THE EFFECTS OF VARIOUS ANESTHETICS ON THE ACTION OF DDT ON *ANOPHELES* LARVAE [1]

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INTRODUCTION. Jones (1958) used a special DDT test for *Anopheles quadrimaculatus* Say (Diptera, Culicidae) in which newly emerged larvae of the fourth stage were exposed for 10 minutes to a massive dose of DDT in order to minimize or avoid the possibility of their eating the particles. He found that when the larvae were anesthetized with half-saturated ether or CO_2 vapours, they were protected during a 5 or 10 minute exposure to .01 percent DDT. Friedman and Chalkley (1962) subsequently reported that if etherized or CO_2-anesthetized (flaccid) *Anopheles* larvae were kept in vigorous and continuous motion at an unspecified rate within a .004 percent DDT suspension, the protective effect of these two anesthetics was abolished. They concluded that the protective effect of anesthesia was ". . . simply one of producing a lower exposure to the insecticide by decreasing the physical activity of the larvae . . ." (p. 1225). Since this conclusion in no way accounts for Jones' observation that quiescent anesthetized larvae continuously drawn through a DDT suspension for 10 minutes did not pick up a significantly greater amount of DDT than those left undisturbed, we re-investigated the problem. Undoubtedly, passively moved or actively moving larvae would automatically contact more DDT particles; what remains in doubt is whether such movements necessarily bring about a greater uptake of DDT.

Jones (1957) had reported that 50 percent of unanesthetized, young fourth stage *Anopheles* larvae pick up a lethal dose of DDT in 5 minutes and 95 to 100 percent of them obtain a lethal dose in 10 minutes at a concentration of .01 percent. In examining the unpublished notes of Jones' anesthesia paper, it was found that *none* of the unanesthetized larvae exposed to DDT for 2½ minutes died (five larvae in each of 3 tests). Thus, one-half of the larvae pick up a lethal dose of DDT between 2½ and 5 minutes. The notes contained records of the number of movements that seven unanesthetized larvae made over precisely this period. During the first 2½ minutes of exposure the larvae made from 0 to 9 movements (3.1 ± 1.3), and between 2½ minutes and 5 minutes the larvae made from 1 to 28 movements (16.4 ± 3.9). Thus, unanesthetized larvae are significantly more active during the time when only one-half of them are obtaining a lethal dose. Unanesthetized larvae begin to exhibit convulsions in about 5 minutes. In the four cases where records were available, the larvae made 40.7 ± 9.7 movements from the fifth through the tenth minute of exposure. All of the etherized larvae remained totally quiescent during exposure to DDT.

METHODS. All but one of the tests reported in the present paper were conducted at $23 \pm 2°$ C with batches of 10 fourth stage *A. quadrimaculatus* (U. S. Naval Medical Research strain). The majority of the larvae were 1 to 4 hours old within the stadium, and none was more than 12 hours old. They were rinsed in distilled water and exposed to

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various anesthetics either while floating on the surface of water in stender dishes or after they had been isolated on a piece of wet filter paper. They were briefly rinsed with distilled water just before placing them in 100 ml. of a freshly prepared acetone suspension of *p,p'*-DDT at a concentration of .01 percent. Unless stated differently, all immobilized larvae were drawn about 250 to 350 times within the DDT suspension with cardboard tips. Most larvae were exposed to DDT for 10 minutes, and all were then rinsed in 2 one-liter changes of distilled water after exposure to DDT before transferring them to individual 30 ml. beakers containing water and food. Unless stated to the contrary, the larvae were always carefully positioned at the air/water interface with cardboard tips and were subsequently held at 28° C. The results were recorded after 24 hours.

RESULTS. Each of nine very different treatments which partially or completely immobilized *Anopheles* larvae afford them a degree of protection against .01 percent DDT which is significantly greater ($P < 0.05$) than for unanesthetized larvae exposed to the same concentration (Table 1). Cold and ether vapor anesthesia provided significantly more protection than anesthesia with procaine, KCl, CO_2, or nitrogen. No significant differences in the degree of protection offered by cold, ether, cyclopropane, or chloroform are apparent in the data presented. Pretreatment with nitrogen clearly gave the least protection, yet this protection is significantly greater than for unanesthetized larvae. Larvae remained completely immobilized during exposure to DDT following treatment with half-saturated ether, procaine, and KCl, whereas larvae recovered and moved about for 4 to 7 minutes during exposure to DDT following anesthesia with cyclopropane, chloroform, CO_2 and nitrogen (Table 1).

Of particular interest are the tests where CO_2 was the anesthetic. In four tests where the larvae were "hosed" with CO_2 and 10 minutes before a 10-minute exposure to DDT in air, the undisturbed larvae actively moved around in the toxicant during the last 4 minutes of the test, yet 45 percent of them survived. Jones (1958) had found earlier that, when the larvae were treated with CO_2 for only 2 minutes, 26 percent survived. In four tests, larvae were hosed with CO_2 for 10 minutes before and throughout a 10-minute exposure to DDT. These larvae remained completely quiescent and were left undisturbed in the DDT; 60 percent of them obtained a lethal dose (Table 1). That is, 40 percent of the completely immobilized larvae survived and this value is not significantly different from that of larvae pre-treated with CO_2 before testing and which moved about in the toxicant after the first 6 minutes of exposure to the DDT (Table 1).

Friedman and Chalkley (1962) found that when CO_2-anesthetized (flaccid) larvae were kept in continuous motion for 10 minutes in .004 percent DDT in a CO_2 atmosphere, only 10 percent of them survived (yet all of their unanesthetized DDT-treated controls died). The CO_2 treatment alone killed 10 percent of their otherwise untreated controls (that is, CO_2 itself has a slight toxic effect). We repeated this experiment in a single test using .01 percent DDT and obtained no survival after vigorously swirling CO_2-anesthetized larvae in DDT for 10 minutes (approximately 150 rotations per minute) versus a 40 percent survival with the unswirled group. Although we agree with Friedman and Chalkley that swirling of CO_2-treated, flaccid larvae greatly increases their mortality to DDT, this particular effect of swirling on mortality clearly does not apply to either unanesthetized larvae or to etherized larvae, as shown below. Further studies are needed to explain the CO_2 effect.

The following experiments were made to test whether prolonged agitation of *Anopheles* larvae in DDT generally leads to a greater mortality. When four batches of young, unanesthetized larvae were placed in DDT and left undisturbed for 4 minutes, most remained at the surface during exposure. The larvae were removed

TABLE 1.—Effects of pretreating fourth stage *Anopheles quadrimaculatus* larvae with various anesthetics on the activity and survival of the larvae in 0.01 percent DDT.

Treatment	No. tests	Larval activity in DDT	Mean 24 hour survival %
10 min. in DDT at 3° C	5	Inactive	96.0±2.0
10 min. in DDT at 22° C	5	Active	0.0
Ether vapors 3 min. before DDT for 10 min.	5	In 2 tests larvae became active after first 5 min.	92.0±2.0
Controls-DDT for 10 min.	5	Active	10.0±0
Cyclopropane vapors 10 min. before DDT 10 min.	5	Mostly active	84.0±6.0
Controls DDT for 10 min.	5	Active	4.0±2.0
3.75% ether 10 min. before DDT 10 min.	7	Inactive	80.0±4.3
Controls-DDT for 10 min.	7	Active	4.3±1.4
Chloroform vapors 4 min. before DDT 10 min.	6	Active after first 5 min.	81.6±6.7
Controls-DDT for 10 min.	6	Active	3.3±1.7
2% procaine 15 min. before DDT 10 min.	6	Inactive	73.3±5.0
Controls-DDT for 10 min.	6	Active	3.3±1.7
1 M KCl 15 min. before DDT 4 min.	5	Inactive	58.0±6.0
1 M KCl 15 min.	5	Inactive	100.0
Controls-DDT for 4 min.	5	Active	28.0±4.0
CO_2 vapors 10 min. before DDT 10 min.	4	Active after first 6 min.	45.0±2.5
Controls-DDT for 10 min.	4	Active	5.0±2.5
CO_2 vapors 10 min. before DDT and during 10 min. exposure to DDT	4	Inactive	40.0±0
Controls-DDT for 10 min.	4	Active	0.0
Nitrogen vapors 15 min. before DDT 10 min.	3	Active during last 7 min.	20.0±0
Controls-DDT for 10 min.	3	Active	6.7±3.3

from the DDT suspension and rinsed in distilled water. Twenty-four hours later 67.5 percent of them were alive (Table 2). When four additional batches of unanesthetized larvae were swirled continuously for 4 minutes (approximately 150 rotations per minute) in the DDT, most larvae fell to the bottom of the test container, and 50 percent survived (Table 2). The survival values of the unswirled and the swirled groups are not significantly different. If Friedman and Chalkley's contention were correct, there should have been a significant difference here as a result of increasing the physical activity of the swirled larvae. It is therefore evident that physical agitation of unanesthetized larvae for 4 minutes did not increase their mortality.

Since a 4-minute swirling of unanesthetized larvae in DDT did not significantly increase the kill, we decided to re-study the effects of swirling on etherized larvae. Larvae were kept for 10 minutes in half-saturated ether before placing them in DDT for 10 minutes. Two batches were swirled continuously in the DDT, and two batches were left undisturbed. Most of

TABLE 2.—Effects of different treatments of unanesthetized and ether-anesthetized *Anopheles* larvae during exposure to 0.01 percent DDT.

Treatment	No. tests	Larval activity	Mean 24 hour survival %
Unanesthetized			
Swirled in DDT 4 minutes	4	Most larvae fell from surface	50.0±10.0
Not swirled in DDT 4 minutes	4	Most larvae at surface	67.5± 7.5
10 min. in 3.75 percent ether before testing			
Swirled 10 minutes in DDT	2	Most larvae fell from surface	60.0±10.0
Not swirled in DDT 10 minutes	2	Most larvae at surface	90.0±10.0
Continuously submerged in DDT 10 minutes	3	.....	53.5±23.3
Not submerged in DDT 10 minutes	3	.....	86.7±10.0

the swirled larvae fell from the surface film,[3] and most of the unswirled ones remained on the surface throughout the exposure. The unswirled larvae were not mechanically drawn about. Sixty percent of the swirled larvae survived (Table 2). This value is strikingly greater than the assumed 10 percent survival in similar tests conducted by Friedman and Chalkley with .004 percent DDT. Ninety percent of our unswirled, etherized larvae survived. The differences between our swirled and unswirled groups are not significant at the 5 percent level (Table 2). It is concluded that physical agitation of etherized larvae in DDT does not increase mortality.

To determine whether submergence of the larvae would affect the results, three batches of etherized larvae were deliberately kept continuously submerged in the DDT throughout a 10-minute exposure to DDT, while the controls were positioned at the surface of the DDT suspension. None of the larvae were swirled. It is evident from Table 2 that the data from continuously submerged etherized larvae are considerably more variable than those in any other series of tests. Nevertheless, no significant differences in mortality are apparent between submerged and unsubmerged groups.

The data presented in Friedman and Chalkley's Table 2 show that the increase in mortality of their CO_2-anesthetized larvae was much greater during the first 3 minutes of continuous swirling (increase of 33.4 percent) than during the 3.1- to 7-minute interval (increase of 16.6 percent), or during the 7.1 to 10 minute interval (increase of 5.5 percent); 68.4 percent of their CO_2-anesthetized larvae obtained a lethal dose during the first 3 minutes of constant swirling in .004 percent DDT. This latter value is essentially equivalent to what we found in 4 minutes with unanesthetized and reactive larvae whether or not they were swirled in .01 percent DDT. The unswirled, unanesthetized larvae are unquestionably coming into much less contact with DDT during the first 2½ minutes of exposure than continuously swirled unanesthetized or etherized groups, yet our data show no significant difference in their obtainment of a lethal dose.

An average of 64 ± 14 percent of those larvae which were anesthetized with ether vapors, cyclopropane, CO_2, and nitrogen

[3] Friedman and Chalkley do not state the position of their CO_2-swirled larvae.

survived a 10-minute exposure to DDT, even though they actively moved about within the suspension for an average of 6 minutes (long enough to kill about 85 percent of the unanesthetized DDT controls).

CONCLUSIONS. From the data presented in this paper, we conclude that agitation of both unanesthetized and etherized *Anopheles* larvae in a high concentration of suspended DDT generally does not lead to a significantly greater mortality. This finding suggests that the uptake of DDT in this special test is not related to a greatly increased contact with DDT when the toxicant is already at a very high concentration. Although agitation of CO_2-anesthetized *Anopheles* larvae leads to a great increase in their mortality from DDT, this effect has not yet been proved to be the result of a greater contact of larvae with the toxicant.

While further studies are clearly needed to determine how many anesthetics protect *Anopheles* larvae from massive doses of DDT, it is evident that the absence or presence of larval movement *per se* is not necessarily involved in the obtainment of a lethal dose in high concentrations of this insecticide.

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