nervous tissue has recently been demonstrated[25], it remains to be solved whether or not DDT detoxication in nerves is capable of explaining comletely the difference of nerve sensitivity between strains.

Though the interest in resistance of house flies to insecticides is increasing in Japan, none of the mechanisms involved has so far been studied using Japanese resistant strains. It is generally believed that the Hikone strain shows resistance against DDT, BHC and dieldrin to some extent. Several studies have been performed on the mechanism of resistance to BHC or dieldrin in countries other than Japan[3,4,28-30,46]. A mechanism similar to that in the DDT-resistant strains seems to be involved because both BHC and dieldrin were shown to act on the nervous system primarily although the nervous symptoms were not quite the same as those caused by DDT[19,46,74].

In view of such evidence, the present investigation was undertaken to gain information concerning the mechanism of DDT, BHC and dieldrin resistance of the Hikone strain of house flies. In the earlier part of the study the values of LD-50 for these insecticides were estimated with both the Hikone and the Takatsuki (so-called susceptible strains), and in the latter part of the study insecticide susceptibilities of nerves from both strains were compared.

Methods

Insects: Both the Takatsuki and the Hikone strains of house flies, *Musca domestica vicina* M., were used. Only females were used in this study. The former strain is known as a susceptible strain to DDT, BHC and dieldrin, and was obtained from the Institute for Chemical Research, Kyoto University, while the latter strain is known as a resistant strain to DDT, BHC and dieldrin, and was obtained from the National Institute of Health. They were reared on a mixture of lees of soy-bean, Japanese name *okara*, and powdered yeast at a constant temperature of about 30°. Adults were given milk made of 10 per cent powdered milk and 5 per cent cane sugar. Female adults 3 to 10 days after emergence were used for experiments.

Toxicity experiments: The values of LD 50 for DDT, BHC and dieldrin in both strains of house flies were determined by topical applications of acetone solutions to the dorsum of the thorax with the aid of a microsyringe of conventional type. The applications of insecticides were made under carbon dioxide anaesthesia. The intoxicated flies were placed in small cages and were left at a constant temperature of 30°. Mortalities were estimated after 24 hours' incubation.

Method of recording action potentials of nerve: There exist at least two limiting factors which should be taken into account in carrying out the present experiments on the nerve susceptibility to insecticides: one is the small size of the house fly, and the other is that it requires a number of experiments to make the comparison between two strains possible. Convulsions of the legs in the fly whose thoracic ganglion was exposed and was treated with the Ringer's solution which contains insecticide may be one of the criteria as the ganglion being affected by the insecticide[38], but this index seems to be more or less rough. Therefore, action potentials were recorded from the nerve under the influence of insecticides.

Various attempts were made on the method of recording the nerve action potentials. They will be described below. In the first place, an isolated central nerve cord was used. In the fly, the thoracic and the abdominal nerve ganglia fused together in the thorax. This fused thoracic ganglion was isolated from the body and mounted on a pair of fine silver wire electrodes. Sponta-

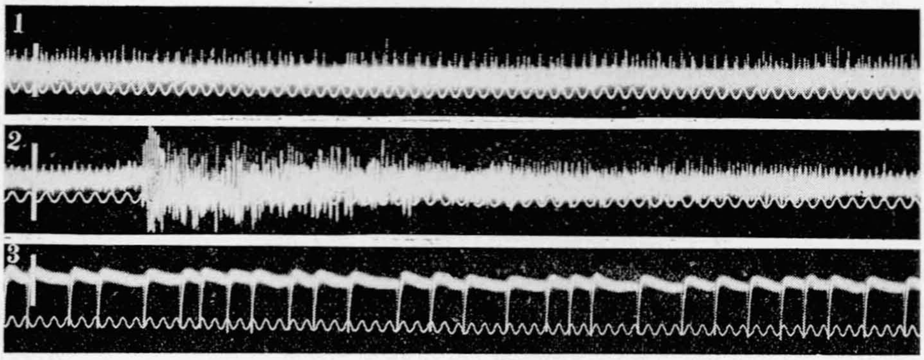

Fig. 1. The action potentials of nerve and muscle of the house fly. Takatsuki strain. Time marker, 50 c. p. s.

1 : Spontaneous discharges in an isolated thoracic ganglion. Voltage calibration, 0.2 mV.

2 : A burst of discharges 14 minutes after application of 10^{-5} DDT. The action potentials were led off by a pair of electrodes inserted near the ganglion in the exposed thorax. Only the nerve action potentials were observable. Voltage calibration, 0.2 mV.

3 : The muscle action potentials led off by a pair of electrodes inserted in the thoracic muscle. The same preparation as that used in record 2. 21 minutes after application of 10^{-5} DDT. Voltage calibration, 2 mV.

neous impulses could be thus recorded (Fig. 1, No. 1), but the amplitude of the action potentials was somewhat small and the level of spontaneous activity was unstable varying with the advance of time. Moreover, it was often found that an outburst of impulses was spontaneously elicited about 20 to 30 minutes after isolation of the ganglion. Though the isolated nerve preparation seems to be most suitable for the present purpose in the sense that the detoxication of insecticide in the tissues other than the nerve need not be taken into account, its unstable activity makes it impossible to apply to the present study.

In the second place, *in situ* recording of the action potentials from the thoracic ganglion was attempted. A fly from which all the legs and wings had been removed was fixed with pins ventral side up on a dissecting dish. Then the sternum of the thorax was carefully removed under a binocular microscope, the thoracic ganglion being able to be observed at the exposed region of the thorax. When a pair of fine silver wire electrodes was inserted in this exposed region, the action potentials of the nerves and muscles were recorded (Fig. 1, Nos. 2 & 3). The amplitude of the nerve action potentials was usually smaller than that of the muscle action potentials, and the former partly depended on the position of the thorax in which the electrodes had been inserted. The discharge activity of the ganglion was stable over a long period of time after excision. However, a difficulty was encountered : the amplitude of the action potentials changed when the short circuit between two electrodesunderwent a change following replacement of Ringer's solution which had been applied to the exposed region of the preparation by a new solution. Moreover, it is necessary for the present purpose to apply a large amount of Ringer's solution containing insecticide to the exposed preparation in order to eliminate the factor of detoxication which may be caused by the tissues other than the nerve. Therefore, this method of recording action potentials could not be used in the present study.

Satisfactory results were obtained by recording the action potentials from the leg. The ganglion-exposed fly preparation was made as described above except for one different point that the left metathoracic leg was left intact. A fine silver wire electrode was inserted in the femur of the intact leg, another fine silver wire electrode being inserted in the intact abdomen. Discharges in both the efferent and the afferent nerves in the leg were recorded. Whether the observed action potentials were originated in the motor or in the sensory neurones was determined by amputating the leg at the peripheral or the central region of the femur (Fig. 6). The action potentials from the efferent nerves were generally larger in amplitude than those from the afferent nerves. Intermittent groups of efferent discharges were usually observed, the frequency of appearance of the grouped discharges or bursts of discharges being higher immediately after operation, but they decreased toward a steady level 10 to 20 minutes later. The action potentials of the muscles were simultaneously recorded: these could easily be distinguished from the nerve action potentials because the duration of the former was much longer than that of the latter. The amplitude of the action potentials recorded by this method was considerably higher than that recorded by the two methods mentioned before and was independent of the amount of Ringer's solution applied to the exposed region of the thorax. Moreover, this operation and recording are very easy to perform, which enable us to repeat a number of experiments. Although the risk of detoxication which may occur in the tissues other than the nerve cannot be excluded completely, it seems not probable that a greater part of the insecticide applied to the preparation is detoxified, because the amount of Ringer's solution containing the insecticide is so large that it occupies almost the same volume as that of the thorax making a huge hemisphere of Ringer's solution on the excised thorax. All the experiments on the nerve susceptibility to insecticides were carried out with this method at room temperatures ranging from 16° to 24°.

Recording apparatus: A pair of fine silver wires was used as recording electrodes as described before. The action potentials were amplified by a CR-coupled amplifier and observed and photographed by a cathode ray oscilloscope. The amplified action potentials were also fed to a speaker to produce sounds which served as an auditory monitor.

Insecticides and solution: Purified *p,p'*-DDT, γ-BHC and dieldrin were tested. The Ringer's solution used contained 214 mM Na^+, 3.1 mM K^+ and 1.8 mM Ca^{++}, its pH being kept at 7.2～7.4 by phosphate buffer. This solution contains more amount of sodium ions than that used in our previous experiments[70-73]. The reason for increased amount of sodium ions will be described elsewhere. The solution applied to the exposed ganglion was prepared by introducing 1 or 2 per cent insecticide stock solution into a Ringer's solution. This stock solution was prepared by dissolving insecticide in a 1 : 1 mixture of ethanol and Emulsifier 1130. Control experiments revealed that these solvents had no effect on the activity of the thoracic ganglion when the same concentration as that used in the present study was applied.

Results

A. Toxicity experiments

p,p'-DDT: DDT was found to be quite ineffective to both strains, so that the calculation of LD 50 could not be made (Table 1). Dosages higher than 5000 γ/g could not be topically applied with considerable accuracy, because there was no evidence that all of the DDT applied to a fly remained on the surface of the cuticle when higher dosages were used.

γ-BHC and dieldrin: Examples of dosage-mortality regression lines for BHC and dieldrin both strains are illustrated by Figs. 2 and 3. The regression lines were calculated by the method of Gaddum[10]. Calculated values of LD-50 are given in Table 2. It can be seen that the Hikone strain exhibits somewhat high resistance to

Table 1. Per cent mortalities following topical treatment of *p,p'*-DDT in the Takatsuki and the Hikone strains of house flies.

Dose (γ/g)	Takatsuki strain			Hikone strain		
	I	II	Mean	I	II	Mean
5000	10.3	13.3	11.8	13.7	16.7	15.2
1000	3.4	10.0	6.7	6.9	16.7	11.8
200	6.9	6.7	6.8	3.4	3.3	3.3
40	3.4	3.3	3.3	3.4	0	1.7

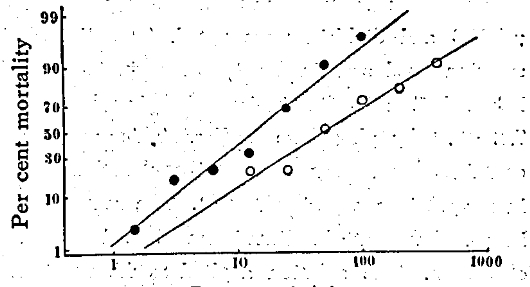

Fig. 2. Dosage-mortality regression lines for BHC in the Takatsuki (closed circles) and the Hikone (open circles) strains of house flies. Topical application.

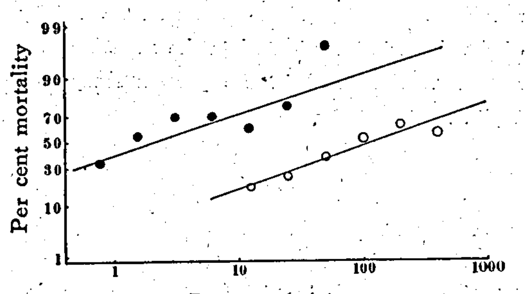

Fig. 3. Dosage-mortality regression lines for dieldrin in the Takatsuki (closed circles) and the Hikone (open circles) strains of house flies. Topical application.

Table 2. Calculated values of LD 50 for topically applied γ-BHC and dieldrin in the Takatsuki and the Hikone of house flies.

Insecticide	Strain	Colony	LD50(γ/g)	Mean
BHC	Takatsuki	I	4.09	10.0
		II	14.1	
		III	10.3	
		IV	11.7	
BHC	Hikone	I	54.8	51.1
		II	27.2	
		III	68.4	
		IV	53.8	
Dieldrin	Takatsuki	I	2.82	2.38
		II	1.93	
Dieldrin	Hikone	I	73.8	96.4
		II	132	
		III	134	
		IV	45.8	

dieldrin and that the resistance of this strain to BHC is not so remarkable.

B. Susceptibility of nerve to insecticides

The effect of DDT on the activity of the thoracic ganglion: The effect of this insecticide on the thoracic ganglion was qualitatively similar between both strains. DDT caused an increase in frequency of motor discharges: the frequency of appearance of motor bursts increased strikingly shortly after application of DDT (Fig. 4, Nos. 1 & 2). The latent period between the application and the appearance of the effect depended upon the concentration of DDT; higher concentrations shorten the latent period to less than 1 minute. The frequency appearance of the bursts also partly depended upon the concentration of DDT, increasing from very few to as high as 50 per minute or more. The frequency of discharges in each burst was not measured exactly, being of the order of 100 to 200 per second. Such bursts caused the leg to initiate convulsions. When a lower concentration just above threshold was used, the bursts which had increased in frequency shortly after application decreased in frequency with the advance of time, a complete recovery down to the normal level being usually observed. However, a substitution of fresh DDT solution for the old one on the ganglion caused an increase in activity again, even though the new solution contained the same concentration of DDT as the old. This second increase in nervous activity was followed by a second decrease. Such a series of events is illustrated by Fig. 5. This result may imply that though the amount of the DDT-Ringer's solution applied was large, DDT was detoxified to such an extent that the concentration of undetoxified DDT decreased below the threshold. This explanation seems to be reasonable because such a reversible action of DDT was observed only when the concentration of DDT was just above threshold. The stimulating effect of DDT usually became apparent within about 10 minutes after application, and the concentration which had not caused any appreciable effect within that period had no effect after a longer period of time.

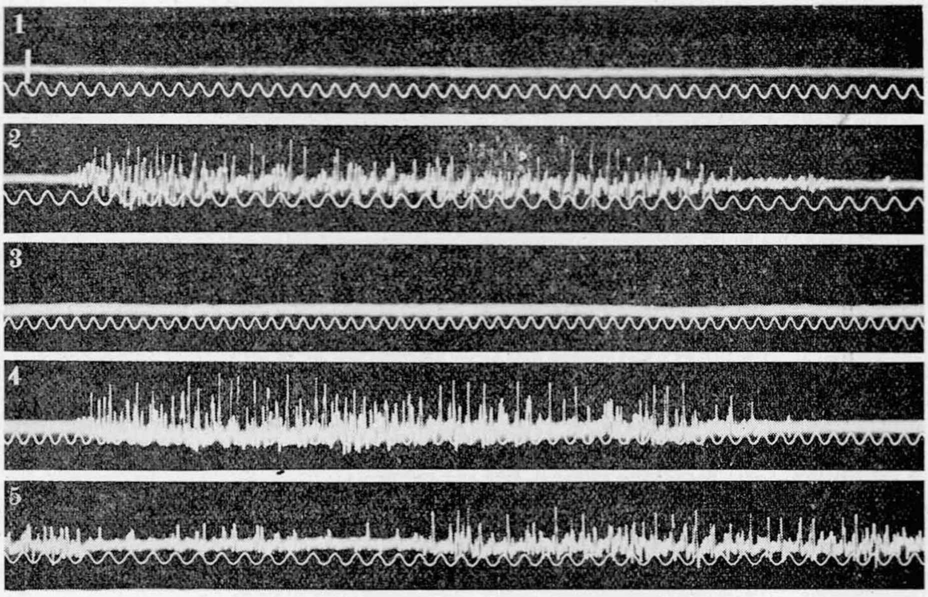

Fig. 4. The effects of DDT and BHC on the exposed thoracic ganglion of the Takatsuki strain of house flies. The action potentials were led off by a pair of electrodes, a different electrode being inserted in the femur while an indifferent one in the abdomen. Voltage calibration of 1 mV shown in record 1 is applied to all records. Time marker, 50 c. p. s.

1 : Normal, few discharges.
2 : 2 minutes after application of 10^{-5} DDT, a burst of motor discharges.
3 : Normal, the other preparation, few discharges.
4 : 5 minutes after application of 5×10^{-5} BHC, a burst of motor discharges.
5 : 32 minutes after application, bursts of motor discharges were still observable.

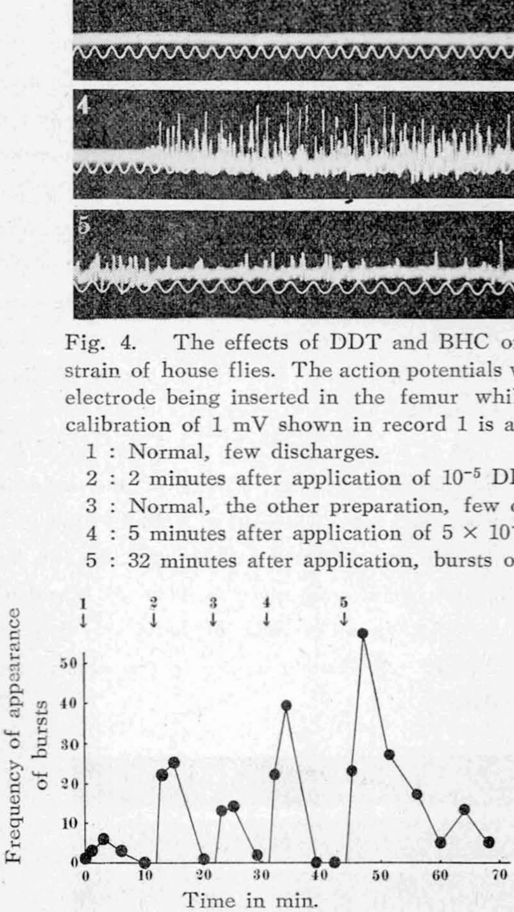

Fig. 5. Reversible action of lower concentrations of DDT on the exposed thoracic ganglion of the Takatsuki strain of house flies. Ordinate indicates the frequency of appearance of motor bursts per minute and abscissa time after application of DDT in minute. Arrows indicate the times of application of DDT. 1, 3×10^{-7}; 2, 3 and 4, 10^{-6}; 5, 3×10^{-6}.

Since DDT was shown to give very low mortality in both strains of house flies, the comparison of the nerve susceptibility to DDT between two strains seems to be meaningless. No detailed experiments were therefore made, the results obtained being given in Table 3. It can be seen that the nerves of the Hikone strain are only slightly less sensitive to DDT than those of the Takatsuki strain.

The effect of BHC on the activity of the thoracic ganglion : The effect of this insecticide on the thoracic ganglion was qualitatively similar between strains. BHC also caused an increase in motor

Table 3. The threshold concentrations of DDT for initiation of motor bursts from the exposed thoracic ganglion in the Takatsuki and the Hikone strains of house flies.

Threshold concentration	Number of insects having the threshold concentration	
	Takatsuki strain	Hikone strain
3×10^{-7}	2	0
1×10^{-6}	2	3
3×10^{-6}	9	4
1×10^{-5}	1	7

activity as in the case of DDT, the frequency of appearance of the bursts increasing markedly shortly after application (Fig. 4, Nos. 3 & 4). However, the latent period was longer than that for DDT. With lower concentrations of BHC, the effect often became apparent more than 10 minutes after application. Although no detailed measurement was made on the relation between the concentration of BHC and the latent period, the latter was usually shorter when higher concentrations were used. The bursts caused by BHC also initiated convulsions of the leg. It was not determined whether the stimulating action could be regenerated by a renewal of the BHC solution as in the case of DDT. But it was usually found that the increased activity of the thoracic ganlion after treatment with a concentration which gave a latent period of less than 10 minutes was maintained for a long period of time (Fig. 4, No. 5).

The ideal way to compare the susceptibility of nerve to any insecticide between two strains is to estimate the relation between the percentage of appearance of stimulating or other action and the concentration of the insecticide with each strain. However, since the latent periods in the case of lower concentrations of BHC are very long, these experiments would be very time-consuming. It would be expected that the latent period is shorter in the strain whose nerve is more susceptible when the same concentration is used. In the light of such a view, the latent period for the increase in the activity of the thoracic ganglion after treatment with 5×10^{-5} BHC was estimated with both strains, and the results obtained are given in Table 4. It can be seen that the mean value of the latent period in the susceptible strain is shorter than that in the resistant one.

Effect of dieldrin on the activity of the thoracic ganglion : The effect of dieldrin on the thoracic ganglion was qualitatively similar with that of BHC, and no difference was found between two strains except for the duration of the latent period. The application of dieldrin caused an increase in frequency of bursts with a longer latent period than that of DDT or BHC even when the same concentration was used. This effect was usually irreversible lasting for a long period of time (Fig. 6).

As in the case of BHC, the latent period for the increase in the activity of the thoracic ganglion after treatment with 5×10^{-5} or 10^{-4} dieldrin was estimated with both strains, and the results obtained are given in Table 5. It will be seen that the mean value of the latent period in the susceptible strain is shorter than that in the resistant one.

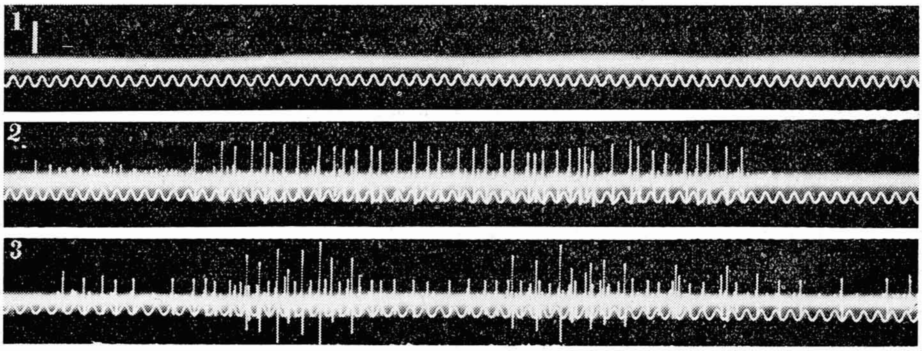

Fig. 6. The effects of dieldrin on the exposed thoracic ganglion of the Takatsuki strain of house flies. The action potentials were recorded as described in Fig. 3. Voltage calibration of 1 mV shown in record 1 is applied to all records. Time marker, 50 c. p. s.

1 : Normal, few discharges.
2 : 12 minutes after application of 10^{-4} dieldrin, a burst of motor discharges.
3 : 94 minutes after application, the femur was amputated at the distal region shortly before recording, bursts of motor discharges were still observable.

Table 4. Latent periods for the initiation of bursts from the thoracic ganglion following application of 5×10^{-5} γ-BHC to the exposed thoracic ganglion of the two strains of house flies.

A. Takatsuki strain

Colony (Temp. °C)	No.	Latent period (min.)
I (22)	1	4
	2	4
	3	4
	4	6
	5	6
	6	6
	7	7
	8	8
	9	25
	10	25
II (24)	1	3
	2	4
	3	5
	4	6
	5	7
	6	12
Mean		8

B. Hikone strain

Colony (Temp. °C)	No.	Latent period (min.)
I (22)	1	4
	2	5
	3	5
	4	8
	5	17
	6	25
	7	33
	8	44
	9	46
II (23)	1	3
	2	3
	3	4
	4	5
	5	6
	6	6
	7	6
	8	7
	9	9
	10	9
	11	15
	12	30
III (23)	1	6
	2	32
Mean		14

Table. 5. Latent periods for the initiation of bursts from the thoracic ganglion following application of dieldrin to the exposed thoracic ganglion of the two strains of house flies.

A. Takatsuki strain

Colony (Temp. °C)	No.	Concentration	Latent period (min.)
I (24)	1	5×10^{-5}	13
	2	〃	13
	3	〃	14
	4	〃	17
	5	〃	20
	6	〃	36
	7	〃	36
I (24)	1	10^{-4}	20
II (20)	1	10^{-4}	17
	2		26
III (20)	1	10^{-4}	14
	2	〃	17
	3	〃	29
	4	〃	>50
IV (18)	1	10^{-4}	44
	2	〃	>30
Mean of 10^{-4}			23*

* Excluding III 4 and IV2

B. Hikone strain

Colony (Temp. °C)	No.	Concentration	Latent period (min.)
I (24)	1	5×10^{-5}	45
II (18)	1	10^{-4}	23
	2	〃	57
	3	〃	63
	4	〃	81
III (19)	1	10^{-4}	26
IV (20)	1	10^{-4}	14
	2	〃	21
	3	〃	36
	4	〃	37
	5	〃	>106
	6	〃	>122
V (19)	1	10^{-4}	16
	2	〃	22
	3	〃	30
	4	〃	30
	5	〃	48
	6	〃	>90
Mean of 10^{-4}			36**

** Excluding IV 5, IV 6 and V6

Discussion

It is generally believed that the Takatsuki strain of house flies is susceptible to DDT, while the Hikone strain is resistant to it. The experimental results described in this paper are to be unexpected from this view. However, these results are rather to be expected for us because the ineffectiveness of DDT against some strains of house flies has already been demonstrated in our laboratory[68]. In that report it was described that the Takatsuki strain of house flies exhibited high resistance to *p,p'*-DDT when it was applied topically at 25°. In that case, though the exact values of LD 50 could not be estimated due to the same reason as that mentioned in the present paper, it can be seen that the degree of resistance to DDT is of the same order as that of the present experiments after looking over the maximum mortalities in several cases: for example 40, 27, 18 and 3 per cent mortalities were obtained in different experiments with the doses of 10,000, 800, 1,600 and 4,000 γ/g respectively. The mortalities of both strains in the present experiments are much lower than the values of LD 50 estimated with American susceptible strains of house flies by topical applications. For example values of LD 50 reported are: 0.05 γ/fly≒2.8 γ/g[9], 0.054 γ/fly≒3 γ/g[32], 20 γ/g[47] and 0.15γ/fly≒8 γ/g[50] respectively. These are to be compared with the values of LD 50 estimated with American resistant strains by topical applications, for example 2.50 γ/fly≒139 γ/g[9], 7.4 γ/fly≒411 γ/g[32], 18,728 γ/g[47] and 25.0 γ/fly≒1388 γ/g[50]. Hence it can safely be said that both the Takatsuki and the Hikone strains are resistant strains at least when tested by the method of topical application.

However, a few factors should be taken into account when we are to decide whether a strain is resistant or not. In the first place, the degree of resistance to any insecticide may depend partly upon testing method. In fact the Takatsuki strain was shown to be susceptible to DDT when tested by contact method[75]. With this method the main route through which DDT enters the body is likely to be through the tarsi of the legs and the mouth. If the rate of penetration of DDT through the tarsus were much higher than that through the dorsum of thorax, and if the detoxication of DDT inside the body played a significant part in resistance, it would be expected that DDT acts less effectively with topical application than with contact application, because DDT which had penetrated into the body slowly through the dorsum of thorax would be detoxified as soon as it entered, while a large amount of DDT which had penetrated into the body rapidly through the tarsi would not be so completely detoxified that the lethal of undetoxified DDT would progress with much higher rate than of detoxication.

It would be worthwhile to cite here the experimental evidence that the injections of *p,p'*-DDT in the Takatsuki strain of house flies caused very low mortalities at 25°[68]. For example, 41, 16 and 7 per cent mortalities were obtained with the dose of 80 γ/g DDT in three cases. These data imply that the Takatsuki strain exhibits higher resistance than the American susceptible strains even though DDT is injected in the former and is applied topically to the latter. In the case of injection the defense mechanism that is operated by the cuticle need not be taken into account, so that our previous experiments cited above clearly demonstrate the high resistance of the Takatsuki strain to DDT.

In the second place, the insecticidal activities are largely different among the analogues of DDT. It was found in our previous report that *o,p'*-DDT was slightly more effective and methoxy analogue DDT or methoxychlor was much more effective than *p,p'*-DDT against the Takatsuki strain when tested by topical application[68]. It was also found that technical DDT which contained several analogues of DDT was more effective than *p,p'*-DDT to some strains when tested by contact method[75]. In the light of such views, laboratory test with *p,p'*-DDT seems to be not always applicable to practical problem where not *p,p'*-DDT but technical DDT is usually sprayed.

In the third place, the insecticidal activity also largely depends both on physiological conditions of insect such as nutrition, age and sex, and on environmental conditions such as temperature and humidity. The dependence of insecticidal activity on nutrition was clearly demonstrated with the house fly[27], but it is extremely unlikely that a great difference in mortality between the Takatsuki or Hikone strain and some American susceptible strains is caused by the difference in nutritional conditions. The same consideration seems to be applicable to the difference in age or sex. Temperature was shown to have a significant effect on the insecticidal action of DDT, lower temperature causing higher toxicity[8,9,12-14,16-18,21,22,36,37,40,41,50,52,53,61,77]. But most insecticidal tests were performed at temperatures ranging from 20° to 30°, so that this factor also cannot explain the great difference in mortality between the Takatsuki or the Hikone strain and some American susceptible strains.

The present results with DDT lead to the conclusion that both the Takatsuki and the Hikone strains of house flies show resistance to the topically applied *p,p'*-DDT when comparisons were made with some American susceptible strains. However, whether or not these Japanese strains are resistant to any other tests remains to be decided at present.

The Hikone strain is more resistant to dieldrin and slightly more resistant to BHC than the Takatsuki strain when tested by topical application. The susceptibilities of the nerves from the Hikone strain to these insecticides are lower than those from the Takatsuki strain, so that this factor is considered to play a part in dieldrin and BHC resistance of the Hikone strain. Such a role of the nerve susceptibility to insecticides in resistance is in accordance with the views offered by other authors with DDT-resistant strains of house flies[20,33,44,54,56]. The detoxications of BHC and dieldrin in the Hikone strain are not yet studied, but it seems probable that the detoxication also plays a part in resistance, for it has been shown that this is actually the case in other BHC-resistant strains of house flies[3,28-30,45].

The mechanism by which the nerves of the Hikone strain show lower susceptibility to dieldrin and BHC than those of the Takatsuki strain is not clear. However, there seem to be at least two mechanisms involved i. e., higher activity of detoxication inside the nerve of the Hikone strain, and other intrinsic factors in the nerves which are related to susceptibility. The former possibility cannot be denied since high activity of DDT detoxication in the brains from DDT-resistant flies has been demonstrated[25] and it has also been suggested by Wigglesworth[57]. The latter possibility seems to exist by virtue of the following evidence: the nerves of resistant flies have been shown to be less sensitive to the direct stimulating action of toluene vapour[54], which indicates that an intrinsic factor is involved, for it is unlikely that toluene is detoxified by the action of DDT dehydrochlorinase. Therefore it is also probable that both detoxication and intrinsic factors are involved together.

Dieldrin exerts an effect on nerve after a longer latent period than BHC. With the Takatsuki strain of house flies the mean values of the latent period for 5×10^{-5} BHC and 10^{-4} dieldrin are 8 and 23 minutes respectively. Since with this strain the insecticidal action of dieldrin is somewhat higher than that of BHC, it seems likely that such difference in the latent period with about the same concentration of both insecticides is not due to a possible difference in threshold concentrations between them which may exist but due to the difference in intrinsic nature between the insecticides. This finding seems to explain the much longer latent period for the increase in respiration following injection of dieldrin than that following injection of BHC[15], and also for the slower and more moderate actions of dieldrin as compared with BHC on cockroach nerves[74].

Summary

The toxicities of *p,p'*-DDT, γ-BHC and dieldrin were estimated by topical application using the Takatsuki and the Hikone strains of house flies. DDT was extremely ineffective against both strains being much more than 5000 γ/g at 30°. Both BHC and dieldrin were less effective to the Hikone strain than to the Takatsuki strain. The values of LD50 for BHC in the Takatsuki and the Hikone strains were 10.0 γ/g and 51.1 γ/g respectively at 30°, and those of dieldrin were

2.38 γ/g and 96.4 γ/g respectively at 30°.

The methods of recording the discharges from the thoracic ganglion were studied. It was found that the recording of the nerve action potentials from the femur is the most suitable method for estimating the activity of the exposed thoracic ganglion under the influence of drugs applied directly to it.

With this method the effects of *p,p'*-DDT, γ-BHC and dieldrin on the activity of the thoracic ganglion were studied. Any of these insecticides caused the ganglion to initiate the intermittent bursts of discharges, the latent period for these effects being dependent on both insecticides and strains.

The latent period for the initiation of the bursts following treatment with dieldrin was longer than that following treatment with about the same concentration of BHC in each strain of house flies.

The nerve of the Hikone strain is only slightly less sensitive to DDT than that of the Takatsuki strain. The nerve susceptibilities of the Hikone strain to both BHC and dieldrin as determined by the latent period for the initiation of the bursts were lower than those of the Takatsuki strain. These differences in the nerve susceptibility were considered to play a part in the resistance of the Hikone strain to BHC and dieldrin.

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