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Effects Of Sublethal Doses Of Ddt On Reproduction And Susceptibility Of Culex Pipiens

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EFFECTS OF SUBLETHAL DOSES OF DDT ON REPRODUCTION AND SUSCEPTIBILITY OF *CULEX PIPIENS*

by

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ABSTRACT

In studying the development of resistance to the insecticide DDT by house flies, Russian workers observed that it was preceded by disturbances in oogenesis leading to degeneration of ovarian follicles, and that once resistance had fully developed these disturbances ceased. They therefore concluded that the insecticide elicited postadaptations to overcome the toxic action and that this and not the selection of preadaptations was the way in which resistance developed.

This investigation studied the matter in 3 strains of Culex, one of which was C. pipiens pipiens and very susceptible, and of the others which were C. p. fatigans one was DDT-resistant and the other slightly tolerant. Each strain was exposed for 6-7 successive generations to the maximum dosage of DDT that caused no significant mortality; this was done by exposing 1-day-old females and males to filter-papers impregnated with the appropriate concentrations of DDT in oil.

The principal effect of the sublethal exposure was to cause the degeneration of the developing eggs (follicles) in certain of the ovarioles; normally 6-8% would degenerate but this proportion was raised by the DDT to 21-28%. The incorporation of the absorbed

insecticide into the ovaries was demonstrated by means of electron-capture chromatography. The hatch rate, determined in terms of larval progeny per egg-raft, was correspondingly reduced by 14-32% in the 3 strains. Effects were also noted on the proportion of females that took a blood-meal and the proportion that oviposited, but these effects varied according to the strain. In the resistant strain both proportions were reduced, in the slightly-tolerant strain the feeding was reduced but the oviposition increased. In the susceptible strain the feeding and oviposition rates were so much increased from their initial levels, which had been low, that the biotic potential was doubled despite the reduction in hatch.

When the sublethal exposures were applied to the 3rd-instar larval stage, the number of basal follicles (ovarioles) developed by the emerging females was increased by 18-19% in the 2 strains (resistant and slightly-tolerant) so studied. But only 46-55% as many offspring hatched from the eggs laid by these females as from those untreated in the larval stage. The sublethal larval exposure also reduced the proportions of pupation, emergence, feeding and oviposition.

As the generations under sublethal adult treatment progressed, their responses to sublethal DDT changed. The susceptible strain became more resistant to the follicular degeneration, which declined to 14%; the other 2 strains became more susceptible to the degeneration, which rose to 45-54%. The numbers of hatched progeny per egg-raft changed in the same way. The post-treatment feeding rate of the 2 non-susceptible strains also declined. The biotic potential of the susceptible strain showed a net increase, that of the slightly-tolerant strain decreased considerably, while in the resistant strain

which also showed less oviposition the net productivity declined almost to nil.

The DDT-resistance level of the resistant strain increased slightly after the 6 generations of treatments. The susceptible strain developed an appreciable resistance, which was not lost when the treatments were discontinued, and which could be demonstrated to involve true resistance genotypes. It was concluded that the hidden selection on the follicles and on the egg embryos was the principal cause of this genotypic change. The slightly-tolerant strain scarcely changed in resistance levels, and immediately returned to the initial level as soon as the treatments were discontinued. It is the experiences with this strain which disproves that postadaptation without selective mortality can cause insecticide resistance to develop.

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INTRODUCTION

The insect vectors of human disease have been combatted with persistent insecticides such as DDT applied to wall surfaces to control adults and to the breeding-places to control the larvae. One effect of these residual deposits has been to select out populations resistant to DDT and other chlorinated hydrocarbons. Another effect has been observed in houseflies by Russian workers, and that is disturbances in oogenesis leading to degeneration of the follicles and death of the developing ova in the ovaries or after oviposition; these workers considered the disturbances in oogenesis to precede the development of resistance, and that postadaptations subsequently led to the disappearance of these disturbances and the development of physiological resistance.

This investigation therefore was designed to elucidate the effects of DDT applied in truly sublethal doses, i.e. causing no significant mortality as compared to the control, not only on one generation but on successive generations. Since it is against the mosquito vectors of diseases such as malaria and filariasis that residual insecticides have been most widely applied, the species selected for this study was the house mosquito, Culex pipiens, with its two subspecies C. p. pipiens and C. p. fatigans.

Adults of both sexes shortly after emergence were exposed to sublethal deposits of DDT, and the females after feeding and mating

were dissected and their ovaries were examined for degenerating follicles. The effect of the DDT on the amount of feeding and ovipositing was also assessed, as was the production of progeny as measured by the number reaching the pupal stage. These parameters were also investigated in the 2nd gonotrophic cycle as well as in the 1st. The effect of a sublethal exposure to DDT in the 3rd-instar larval stage was also assessed. For these studies a susceptible strain of C. p. pipiens, a slightly-tolerant strain of C. p. fatigans, and a DDT-resistant strain of C. p. fatigans were employed, and sublethal exposures were repeated for 6-7 generations. The DDT-susceptibility levels of each generation of adults, and of the larvae at the beginning and the end of the investigation, were also determined.

REVIEW OF LITERATURE

Effect of Insecticide Application on Susceptibility Levels

The development of insecticide resistance, which is now accepted as being due to a selective process favouring the resistant genotypes carrying the resistance genes or preadaptations (Crow, 1957), was initially considered to be a result of postadaptation without genetic change; and this view still persists in certain quarters. The development of resistance to DDT in the USSR observed by Derbeneva-Uhova and Morozova (1950) and Derbeneva-Uhova and Lineva (1951) was regarded by them as an instance of postadaptation. Lineva (1955) writes (translated): "The results of testing house flies for immunity to DDT showed once again in the present work that, in the reproduction process of the adult, a gradual adaptation (growing stronger with each succeeding generation) to the action of the insecticide is observed."

Nabokov (1958) goes on to state: "The explanation of the arising of resistant forms which are referred to by the foreign authors who stand on the platform of formal genetics and give fundamental significance for selection of already resistant forms, is rejected in our time by Soviet research workers Derbeneva-Uhova and Morozova (1950), Derbeneva-Uhova and Lineva (1951) and others. These workers

substantiate the point of view of Michurinistic biology, according to which the resistant forms arise as the result of the influence of the environment and adaptation to new conditions under the immediate influence of insufficiently high doses of insecticides." More recently Derbeneva-Uhova, Lineva and Drobzina (1966), in discussing the results of a 7-year programme of DDT-spraying against house flies in a Russian village, concluded that: "All this leads to the assumption that as a result of sublethal insecticide exposure of a number of generations of flies there may occur not only signs of delayed toxic action but also adaptations to overcome that action (activation of the detoxication systems), which, like the delayed toxic effects, maintain themselves over a number of generations and may become more intense from one generation to another if contact with the insecticide continues."

The results of the first artificial selection with DDT to be made on the mosquito Anopheles quadrimaculatus Say in the United States were interpreted as involving postadaptations or "Dauermodificationen" by Fay, Baker and Grainger (1949). Confronted with the immediate return of full susceptibility immediately after the selective pressure was discontinued, they considered that the resistance which had developed in the F_1 and had not increased thereafter was due to "Dauermodificationen" induced by the dose of DDT applied to the parental generation.

Eddy et al. (1955) reported that a strain of the body louse Pediculus humanus humanus L. maintained on cloth impregnated with 0.001% DDT, a level at which only a lower egg-production and longevity

could be detected, succeeded in developing a very high DDT resistance by the 16th generation; since there had been an insignificant increase in mortality in this strain, they considered the evidence insufficient to conclude that resistance was due to selection alone. However, Cole et al. (1957) obtained firm evidence that maintenance of the body lice at 0.0001% DDT, where mortality was definitely no greater than on untreated cloth, did not induce more than a 2-fold DDT-resistance in 26 generations. Moreover, Luers and Bochnig (1963) reared a strain of the pomace fly Drosophila melanogaster Meig. in a medium containing truly sublethal concentrations of DDT for as many as 150 generations and found that it did not develop any DDT-resistance.

Following the establishment by Campbell (1926) with sodium arsenate on the silkworm Bombyx mori L. and by Moore (1933) with HCN on the California red scale Aonidiella aurantii (Mask.) that truly sublethal doses applied to individual insects did not increase their resistance but actually reduced it, such investigations were extended to DDT. Hoffman et al. (1951) found that females of the house fly Musca domestica L. which had survived a series of 6 sublethal doses of DDT over a 3-day period showed a higher mortality than untreated flies when ultimately challenged with a high dosage of DDT, and suggested that the reduction in resistance was due to accumulation of DDT in the pretreated flies. Similar experiments were performed by Beard (1952) on larvae of the wax moth Galleria mellonella (L.) and adults of the milkweed bug Oncopeltus fasciatus (Dall.) with

sublethal doses of DDT, as well as nicotine, arsenic and pyrethrum. When he tested the susceptibility levels to a decisive dose 1 week later the treated insects were found to be no more resistant and in some cases had become more susceptible. Hadaway (1956) treated house flies with a series of 6 sublethal doses of DDT, gamma-BHC, dieldrin and diazinon over a 6-day period, and the survivors were found to be more susceptible than the untreated flies.

Evidence for postadaptative effects is more valid in the case of dieldrin. Afifi and Knutson (1956) found that dieldrin treatments that did not affect the fecundity of the treated house flies did however greatly increase the fecundity of their untreated offspring. This increase, amounting to 70% above the normal egg production, had disappeared by the F_2 and F_3. This apparent "Dauermodification" was however interpreted by the authors to be due to the selection of more prolific females. This effect on the untreated F_1 from treated parents was confirmed for Liberian flies in the laboratory (Gratz, 1961) and corresponded with the field observations that dieldrin application led to an abundance of flies greater than that explicable by the simple survival of the resistant portion of the population (Kilpatrick and Schoof, 1956). Bridges and Cox (1959) were so impressed with the loss of dieldrin-resistance when flies were bred in a dieldrin-free medium and with the immediate recovery of resistance on the readdition of dieldrin that they took this phenomenon to suggest that it "was not controlled by any genetic factor". Some indication of post-adaptation to dieldrin was found by Khan and Brown (1966) in the larvae

of a resistant strain of Aedes aegypti (L.); after exposure to truly sublethal concentrations of dieldrin for 2 days of their early development, they accumulated 10% more total lipids in their bodies than unexposed larvae. The evidence for dieldrin-induced lipogenesis however was just short of statistical significance.

Persistent insecticides such as the chlorinated hydrocarbons often cause delayed mortality or after-effects; for example it is well known that doses of DDT taken in by the larvae of house flies may kill the adults when they emerge from the pupa. Kalina (1950) reared a culture of Drosophila melanogaster on a medium containing 5 p.p.m. DDT, a dose which had no effect on the larval development, and found that the emerging adults died within 24 hours, even if the pupae had been transferred to a DDT-free medium. He suggested that DDT absorbed by the larvae was stored in the fat body and later released in sufficient quantities during metamorphosis to poison the adults. In house flies, the discovery by Moorefield and Kearns (1957) that the adults contain only half as much of the DDT-detoxifying enzyme (DDT-dehydrochlorinase) as the larva provides an explanation of this phenomenon.

Garms (1961) found that a dosage of 0.005 p.p.m. dieldrin that caused only 1.7% mortality among the larvae of Anopheles stephensi Liston in 24 hours went on to cause them to show a mortality of fully 47% in females and 61% in males when they emerged as adults; DDT did not show such after-effects.

The ability of an insecticide applied to the larvae to cause

after-effects on the oriental fruit fly, Dacus dorsalis Hendel, in the adults was termed "latent toxicity" by Tamashiro and Sherman (1955). They found that aldrin, isodrin, dieldrin, endrin and chlordane exhibited this latent toxicity. In a DDT-susceptible strain of the house fly, Sherman and Sanchez (1964) found that DDT as well as dieldrin, aldrin and heptachlor showed a significant latent toxicity, whereas in a resistant strain only aldrin and heptachlor showed it, and to a very slight extent. Methoxychlor, lindane, malathion and diazinon showed no latent toxicity to either strain. The cause of the latent toxicity was established by Sanchez and Sherman (1966) in the case of DDT, whereas in the resistant flies only DDE was present in the emerging adults, in the susceptible flies there were still appreciable amounts of undetoxified DDT.

Effect of Insecticide Application on Egg-Production

The effect of sublethal concentrations of insecticides on the fecundity of female insects has been studied by many investigators. Kalina (1950) reported that the rearing of Drosophila melanogaster in a medium containing a truly sublethal dose of DDT did not result in any decrease in egg-production or hatching rate. When the adults were treated with high doses (LD_{66} - LD_{99}) of dieldrin (Knutson, 1955) the survivors showed an 8% increase in egg-production, and the offspring reaching the adult stage were 6% more numerous than those from untreated controls.

DeCoursey and Webster (1952) found that dust formulations of several insecticides all had the effect of reducing egg-production of blood-fed females of the salt-marsh mosquito Aedes sollicitans Walker. DDT reduced it by 46%, and dieldrin and chlordane as much

as 86% and 44% respectively. With Anopheles quadrimaculatus however (DeCoursey et al., 1953) the DDT and other insecticides did not significantly reduce the oviposition on wet media (damp filter-paper), but the forced-laying effect of the chlorinated-hydrocarbon insecticides resulted in oviposition on dry filter-papers being considerably higher than normal. Duncan (1963) treated 3 groups of blood-fed females of Aedes aegypti topically with graded sub-lethal doses of dieldrin sufficient to cause respectively 8%, 2% and zero mortality. All the groups laid a full batch of eggs 3-4 days after treatment, but subsequent oviposition was normal only in the third group, whereas the second group laid much smaller egg-batches, and the first group produced only few eggs without an ovipositional rhythm. Sutherland et al. (1967) exposed larvae of Aedes aegypti to the $LD_{0.1}$ of DDT, dieldrin and malathion during their entire larval life, starting 2-3 hours after hatching. The adult females resulting from the DDT treatment showed an increase of 9% in the number of basal follicles, while 14% increases were shown by adults emerging both from the dieldrin and the malathion treatments. The authors suggested that the increase in the number of basal follicles was due to the effect of the insecticides on the larval hormonal system to increase either the proportion of the rudimentary ovarioles that matured or the number of ovarioles that developed at an earlier stage of development.

Lineva (1955) found that exposure of female house flies to truly sublethal doses of DDT throughout their adult life results in a slowing in the development of the follicles, and the appearance of degenerating follicles in one-third of the adult ovaries. Lineva (1966) went on to

report that these follicular disturbances were found in most of the surviving females whereas normal oogenesis was found in most of the females which succumbed to the DDT. Hunter et al. (1958) treated normal female house flies with the LD_{50} of DDT daily for 2 or 3 successive days, and found that the survivors showed a 13% increase in egg-production per female, and an 18% increase in the total number of F_1 flies ultimately emerging. DDT-resistant flies similarly treated with their LD_{50} showed a 17% reduction in egg-production per female, and a 34% reduction in the number of F_1 flies ultimately produced. A similar reduction was observed with two other resistant strains of house flies (Hunter et al., 1959). Beard (1965) fed susceptible female house flies during the 5-day preoviposition period with a truly sublethal dose of 200 p.p.m. C^{14}-DDT and found a reduction of 74% in the total egg-production. The eggs deposited were found to be highly radioactive, indicating the presence of DDT or its metabolites. Baldry (1964) found that DDT reduced the number of pupae laid by females of the tsetse fly Glossina palpalis (R-D), exposed when 1 day old to deposits for different periods; the reduction ranged from 54% for a nearly sublethal exposure to 13% for an almost completely lethal period.

With dieldrin, however, application of the LD_{60} - LD_{90} to susceptible house flies resulted in a 17% increase in adult progeny (Afifi and Knutson, 1956). With diazinon, 2-3 successive applications of the LD_{50} to susceptible house flies increased the adult progeny by only 1%, and the higher LD_{50}'s similarly applied to diazinon-resistant flies decreased the adult progeny by 21% (Hunter et al., 1958). Lineva (1966) observed that chlorophos (= Dipterex, trichlorofon)

applied to susceptible flies reduced the number of eggs laid because of its effect not only on the act of oviposition itself, but also on the formation of follicles in the ovaries.

With the carbamate Isolan, topical application of the LD_{25} to susceptible house flies (Georghiou, 1965) reduced the egg-production by 36% if they had not yet been mated, and by 46% if they had already mated. Other carbamates as dimetilan, carbaryl, arprocarb and Hercules 5727 were found to reduce the fecundity of the flies, while pyrolan had no effect.

With agricultural insect pests, Hrdy (1955) found that females of the cockchafer Melolontha melolontha (L.) that had survived field-control application of DDT manifested no significant reduction in egg-laying, whereas BHC application caused a 47% reduction as compared to the control. Lakocy (1960) reported a two-fold increase in the egg-production of the Colorado potato beetle Leptinotarsa decemlineata Say following exposure to truly sublethal doses of DDT. Similarly an increase of 10-20% in egg-production was observed by Kuipers (1962) following the application of sublethal doses of DDT topically to this species. When low doses of parathion were applied to the insects, no change was observed in the egg-production until a 10% increase occurred during the 4th week.

Loschiavo (1955) exposed adult females of the flour beetle Tribolium confusum Duval to a sublethal concentration of DDT which caused a 20% mortality, and found that the survivors laid a significantly lower number of eggs than the controls. On the other hand, lower concentrations of DDT had not caused any significant reduction. Topical application

of DDT at the LD_{34} level to the lady beetle Coleomegilla maculata DeGeer was found by Atallah and Newsom (1966) to increase the egg-production by 67%. On the other hand, only 21% of the larvae from the treated beetles had pupated as compared to 53% in the control. Also the percentage of the pupae that gave rise to adults was 89% in the treated group and 96% in the control. Lakocy (1964) reported that sublethal doses of endrin, DDT and dieldrin increased the egg-production of the granary weevil Sitophilus granarius L. by 46%, 29% and 4% respectively, whereas lindane had no effect. When DDT and endrin were applied to the flour moth Ephestia kuehniella (Zell.), a respective increase of 61% and 30% was observed in their egg-production.

Among the lepidopterous larvae, it was found by Adkisson and Wellso (1962) that a partially lethal level of DDT decreased the egg-production of the pink bollworm Pectinophora gossypiella (Saund.). Unmated females treated with the LD_{20} concentration laid 50% fewer eggs than the control, and their incidence of mating was reduced by 23%. The LD_{10} dose did not reduce either the mating or the egg-production. Chauthani and Adkisson (1966) treated the moths of the bollworm Heliothis zea (Boddie) and the tobacco budworm Heliothis virescens (F.) topically with partially lethal doses of endrin, azinphosmethyl and azinphosethyl. The corresponding mortalities among the budworm moths were 16% for endrin, 31% for azinphosmethyl and 40% for azinphosethyl; the reductions in oviposition were 10%, 21% and 29% respectively. Similar results were obtained with the tobacco budworm moths.

Observations were also carried out with field populations of house flies to detect any changes occurring in their susceptibility levels and

in egg-production as a result of insecticidal application. Knutson et al. (1958) found that flies from an area which had received dieldrin application for 3 years showed no decrease in their biotic potential during the first two years, during which they had developed a 4-fold increase in their resistance to dieldrin. In the third year, in which a 40-fold increase in dieldrin-resistance developed, the biotic potential dropped to one-half the normal. The reproductive rate proceeded to recover after the cessation of treatment.

Derbeneva-Uhova et al. (1966) observed that changes in DDT-susceptibility of house flies in an area sprayed with DDT for 7 years were accompanied by changes in the percentage of females which showed disturbances in oogenesis. In the second year of spraying the percentage of such females still remained at about 3% which was the normal figure for untreated flies; but then it gradually increased, to reach 30% by the fourth year. It was in this year that the resistance increased quite sharply, having remained stable or even decreased during the previous 2 years. In the fifth and succeeding years, the percentage of females which showed oogenesis disturbances gradually decreased, although the resistance continued to increase slightly.

Lineva (1966) observed similar changes in a DDT-resistant house fly population in an area sprayed for 8 consecutive years with chlorophos (Dipterex). The susceptibility of the flies to chlorophos gradually increased in the first 4 years of application, so that the LD_{50} fell to one-tenth of the initial level. Subsequently the susceptibility gradually decreased, so that the LD_{50} recovered to its initial level in the 8th year. During this period of annual

treatments with this organophosphorus insecticide, disturbances of oogenesis were common among the female flies. The first type of disturbance was a relative inability to perform the act of oviposition, but the frequency of this adverse symptom was inversely proportional to the LD_{50} level, decreasing from 65% of the females in the 1st year down to 10% in the 4th year when the susceptibility of the flies had reached its maximum. Subsequently this percentage gradually recovered to about 60% in the 8th year when the initial tolerance level of the flies had returned. The second type of oogenesis disturbance was the inability of certain females to achieve the normal formation of follicles; the incidence of this was 3% in the 1st year increasing sharply thereafter, reaching 50% in the 4th year, and thereafter decreasing towards the initial level.

A certain amount of information is available on the effect of an insecticide applied to the parent female on the fecundity and susceptibility level of her untreated offspring. Lakocy (1964) treated females of the flour moth Ephestia kuehniella with what must have been a partially lethal dose of DDT (0.2% concentration), and found that although their egg-production was reduced by 19%, their untreated offspring laid 7% more eggs than normal. When the DDT concentration was only 0.008% (presumably truly sublethal), the parental fecundity was increased as much as 61%, but the egg-production of the untreated offspring was 12% lower than the normal. The same result was given by endrin treatments, the higher dosage reducing parental fecundity but increasing filial fecundity, and vice versa with the lower dosage. Afifi and Knutson (1956) obtained a similar result with dieldrin on

house flies, in which a dose that caused 60-90% mortality in female parents, did not change the fecundity of the survivors but nevertheless induced a filial fecundity (egg-production) that was fully 69% greater than the control flies. However with DDT on house flies, it was found by Lineva (1955) that the offspring of survivors of a truly sublethal exposure showed a greatly reduced biotic potential. Some of these filial flies could not lay eggs at all, while others oviposited but the eggs did not hatch, or these larvae that did hatch seldom developed successfully to the adult stage.

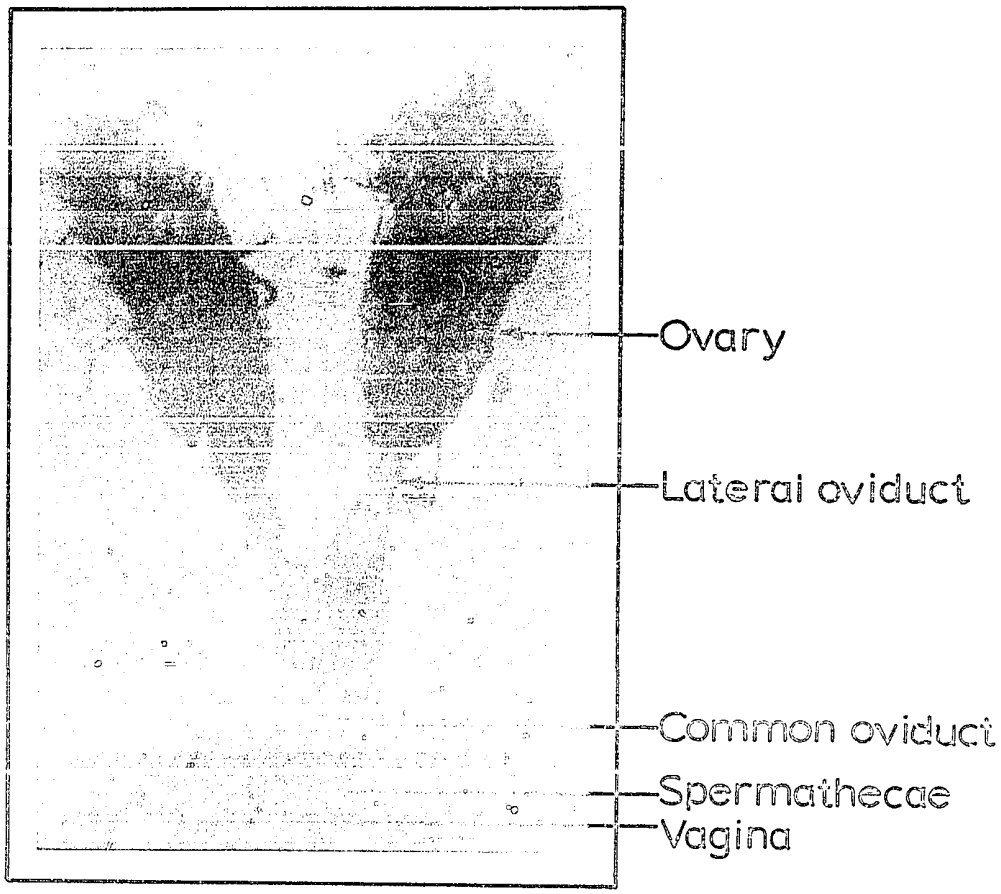
There is some evidence of a non-selective effect of a parental application on the susceptibility level of the offspring. Beard (1965) fed susceptible adult house flies truly sublethal doses of DDT, and found that the untreated first filial generation became 4-10 times more DDT-tolerant than the normal control. In the early experiments conducted by Fay et al. (1949), adult Anopheles quadrimaculatus were exposed to DDT deposits at a concentration sufficient to cause a 66% mortality in 4 successive generations, but not to the adults of the fifth and sixth generation. The susceptibility fell abruptly in the F_1 after the initial selection of the parental generation, and remained at this lower level for the next 3 generations (the F_2 to F_4) each of which had been preceded by a selection; however the full susceptibility returned in the F_5 which had not been preceded by a selection applied to the adult parents. The authors therefore suggested that the DDT was capable of inducing somatic changes in the sperm or egg cell of the adult mosquitoes and these changes were carried only through the next generation.

The Process of Egg-Production in Mosquitoes

Imms (1908) was the first to report that the rudimentary ovaries could be seen in the larvae of Anopheles maculipennis Meig. and that the follicles start to form in the pupal stage. However in Aedes aegypti the follicles may appear as early as the 4th-instar larva (Christophers, 1960). In Culex pipiens pallens Coq. the follicles are still so small in the pupal stage that they can hardly be counted (Hosoi, 1954a).

The structure of the reproductive organs of various species of Anopheles has been described by Nicholson (1921) in An. maculipennis; by Mer (1936) in An. elutus (=sacharovi); and by Giglioli (1959) and Bertram (1961) in An. gambiae. The reproductive organs in Aedes aegypti have been described by Christophers (1960), Curtin and Jones (1961) and Bertram and Bird (1961), while Lecallion (1900) has described them in Culex pipiens. In all these species of mosquitoes the female has two ovaries situated one on each side and dorsally in the posterior portion of the abdomen; their two lateral oviducts connect to a common oviduct, which continues down to the vagina and finally to the atrium (Plate 1). The ovary is enclosed in a thin elastic membrane known as the ovarian sheath, which thus forms an elongate ovoid chamber. Packed tightly together within this chamber and radiating around the internal oviduct (calyx) are the ovarioles, the sources of the eggs (Plate 2). Each ovariole consists of a follicular tube, the apex of which contains the germarium or growth zone, where the follicle originates before breaking out, while below the germarium there is a succession of developing follicles. Each follicle consists of 8 cells

Plate 1. The pair of ovaries in female Culex p. fatigans (40 X) showing the lateral oviducts, common oviduct, vagina and spermathecae


Ovary
Lateral oviduct
Common oviduct
Spermathecae
Vagina

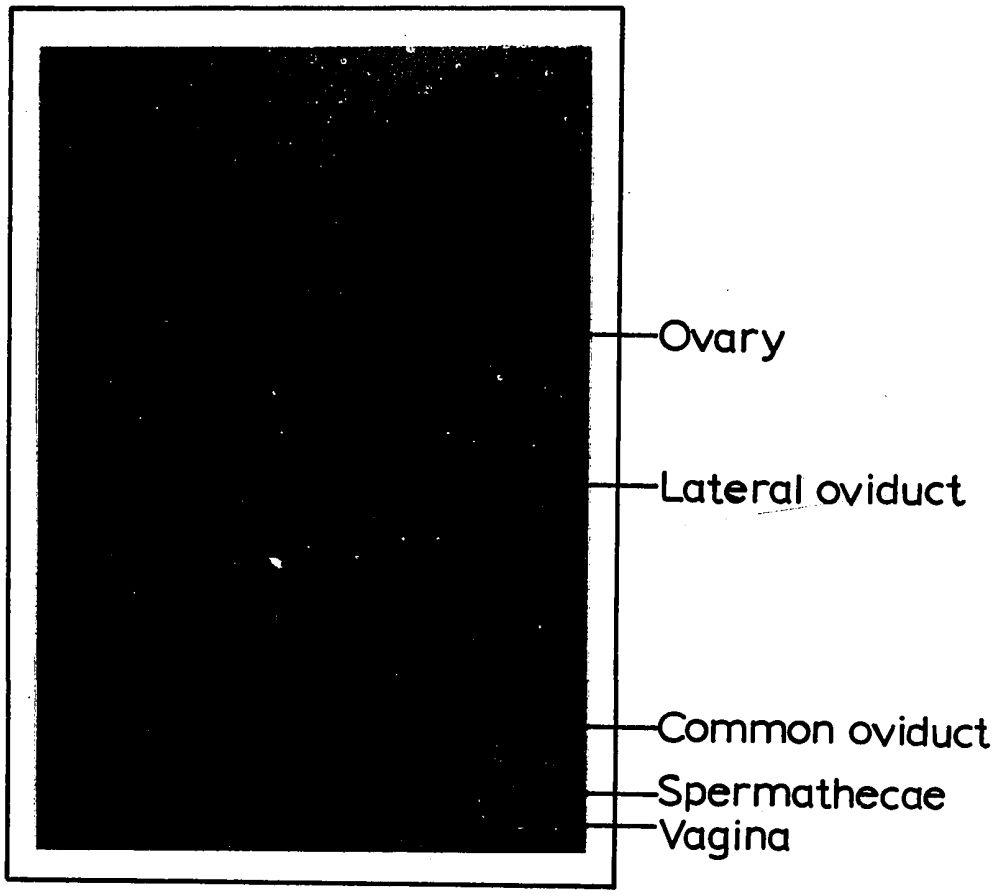
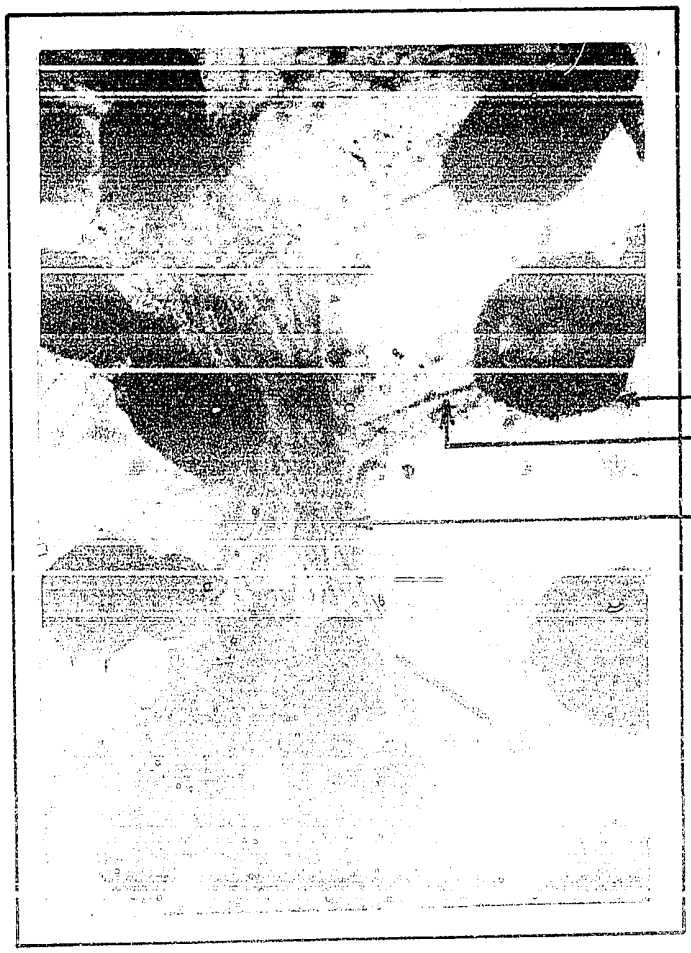
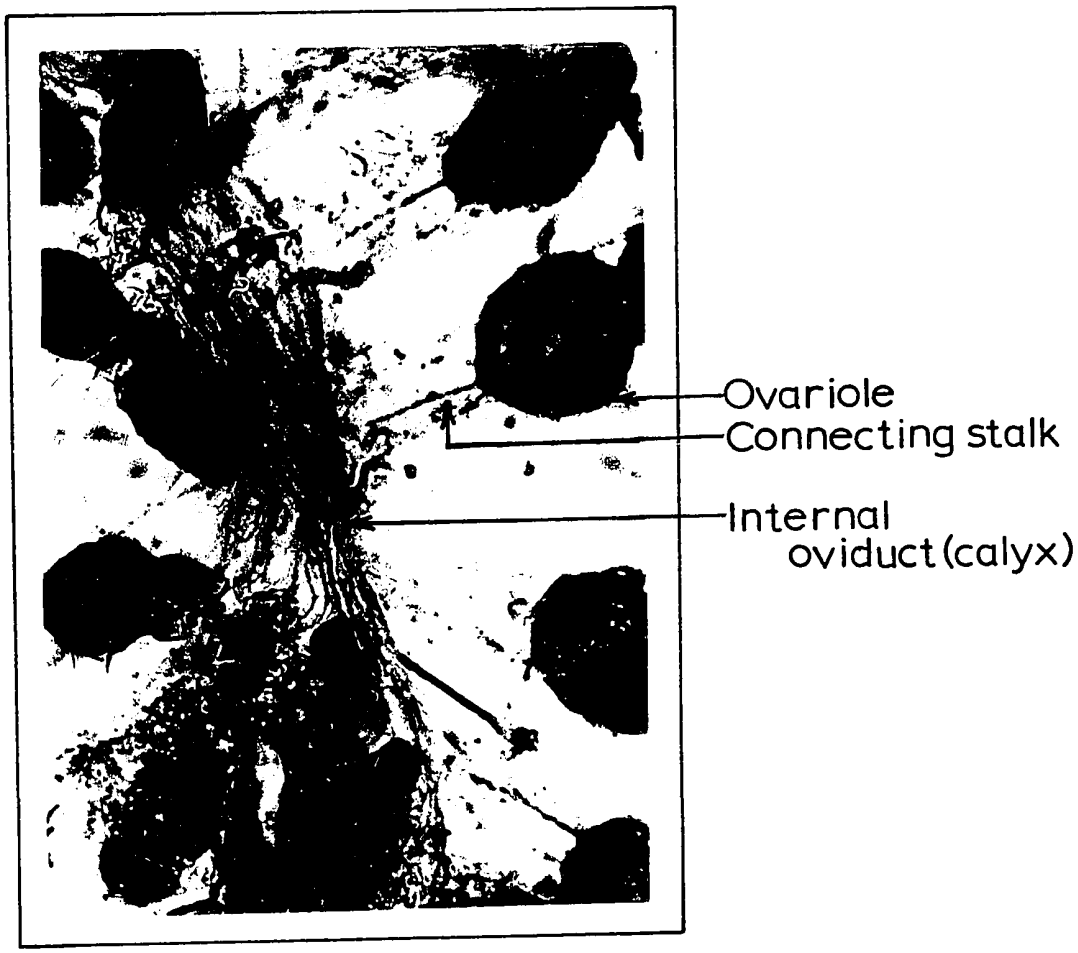

Ovary
Lateral oviduct
Common oviduct
Spermathecae
Vagina

Plate 2. Ovarioles leading into the internal oviduct of the ovary (300 ×)


Ovariole
Connecting stalk
Internal oviduct (calyx)


Ovariole
Connecting stalk
Internal
oviduct (calyx)

surrounded by a single cell layer, the follicular epithelium; 7 of these are nurse cells, and it is the eighth, the most distally situated in the follicle, which is the oocyte or future ovum (Christophers, 1960). The oocyte becomes conspicuous when the adult emerges or shortly thereafter, and it increases in size as development proceeds in the adult mosquito.

Christophers (1911) distinguished 5 stages in the development of the egg follicle in Anopheles maculipennis as follows:

Stage 1. Cytoplasm of the oocyte free from granules; this stage is only seen in the newly emerged mosquito.

Stage 2. Yolk granules present in the cytoplasm of the oocyte, but not entirely hiding the nucleus; this stage may be seen in a mosquito that has emerged some time previously but has not yet fed on blood, or one that has fed but oviposited and has not since had a blood meal.

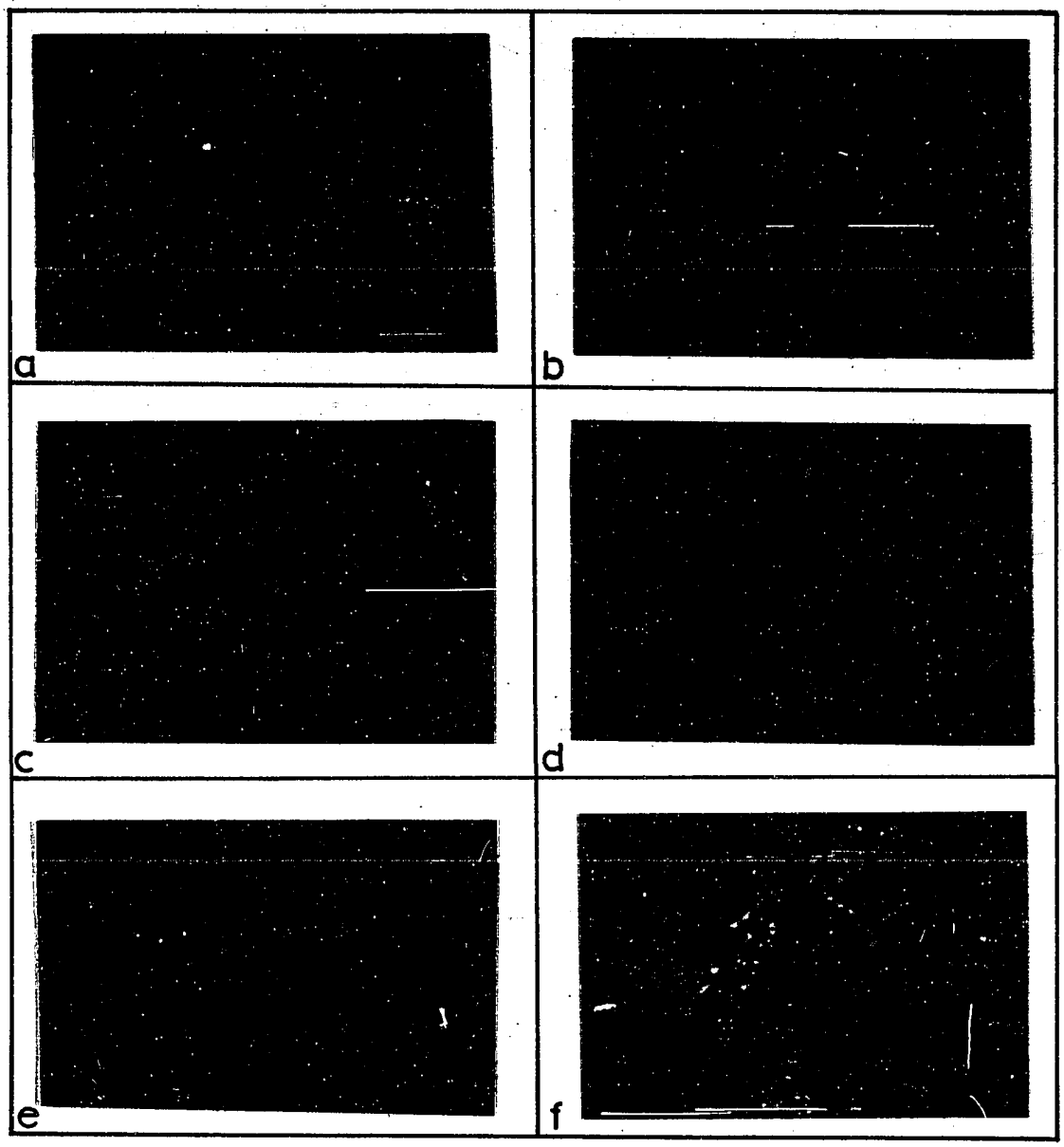
Stage 3. The nucleus of the oocyte is obscured by yolk granules and the oocyte occupies three-quarters of the follicle; usually this stage is attained after a blood meal has been taken.

Stage 4. The follicle is elongate and more or less the shape of the future egg, and the nurse cells become eventually inconspicuous.

Stage 5. Chorionic structure is developed. (Plate 3).

Clements (1963) pointed out that the development of the egg-follicles essentially consists of just 2 stages: first, development up to a resting-stage corresponding to the 2nd stage of Christophers at which point it may show an ovarian diapause; and subsequent

Plate 3. Follicular development, during the maturation of ova, according to the 5 stages of Christophers (1911) (a) Stage 1; (b) Stage 2, yolk developing; (c) Stage 3, yolk engulfing three-quarters of the ovum; (d) Stage 4, yolk engulfing nine-tenths thereof; (e) Stage 5, mature egg; (f) Egg has passed down connecting stalk, and new ovum (follicle) is developing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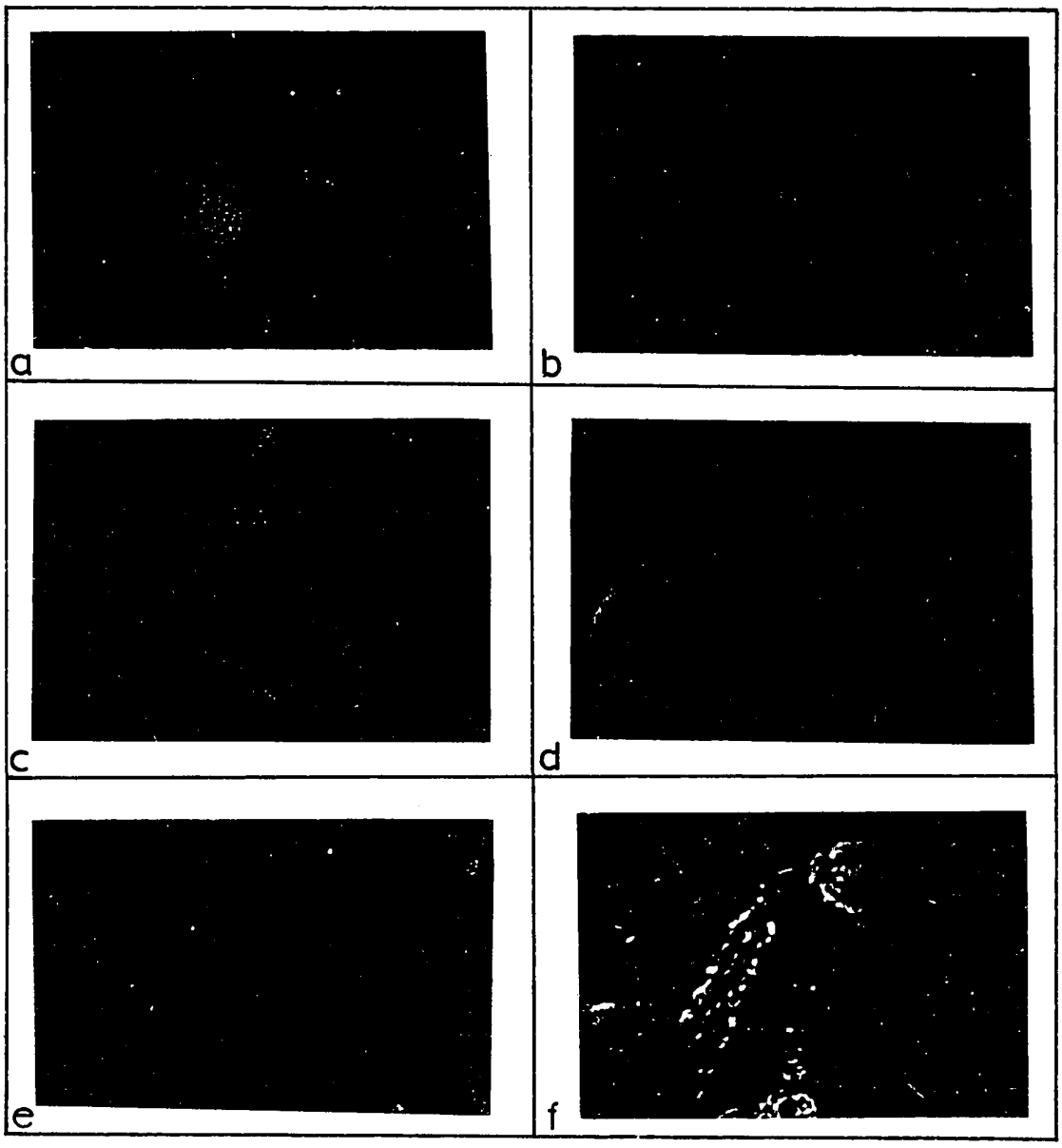
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development to the mature egg, which does not normally start until the mosquito has taken a meal of blood. Since the first stage is completed automatically after emergence, mosquitoes are therefore generally able to develop a batch of eggs after each blood meal.

Autogenous development of ovaries was firstly reported by Theobald (1901), who noticed that some individuals of Culex pipiens were able to lay eggs without feeding on blood. The term autogeny was introduced by Roubaud (1929) to describe the ability of certain genetically-distinct populations of C. pipiens able to lay their first egg-raft without feeding on blood, although still requiring a blood meal before they can subsequently develop more eggs. Most early workers considered that the autogenous females had accumulated an extra amount of metabolic reserves during their larval life, sufficient to cause the follicles to complete their development without an adult blood meal. Evidence against this was advanced by Clements (1965a) and by Twohy and Rozeboom (1957) when they found little difference in the type or amounts of reserves between the autogenous and anautogenous females of Culex pipiens. They therefore postulated that a gonadotrophic hormone controlling the growth of the ovaries was automatically secreted after emergence in the autogenous females but was not secreted in the anautogenous females without the stimulus of the blood meal. Autogeny is now known to occur very widely in mosquitoes and has been recorded in the following genera: Anopheles (Markovitch, 1941), Aedes (Vermeil, 1953), Culiseta (Marshall and Staley, 1936) and Mansonia (Anon, 1960).

It has been established in a great variety of insects that the development of sexual organs and gametes depends on the activity of

the corpora allata. In the female mosquito the paired corpora allata are situated behind the brain, where the neck joins the thorax. They secrete the juvenile hormone, neotenin, which serves to maintain larval characters in the young insect, ensures the deposition of yolk in the developing egg, induces the type of behaviour appropriate to the reproductive activity and has some general effects on metabolism (Wigglesworth, 1965).

It was Mellanby (1939) who first found that a blood meal stimulates the corpora allata to secrete a hormone which in turn causes the ovaries to develop in a fertilized female of the bed-bug Cimex lectularius. During the first 3 hours after the meal there was too little hormone to cause the ovaries to develop, but the glands produced the necessary amount within 24 hours. In mosquitoes, Gillett (1955) demonstrated that the hormone produced in Aedes aegypti enters the blood during the period between 8 and 14 hours after the blood meal, while Detinova (1945) found that an adequate quantity of the hormone was secreted by Anopheles maculipennis messae after more than 6 hours had elapsed following the blood meal. In anautogenous females of Culex pipiens berbericus the hormone accumulated immediately after the blood meal (Clements, 1945) and the same result was noted in Aedes aegypti and An. labranchiae atroparvus.

Proof that the deposition of yolk in the mosquito oocytes is dependent on the presence of the hormone secreted by the corpora allata was reported by Larsen (1958). Subsequently Larsen and Bodenstein (1959) transplanted the corpora allata from autogenous females of Culex pipiens var. molestus less than 1 day old into

anautogenous females of C. pipiens and Aedes aegypti which had never taken a blood meal, and found that in a few cases the host's ovaries could then develop to maturity. They also obtained similar results by injecting their mosquitoes with extracts from the corpora allata of the cockroach Periplaneta americana.

Detinova (1953) studied the connection between the distention of the stomach and the discharge of the hormone in female Anopheles maculipennis infected with Microsporidia. The infection of the gastric epithelium caused it to thicken and project into the gastric cavity, leaving the stomach with insufficient capacity for a full blood meal. Accordingly the nerve impulse fails to stimulate the hormone secretion, and as a result the follicles fail to complete development.

One of the factors found to determine the number of developing follicles in mosquitoes is the size of the female. A positive correlation was found in Anopheles stephensi by Roy (1931), An. maculipennis by Detinova (1955) and Aedes aegypti by Colless and Chellapah (1960). A similar correlation between the number of developing follicles and the wing length was reported by Hosoi (1954) in Culex pipiens pallens.

Roy (1963) and Woke et al. (1956) detected a positive correlation between the number of eggs laid per batch by Aedes aegypti and the amount of blood ingested. Beklemishev (1957)) reported that in An. maculipennis any increase in the size of the blood meal over the amount necessary to initiate the gonotrophic cycle, which happens to be roughly equal to the body weight, served to increase the number

of follicles which begin and complete development. Autogenous females of Culex pipiens var. molestus from undernourished larvae laid fewer and smaller eggs than normal (Oelhafen, 1961).

The nature of the blood ingested exerts a strong effect on the number of eggs laid. In C. pipiens, more follicles develop when the blood meal is taken from a bird rather than a mammal, the yield per milligram of blood taken being 82 eggs for canary blood as compared to 40 eggs for human blood (Woke, 1937). Females of C. p. pallens matured an average of 310 follicles when fed on chicken blood, as compared to 224 and 112 when fed on mouse and human respectively (Hosoi, 1954).

The number of eggs laid in each batch decreases with the succession of gonotrophic cycles. Putnam and Shannon (1934) found that each successive egg-batch of female Aedes aegypti contained 15 per cent fewer eggs than the one preceding it. In female An. maculipennis the average number of developing eggs observed by Detinova (1955) steadily decreased from 276 in the first gonotrophic cycle down to only 12 in the 13th gonotrophic cycle.

There are seasonal variations in the number of eggs developed. In An. maculipennis, Detinova (1955) found that females which had overwintered laid an average of 195 eggs in April, then 172 in May and 149 in June. On the other hand, the offspring of these overwintering females laid an average of 289 eggs in June, 263 in July, 255 in August and 180 in September. In the laboratory, Hosoi (1954) observed that in C. pipiens the number of first stage ovarian follicles per unit dry weight of mosquito decreased when the larvae were reared at

low temperatures. On the other hand, in Aedes aegypti the number of follicles decreased with the increase of the rearing temperature (Van Den Heuvel, 1962).

Degeneration of a certain number of follicles seems to be a normal occurrence in mosquitoes throughout their ovarian development. This phenomenon was first observed by Nicholson (1921) in his histological studies on egg formation in An. maculipennis, and it has been more recently described in this species by Polovodova (1947) and Detinova (1949). It has also been noted in other mosquitoes such as Mansonia (Mansonioides) uniformis (Samarawickrema, 1962) and C. pipiens pallens (Hosoi, 1954). This follicular degeneration has also been described in other Diptera, namely the sandfly Phlebotomus papatasii (Delmatova, 1949) and the house fly Musca domestica (Lineva, 1955).

The process of follicular degeneration, as studied in detail in An. maculipennis by Detinova (1942, 1949), Polovodova (1947) and Markovich (1951), consists of a gradual decomposition of the protoplasm of the oogonium, nurse cells and follicular epithelium, and later of the yolk accumulated in the oogonium. As degeneration sets in, the shape of the follicle begins to change and its outline becomes irregular. Degeneration takes place most frequently in the early stages of follicular development, but sometimes occurs in the later developmental stages. When the follicles next in line break away from the germarium, they may either complete their development or degenerate like their predecessors at one of the growth stages.

The presence of retained mature eggs in the ovarioles causes the follicles lying above them to degenerate. Detinova (1953) dissected

female An. maculipennis after they had oviposited and taken the second blood meal, and found that where mature eggs from the first cycle had been retained the second follicles lying above them had degenerated. The number of non-functioning ovarioles with degenerating follicles was observed by Detinova (1955) to increase with the physiological age of the female of this species. In Mansonia uniformis, whereas females in their second gonotrophic cycle showed degeneration in only 3.6% of their follicles, in the 3rd gonotrophic cycle they showed 15.6% (Samarawickrema, 1962).

An insufficient blood meal was observed by Roy (1936) in Aedes aegypti to cause the decreased egg production which is the outcome of increased follicular degeneration. Nicholson (1921) considered that the degeneration of a proportion of the follicles when the blood meal is inadequate is a useful process, since it leaves more nutrients for other follicles to complete development. When Hosoi (1945) fed C. pipiens pallens on casein instead of blood, he found that although many follicles started to develop most of them later degenerated. However, when he supplemented the casein meal with small amounts of extracts of chicken testis or ovary, the mosquitoes developed their follicles normally. These results suggested that factors promoting the later-stage development of the ovaries are present in chicken testis and ovary as well as in the blood. Presumably such factors may also be responsible for the different degrees of egg production from various sources of blood.

The Population Structure of the Culex pipiens Complex

Members of the pipiens complex are closely associated with man and have been distinguished for a very long time. Culex pipiens was firstly described by Linnaeus from North Europe in 1758, C. molestus by Forskal from Egypt in 1785 and C. fatigans by Weidemann from the East Indies in 1828. Culex pipiens is largely a North Temperate form, though it occurs in East and South Africa; C. fatigans, known in the American literature as C. quinquefasciatus Say, is distributed throughout the tropics; C. molestus is known from Western and Central Europe, the Near East, North Africa and Sudan, and occurs widely in the United States. In regions where C. fatigans and pipiens occur together, such as the southern United States, Africa, China and Australia, hybridization takes place freely (Mattingly, 1953). The population known as C. p. pallens distributed throughout Japan has been found to be the result of hybridization between C. fatigans and pipiens (Bekku. 1956; Kamura and Bekku, 1957).

Although the attempts to establish significant biological differences between pipiens and fatigans have so far failed, it might be objected that a biological difference of no small magnitude is already known to occur, namely that fatigans readily bites man while pipiens does not (Mattingly, 1951). It should be noted that fatigans does not always bite man; Guillard (1936) reported that at Hanoi some fatigans fed indiscriminately on bird, guinea-pig or man, while others would not bite man.

Females of autogenous and anautogenous pipiens each produce a characteristic number of eggs. An autogenous strain in Boston oviposited a mean of 243 eggs, while on the other hand, autogenous

females from the same location produced a mean of 68 eggs (Spielman, 1964). Although subsequent ovarian cycles require blood meal stimulation, the egg clutches then do not appear to be significantly larger than the first autogenous clutch (Christophers, 1951; Dobrotworsky, 1954; Kal'chenka, 1962).

When crossing experiments were made between autogenous and anautogenous strains of C. pipiens, autogeny disappeared in the F_1 but reappeared in the F_2 generation (Roubaud, 1930, 1933). It was postulated that autogeny was a result of a single recessive gene in a homozygous condition, and that therefore anautogeny was completely dominant. However it seems to be characteristic of all crosses between the two strains that autogeny appears irregularly in the succeeding generations (Kitzmiller, 1953; Krishnamurthy and Laven, 1961). The autogenous biotype is unable to hibernate and survive a cold winter (Roubaud, 1933) whereas anautogenous females are able to develop a large fat body and successfully overwinter (Spielman, 1957). Further ecological requirements serve to separate the two types since the anautogenous form requires exposed breeding sites such as open ditches and rain barrels while the autogenous form is commonly found in sewers and covered wells (Shute 1951; Kamura, 1959).

MATERIAL AND METHODS

Biological Material

The studies were performed on 2 strains of Culex p. fatigans of different degrees of DDT-resistance as compared with a susceptible strain of Culex p. pipiens. These strains had been maintained for several years in the insectary of the Department of Zoology, University of Western Ontario, London, Ontario. Their description follows:

Guelph. A DDT-susceptible strain of C. pipiens (LC_{50} = 0.002 p.p.m.) derived from females collected in the basement of the Entomological Research Laboratory, Guelph, Ontario in 1956; from a colony cultured there, a substrain was received by our laboratory in 1960.

Rangoon-LP. A DDT-tolerant strain of C. fatigans (LC_{50} = 0.13 p.p.m.) derived from a large pool of about 100 females collected in Rangoon, Burma and received at this laboratory in December 1963.

Rangoon-DDT. A DDT-resistant strain of C. fatigans (LC_{50} = 6.88 p.p.m.) previously developed from the Rangoon-N strain by larval selection with DDT for 15 consecutive generations (Tadano and Brown, 1966). This strain had originated from 4 females received from Rangoon in May 1963.

Chemical Material

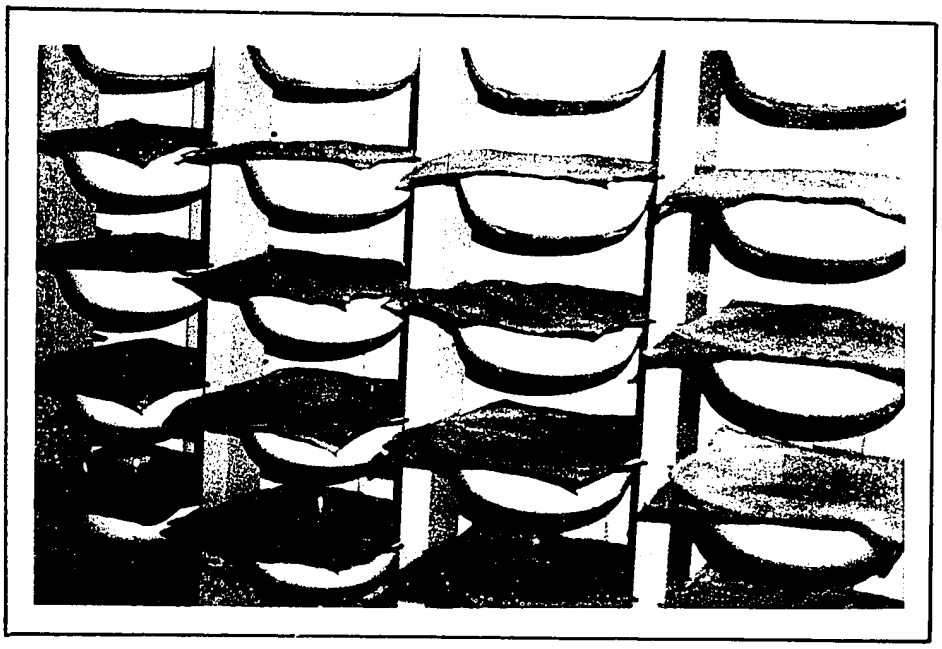
DDT: 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane, supplied by Geigy Agricultural Chemicals, Yonkers, N.Y. and recrystallized by Mr. E.E. Inwang of this laboratory.

Test papers: Standard Whatman No 1 filter-papers impregnated at 3.6 mg/cm^2 with solutions of DDT (p,p' isomer) in mineral oil (Risella 17, Shell Corp.) at concentrations of 0.25%, 0.5%, 1.0%, 2.0% or 4.0%. These papers were supplied by the Division of Environmental Health, World Health Organization, Geneva, Switzerland.

Rearing Methods

Groups of 3-4 egg-rafts obtained from the stock culture were placed to hatch in 1500 ml distilled water in 14 x 9 x 2 inch enamel pans. These rearing pans were covered with wire screening (Plate 4) and kept in the insectary maintained at 24°C. At the time of hatching and every 2nd day thereafter the larvae were fed small amounts of 5:2:1 mixture of brewer's yeast powder, blood albumin and ribonucleic acid stirred gently into the water. Periodically the water surface was freed of scum by dragging strips of paper towelling across it. On the 8th or 9th day the pupae were collected with a scoop, and sorted into males and females on the basis of size by means of a "pupae-picker" (an apparatus allowing a stream of water to cause pupae to flow between two glass plates the distance between which could be controlled by means of a screw). Lots of approximately 50 pupae of one sex or the other were placed in water in gauze-covered 1000-ml beakers, and the adults which emerged were checked for uniformity of sex, in order to ensure that all those taken for study were unmated.

Plate 4. Rearing pans for the larvae, covered with wire screening



Toxicological Methods

Susceptibility Levels. Adult susceptibility levels to DDT were determined according to the WHO method for adult mosquitoes (World Health Organization, 1960). Lots of 25 unfed and unmated 1-day-old males or females were made ready in plastic tubes lined with plain paper. They were then transferred to the exposure tubes lined with filter-papers impregnated with the appropriate concentration of DDT, and left there for an exposure period of exactly 1 hour. They were then returned to the holding tubes, pads of wet cotton-wool were placed on the screen tops of the tubes, and the entire set of tubes was covered with a plastic sheet to protect the mosquitoes from desiccation. Following a 24-hour holding period at 24°C, the mortality was determined for each concentration, the counts of moribund individuals being added to the dead. At least 100 adults of each sex were exposed to each concentration, which ascended in multiples of 2, and a similar number was exposed to control papers impregnated with the oil solvent only. The percentage mortalities were plotted on a probability scale against the DDT concentrations on a logarithmic scale (Keuffel and Esser graph No 46-8080), the dosage-mortality regression lines were fitted by eye, and the LC_{50} levels were determined as the median lethal concentrations.

With the resistant strains, the concentration of DDT in the paper was restricted to 4%, and the hours of the exposure periods ascended in multiples of 2; to avoid desiccation, the tubes were kept in a moist chamber throughout the exposure period. The percentage mortalities were plotted on a probability scale against the hours of exposure on

an arithmetic scale, and the LT_{50} was determined as the median lethal time.

Larval susceptibility levels to DDT were determined according to the WHO standard method for mosquito larvae (World Health Organization, 1960). Lots of 25 larvae in the 3rd instar were made ready in beakers with 25 ml distilled water, while 1 ml of the DDT solution in 95% ethanol was added to 225 ml distilled water in 16-oz. jars. After the treated water had been stirred with a glass rod, the larvae in the 25 ml water were added to the jars. Tests were made with 100 larvae for each of the concentrations, which ascended in multiples of 2, and a similar number of larvae were treated with the ethanol solvent alone as a control. After 24 hours at 24°C, the dead and moribund larvae were counted; the percentage mortality figures included both categories together, and the dosage-mortality regression lines were plotted and the LC_{50} determined as before.

Histological Methods

Approximately 100 female pupae were placed in the 1-litre beakers, and as soon as they started to emerge a sample of 10 females was taken by means of an aspirator and anaesthetized with chloroform. After the wings and legs had been removed, each female was placed in a drop of physiological saline solution (0.9% NaCl) on a glass slide. With fine dissecting needles, the intersegmental membrane anterior to the last abdominal segment was cut; then with the last segment firmly held by a needle, the anterior of the body was pulled away, leaving the exposed ovaries attached to the last segment. The ovaries

were then transferred to a fresh drop of saline, and by means of the needles the ovarian sheath was cut open to expose the ovarioles and facilitate the examination of the follicles.

The remainder of the emerging females were transferred to an 18 x 12 x 12 inch cage to mate with an equivalent number of males; it was supplied with cotton pledgets thrust into small bottles containing a 10% sucrose solution to provide food for the males, as well as a bowl full of water to attract oviposition. The cages were kept in the insectary maintained at 24°C and 75% relative humidity. On the following day, the females were offered a blood meal on a shaved guinea-pig immobilized by wrapping it in a cylinder of rat wire and introduced for 1 hour in the cage while the room was completely dark. Batches of these females were taken for dissection at graded time intervals after this blood meal and their follicular development was examined. The last batch of females were left in the cage to oviposit and then were dissected directly thereafter. On the successive samples of 10 females taken, counts were made of the degenerating follicles and developing follicles in each individual. The dissected females were all examined to ascertain the stage of follicular development (Plate 3) that they had reached, using the classification of stages proposed by Christophers (1911).

Sublethal Exposure to DDT

Groups of 25 females taken 1 day after emergence were exposed to papers impregnated with the maximal sublethal concentration of DDT for a period (1 to 8 hours) that gave no higher mortality than that of the control; this sublethal exposure had been established by means

of previous adult susceptibility determinations. They were then placed in cages to mate with an equivalent number of similarly treated males. To test the effect of these exposures on the feeding proclivity of the females, a mouse was placed overnight in the cage, and those females which succeeded in feeding were counted and compared with a similar group which had been exposed to control papers impregnated with the oil solvent alone.

Those females which had fed were transferred the next morning by means of an aspirator onto the DDT-impregnated papers for a further exposure period (24 to 72 hours) that gave no higher mortality than the re-exposed controls, in order to assess the effect of the sub-lethal DDT exposure on their essential fecundity. Each group, treated and untreated, of mated fed females was then placed in a cage supplied with an oviposition dish; counts were made of the egg-rafts produced and of the pupae obtained from the larvae which hatched from them, and thus the effect of sublethal exposure to DDT on the egg-raft production and the pupal production could be compared with the untreated control.

For determining the effect of exposure on the number of developing and degenerating follicles, samples of females which had been subjected to the 2 successive exposures to the DDT-impregnated or control papers were taken immediately from the oviposition cage, before they had themselves oviposited, and were dissected by the method described above.

When the effects of sublethal exposure were studied in the successive filial generations obtained from the treated parents, some of the females in each generation were tested for their susceptibility

levels, while others were treated with the sublethal concentrations of DDT for testing their feeding proclivity, egg-raft production and pupal production, and for the number of developing and degenerating follicles in their ovaries. It was from this latter group that the eggs and pupae of each subsequent generation were obtained.

The effect of larval exposure to truly sublethal concentrations of DDT on follicular development and on egg and pupal production was also investigated. After the larval susceptibility levels had been determined, batches of larvae of the slightly DDT-tolerant and of the DDT-resistant strain of C. fatigans in the 3rd instar were exposed for 24 hours to truly sublethal concentrations of DDT in 95% ethanol for 24 hours at 24°C. Two other groups of larvae, one in water treated with the ethanol solvent and the second in untreated water, served as controls. After a 24-hour exposure period, the dead larvae in the treated groups were counted and discarded, and the survivors were carefully rinsed with tap water and placed in clean water for pupating. The pupae were counted and transferred to gauze-covered beakers for emerging, and thereafter the adults were counted and released in cages with water and sugar solution. Three days after emergence a number of females was taken from each group for dissection, and the numbers of basal follicles in their ovaries were determined. The females left in the cages were then offered a blood meal on a mouse, and those that succeeded in feeding were counted and transferred to new cages with oviposition dishes. The egg-rafts obtained were counted, as were the pupae obtained from the larvae which hatched.

Detection of DDT in Ovaries of Sublethally-Treated Females

Samples of 10-13 females exposed to sublethal DDT in the adult or larval stages were immersed for 30 seconds in acetone to dissolve any external DDT residues. The ovaries were pulled out and dried, and ground in a mortar with anhydrous Na_2SO_4. The mass was extracted for 4 hours in a micro-Soxhlet apparatus with 20 ml n-hexane, and the extract was evaporated to 8 ml.

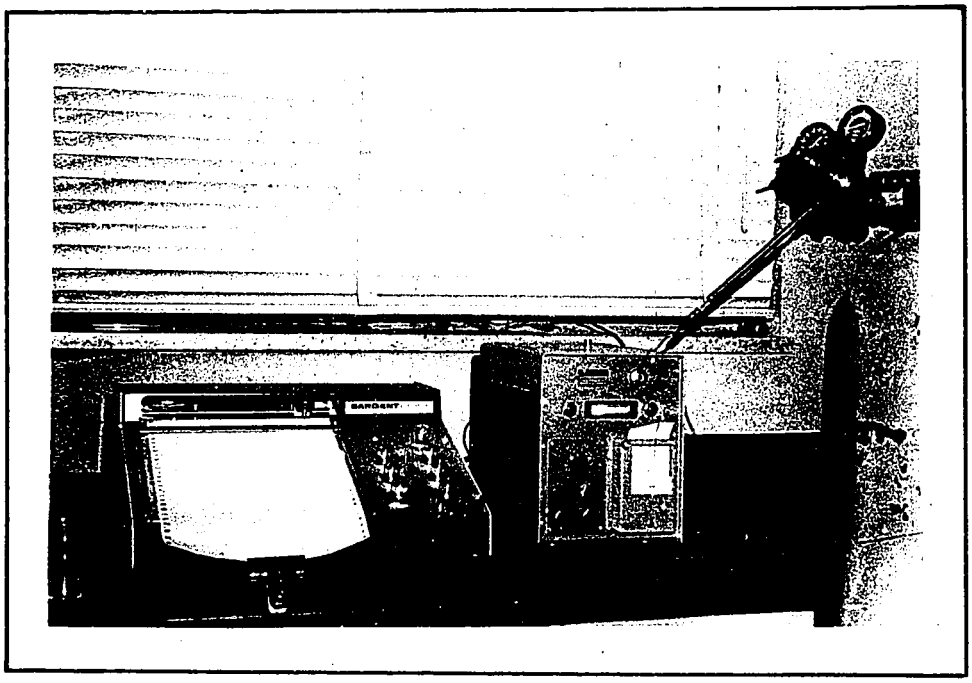
A sample of 1 microlitre of the extract was injected into a gas chromatography apparatus (Wilkens Aerograph Pestilyzer, Model 680). The chromatographic column was packed with 5 per cent of the polar fluorinated silicone QF-1 on 60/60 Chromosorb-W, HMDS treated and conditioned at 100°C for 48 hours before use. The operation was performed under the following conditions:

Column dimension	5 feet x 1/8 inch
Carrier gas	N_2 at 20 psi
Oven temperature	185°C
Attenuator	16

For accurate recording of the gas chromatograms, the Pestilyzer was connected to a Sargent Model-SR Recorder. (Plate 5).

For identifying the DDT (and some DDE) and calibrating the amounts involved, a solution of 0.1 p.p.m. DDT in n-hexane was employed. Injection of 1 microlitre of this solution, containing 0.0001 micrograms (= 0.1 nanograms = 100 picograms) gave a peak occupying an area of 288 units on the recorder chart employed (S-72166).

Plate 5. Aerograph Pestilyzer - Model A-680, connected to a nitrogen cylinder and a Sargent Recorder - Model SR, S-72180-05


SARGENT

RESULTS

In assessing the effect of sublethal exposures to DDT on adults, the 3 strains studied were a susceptible strain of C. p. pipiens, a slightly DDT-tolerant strain of C. p. fatigans and a fully DDT-resistant strain of C. p. fatigans. The maximal sublethal levels were established from susceptibility level determinations for unfed and unmated adults, which proved to be identical in either sex. These sublethal exposure levels were determined to be as follows:

Susceptible strain	1 hour on 0.25% DDT papers
Slightly tolerant strain	2 hours on 4.0% DDT papers
Resistant strain	8 hours on 4.0% DDT papers

These sublethal exposures were sufficient to give a verdict on their effect upon subsequent feeding proclivity of females. However, they had very little effect on either the follicular development or the egg-raft and pupal production, as shown by the following figures for experimental exposures to the sublethal dosage as compared to control exposures to papers impregnated with oil alone:

	Developing follicles per female		Degenerating follicles per female		Per Cent oviposition		Pupae per egg-raft	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
Susceptible	149	160	14	10	50.0	45.8	52	50
Slight. tolerant	131	128	14	11	51.2	48.1	115	111
Resistant	101	105	12	7	59.4	66.7	73	77

Accordingly, in order to investigate the fullest possible effect that sublethal exposures could have on the follicular development and egg production, it was necessary to re-expose those females that had taken a blood-meal to the sublethal doses for a further and lengthened period. The small percentages of mortality involved in this second exposure are set forth in the text, and in sum the average re-exposure mortalities for the various generations, compared to re-exposure mortalities on papers impregnated with oil alone, were as follows:

	Average of P to F_6		Control	
	Numbers	%	Numbers	%
Susc. C. p. pipiens	12/257	4.7	3/33	9.0
Sl. toler. C. p. fatigans	45/610	7.4	5/71	7.0
Resist. C. p. fatigans	43/542	7.9	3/63	4.8

Since the mortalities on the sublethal DDT re-exposures were quite constant from one generation to another, thus allowing the figures for the P to the F_6 generation to be averaged together, it is clear from the above table that this re-exposure to DDT papers for the lengthy post-feeding period did not induce any greater mortality than the controls on oil-impregnated papers alone.

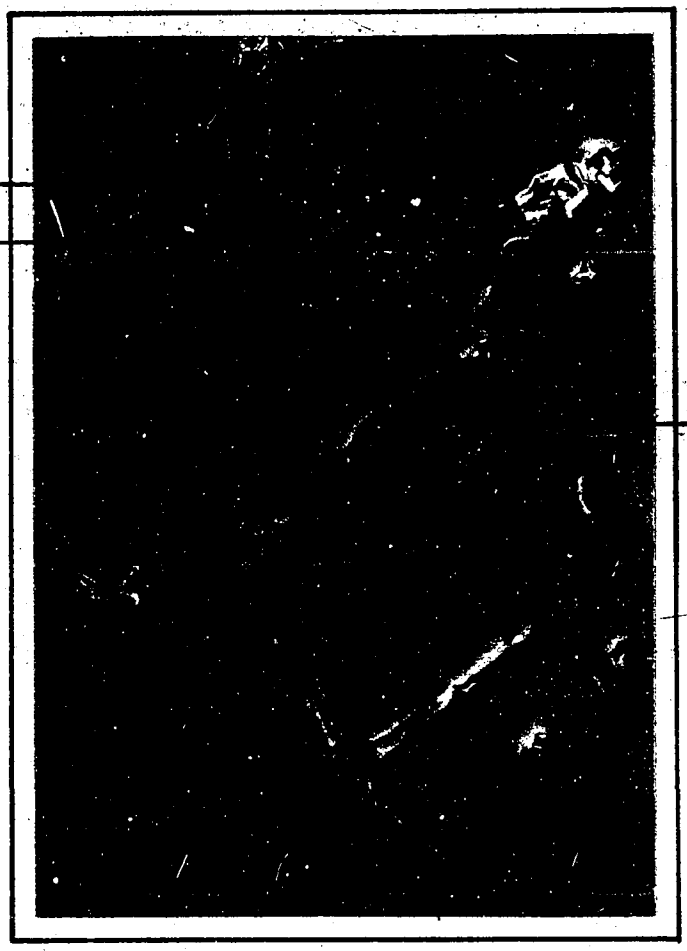
When the effect of sublethal exposures upon several successive generations of a strain was assessed, the females that laid the eggs taken for the next generation had been exposed not only for the short pre-feeding period but also for the prolonged post-feeding period.

When dissections were performed these successive sublethal exposures to DDT were found to cause disturbances of oogenesis in the treated females which inhibited the normal follicular development. The process of follicular degeneration did not seem to differ qualitatively from the normal degeneration which takes place at low frequency in untreated females.

These histological disturbances in oogenesis resulting from the sublethal treatments were as follows:

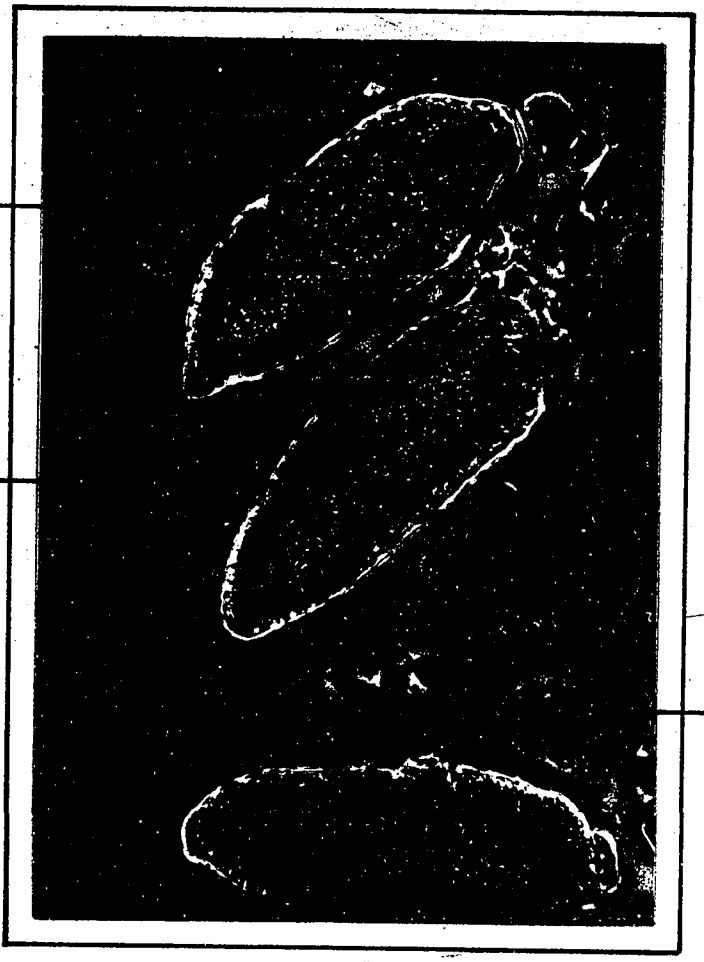
1) The follicles may degenerate during the first gonotrophic cycle and this degeneration may occur at any stage of follicular development (Plate 6).
2) Follicular degeneration is more frequent during the second gonotrophic cycle (Plate 7) than during the first cycle.
3) The normal synchrony in the development of the follicles was destroyed since while some attained normal development, others developed slowly and the remainder failed completely and started to degenerate (Plate 8).
4) A few follicles in each ovary may attain complete development, while the majority of follicles in the same ovary suffer developmental arrest at an early stage of their development (Plate 9).
5) Occasionally the follicles in the ovary may develop mature eggs, but they may be so deformed that they are not oviposited (Plate 10).

Plate 6. Degeneration of 2 follicles under the influence of sublethal DDT; the ovarioles were in the first gonotrophic cycle since the connecing stalks show no evidence of a previous ovum having passed down them


Developing follicle
Connecting stalk
2 Degenerating follicles

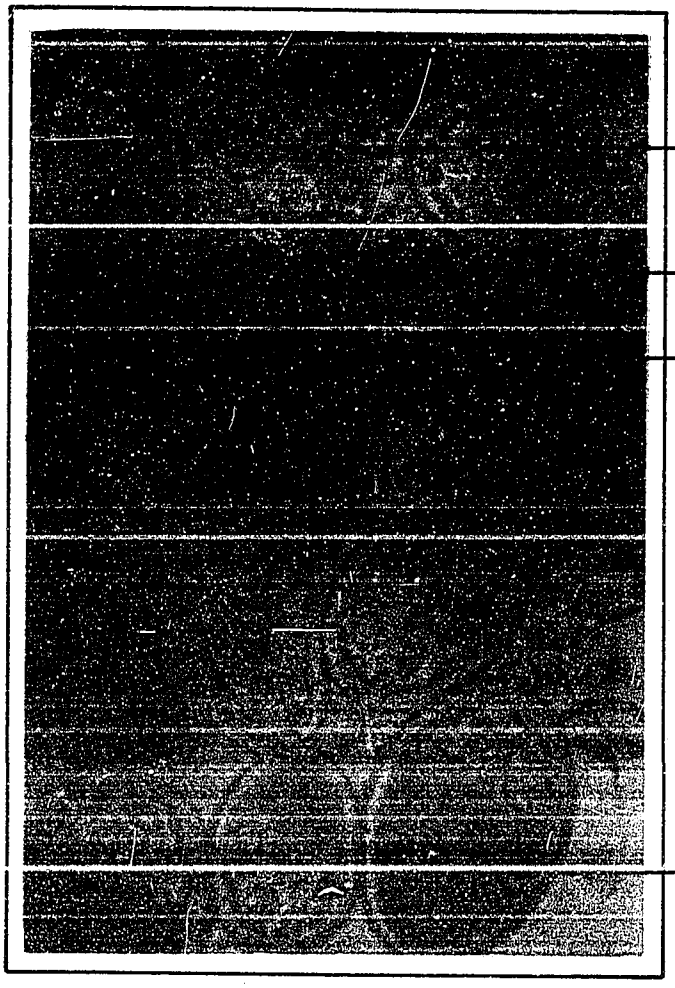

Developing follicle
Connecting stalk
2 Degenerating follicles

Plate 7. Degeneration of follicle under the influence of sublethal DDT in the second gonotrophic cycle; connecting stalks of normal follicles having 1 dilat indicating that one ovum has already been delivered


Developing follicle
1 Dilatation
Degenerating follicle in 2nd gonotrophic cycle


Developing follicle
1 Dilatation
Degenerating follicle in 2nd gonotrophic cycle

Plate 8. Follicles (developing ova) of a female sublethally treated with DDT in which only 2 have reached stage 3 and the remainder will either degenerate or be delayed in their development


Degenerating follicle
Follicle in stage 2
Follicle in stage 1
Follicle in stage 3

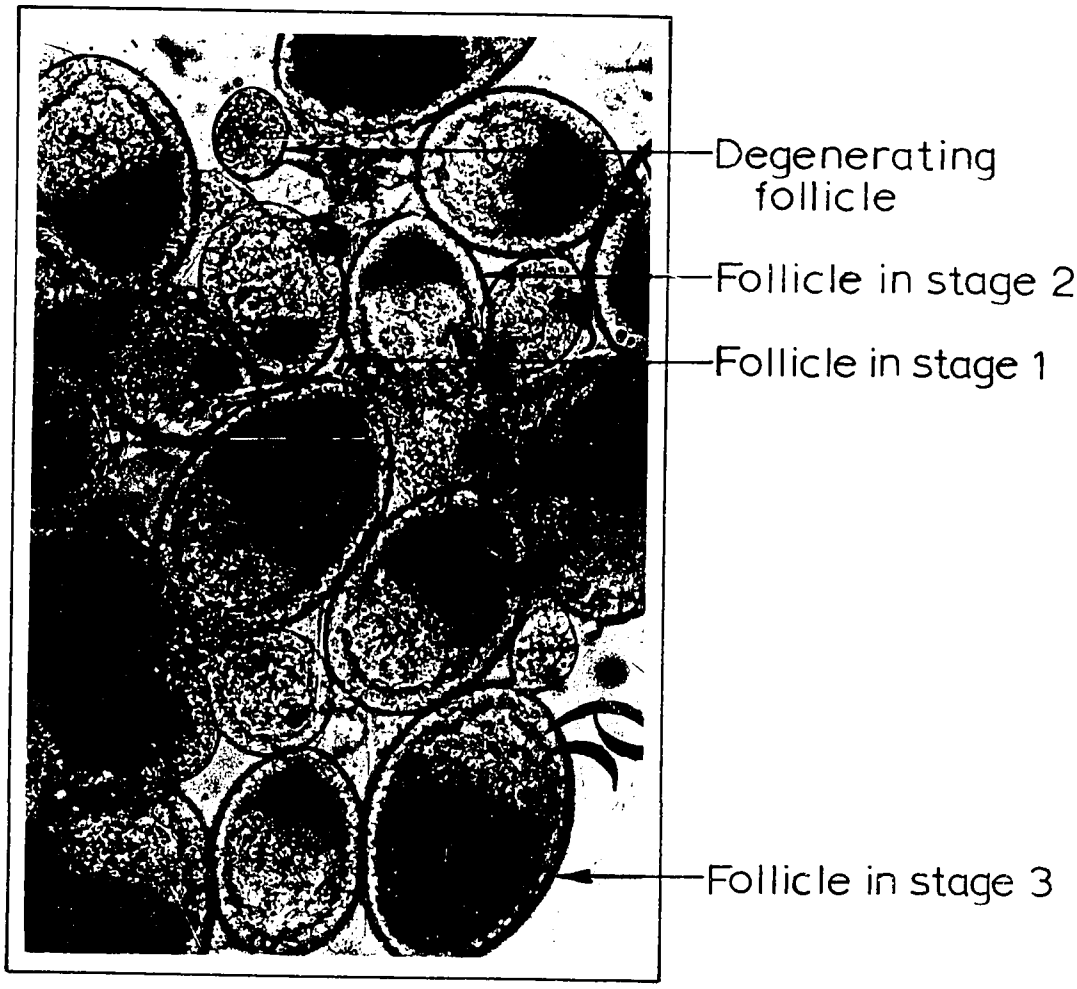
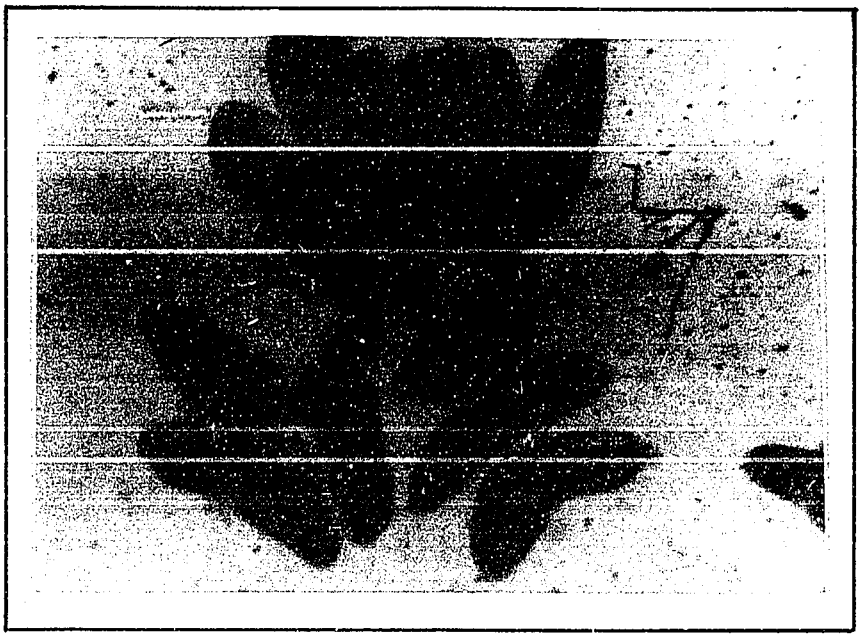

Degenerating follicle
Follicle in stage 2
Follicle in stage 1
Follicle in stage 3

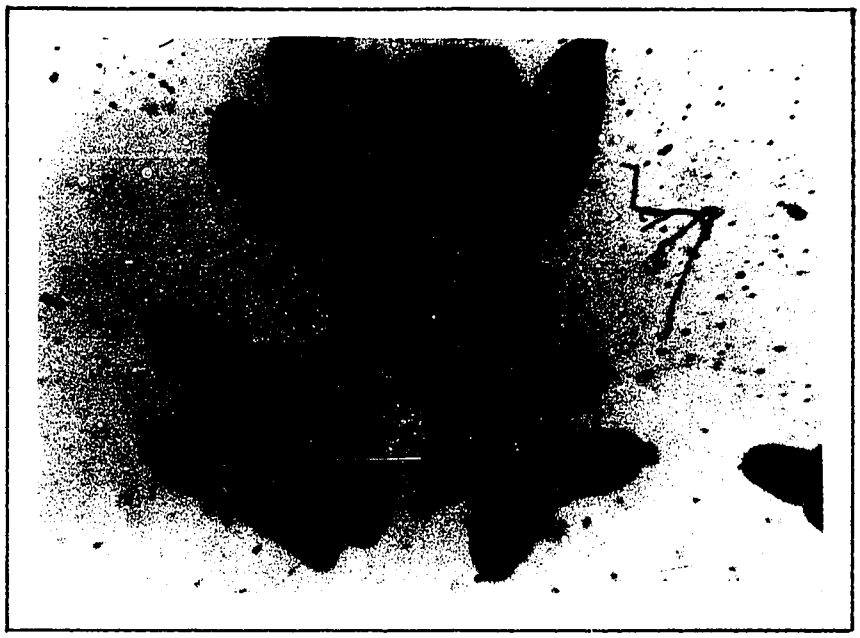
Plate 9. Numerous follicles that failed to develop in early stage, as a consequence of sublethal DDT, along with 3 follicles that developed to the mature egg


Follicle which failed to develop
Follicle developed to mature egg


Follicle which failed to develop
Follicle developed to mature egg

Plate 10. Mature eggs dissected from the ovary, showing deformation due to a sublethal dose of DDT





Effect of Sublethal Exposure to DDT on a Susceptible Strain of C. p. pipiens

The susceptibility level of the Guelph strain of C. p. pipiens, a very susceptible strain taken as a counterpart of the DDT-resistant strain of C. p. fatigans, was determined by the standard WHO method, in which the adults are exposed to DDT-impregnated papers for 1 hour (Table 1). Since the mortality on papers impregnated with 0.25% DDT was not significantly higher than on the control papers impregnated with oil alone, a 1-hour exposure to 0.25% DDT was concluded to be the maximal sublethal dose for this strain.

1. Direct Effect on the Treated Parents

Feeding Proclivity. Adult females taken 1 day after emergence were exposed for 1 hour to the sublethal concentration of 0.25% DDT, and then placed together (after the 24-hour holding period) in cages where they could mate with similarly treated males. A similar group of females and males was exposed to papers impregnated with the oil solvent alone as a control without DDT. When the mated females were offered a blood meal on a mouse, the feeding proclivity of the two groups proved to be as follows:

Exposure	No. offered blood	No. taking blood	Per Cent Feeding	t*
Control papers	198	86	43.3	
0.25% DDT papers	237	143	60.3	3.6

* $t_{0.05} = 1.96$

These results show that the exposure of adults to 0.25% DDT paper had the effect of significantly increasing the proportion of females that succeeded in taking the blood meal.

Table 1. Susceptibility levels to DDT of adults of the Guelph strain of *C. pipiens*: 1-hour exposure period

DDT Concentrations:-	0.25%		0.5%		1.0%		2.0%		4.0%		Control	
	No. Tested	% Mortal.	No Tested	% Mortal.	No Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.
Males	250	8.0	175	21.1	175	58.3	175	80.6	92	95.7	1149	1.7
Females	250	4.0	190	11.8	225	45.8	195	74.4	177	93.8	939	1.0

Follicular Development. The females that had taken the blood meal were promptly re-exposed to 0.25% DDT papers; this second exposure lasted for 24 hours and had caused an average of 9% mortality, similar to that which occurred on papers impregnated with the oil solvent alone. A sample of these females which had been subjected to the two successive DDT exposures were dissected, and the developing and degenerating follicles in their ovaries were counted. Females re-exposed to the control papers were also dissected, and the study was restricted to the 1st gonotrophic cycle. The average numbers of developing and degenerating follicles were as follows:

Exposure	No. females tested	Developing follicles per female	Degenerating follicles per female
Control papers	20	160	10
0.25% DDT papers	26	105	30

It is seen that the double sublethal DDT treatment reduced the number of developing follicles by 30 per cent, while the number of degenerating follicles was increased by fully three times. The total of developing plus degenerating follicles was decreased by 20 per cent by the DDT treatment.

Egg-Raft Production and F_1 Pupal Production. The remainder of the fed females which had been subjected to the 2 successive DDT-exposures of 1 and 24 hours were put in cages with oviposition dishes, the egg-rafts obtained were counted, and the larvae which hatched from them were taken to the pupal stage and then counted. Results were obtained (Table 2) not only for the 1st gonotrophic cycle but also for the 2nd cycle some 3 days later.

Table 2. Effect of sublethal exposure of adults to DDT on egg-raft production and on the numbers of F_1 pupae eventually obtained in the Guelph strain of C. pipiens

Type of exposure	No. females tested	First gonotrophic cycle			Second gonotrophic cycle		
		No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft	No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft
Control Papers	48	22	45.8	50	11	22.9	42
0.25% DDT Papers	63	46	73.0	34	54	85.7	21

These results indicate that the DDT-treated females showed a 27% higher oviposition rate in the 1st gonotrophic cycle and 63% in the 2nd cycle as compared to the control. On the other hand, the average number of F_1 pupae eventually produced was little more than one half of those produced by the control females, the decrease being especially marked in the 2nd gonotrophic cycle.

2. Effect of Treatments Applied in Successive Generations

Some of the F_1 adults obtained from the previous experiments were tested for their susceptibility level; others were tested for their feeding proclivity after the first DDT-exposure and after the second lengthened DDT-exposure for their production of eggs and pupae of the F_2 generation. The susceptibility level tests and the sublethal treatments were repeated with the F_2 adults obtained from their pupae, and again with adults from the F_3 to the F_6 generations (Table 3). Due to the steady increase in tolerance, the sublethal concentration applied to the F_2 and F_3 adults was set at 1.0% DDT, a concentration that caused a mortality no more than 2.5% in the males and 2.2% in the females as compared to 58.3% and 45.8% respectively in the P generation. In the F_4 generation the sublethal concentration was raised to 2.0% DDT, a dose which caused a mortality of 4.7% in the males and 2.2% in the females; in the F_5 generation it was doubled again to 4.0% DDT, which caused a mortality of 2.0% in the males and 1.0% in the females. In the F_6 generation the sublethal exposure was further increased by prolonging the exposure period on 4.0% papers to 2 hours, which proved to cause exactly 0% mortality in both sexes (Table 3a).

Table 3. Susceptibility levels to DDT of adults of the Guelph strain of *C. pipiens* exposed in all preceding generations to sublethal DDT concentrations: 1-hour exposure period

DDT Concentrations:-		0.25%		0.50%		1.0%		2.0%		4.0%	
Generation	Sex	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.
P	Males	215	8.0	175	21.1	175	58.3	175	80.6	92	95.7
	Females	250	4.0	190	11.8	225	45.8	195	74.4	177	93.8
F_1	Males	215	4.6	150	13.3	75	50.7	75	77.3	100	76.0
	Females	139	2.9	100	7.0	75	32.0	100	60.0	75	74.7
F_2	Males	-	-	92	3.3	79	2.5	100	44.0	80	53.8
	Females	-	-	80	3.7	92	2.2	97	22.7	80	45.0
F_3	Males	-	-	-	-	125	1.6	125	26.4	85	87.1
	Females	-	-	-	-	150	2.0	100	16.0	96	63.5
F_4	Males	-	-	-	-	86	4.7	105	4.8	115	95.7
	Females	-	-	-	-	89	2.2	70	1.4	98	37.8
F_5	Males	-	-	-	-	-	-	100	2.0	200	1.5
	Females	-	-	-	-	-	-	100	1.0	128	4.9

Table 3a. Susceptibility levels to DDT of adults of the Guelph strain of C. pipiens exposed in all preceding generations to sublethal DDT concentrations (cont'd): 4% DDT papers

Exposure periods:-		2 hours		4 hours		8 hours		16 hours	
Generation	Sex	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.
F_5	Males	65	10.8	121	100.00	-	-	-	-
	Females	64	7.8	94	73.4	-	-	-	-
F_6	Males	100	0	100	8.0	125	13.6	150	88.7
	Females	75	0	100	11.0	125	40.8	100	68.0

Susceptibility Level. Tests performed on unfed adults 1 day after emergence (Table 3) showed that the F_1 was considerably less susceptible than the P generation, and the following generations even less so. The dosage-mortality regression lines (Figure 1) reveal a progressive increase in the female LC_{50} from 1.2% in the P generation to 1.8% in the F_1 and 7.0% in the F_4 generation. A similar pattern of increase in the LC_{50} was observed in the males of successive generations (Figure 2). The DDT-tolerance on the other hand increased so markedly after the F_4 that in the F_5 generation only 1.5% males and 4.9% females died after 1 hour-exposure to 4% papers, as compared to 95.7% and 37.8% respectively in the preceding generation. Moreover, in the F_6 generation the exposure to 4% papers for 2 hours caused no more than zero mortality in both sexes (Table 3a).

Feeding Proclivity. Samples of females from each successive generation were taken after their initial 1-hour exposure to the sublethal concentration and tested for their proclivity to take blood. The results were as follows:

Generation	Exposure: Per Cent DDT	No. offered blood	No. taking blood	Per Cent Feeding
P	0.25	237	143	60.3
F_1	0.25	123	50	40.7
F_2	1.0	90	65	72.2
F_3	1.0	147	29	19.7
F_4	2.0	117	63	53.8
F_5	4.0	122	49	40.1
F_6	4.0	75	32	42.7

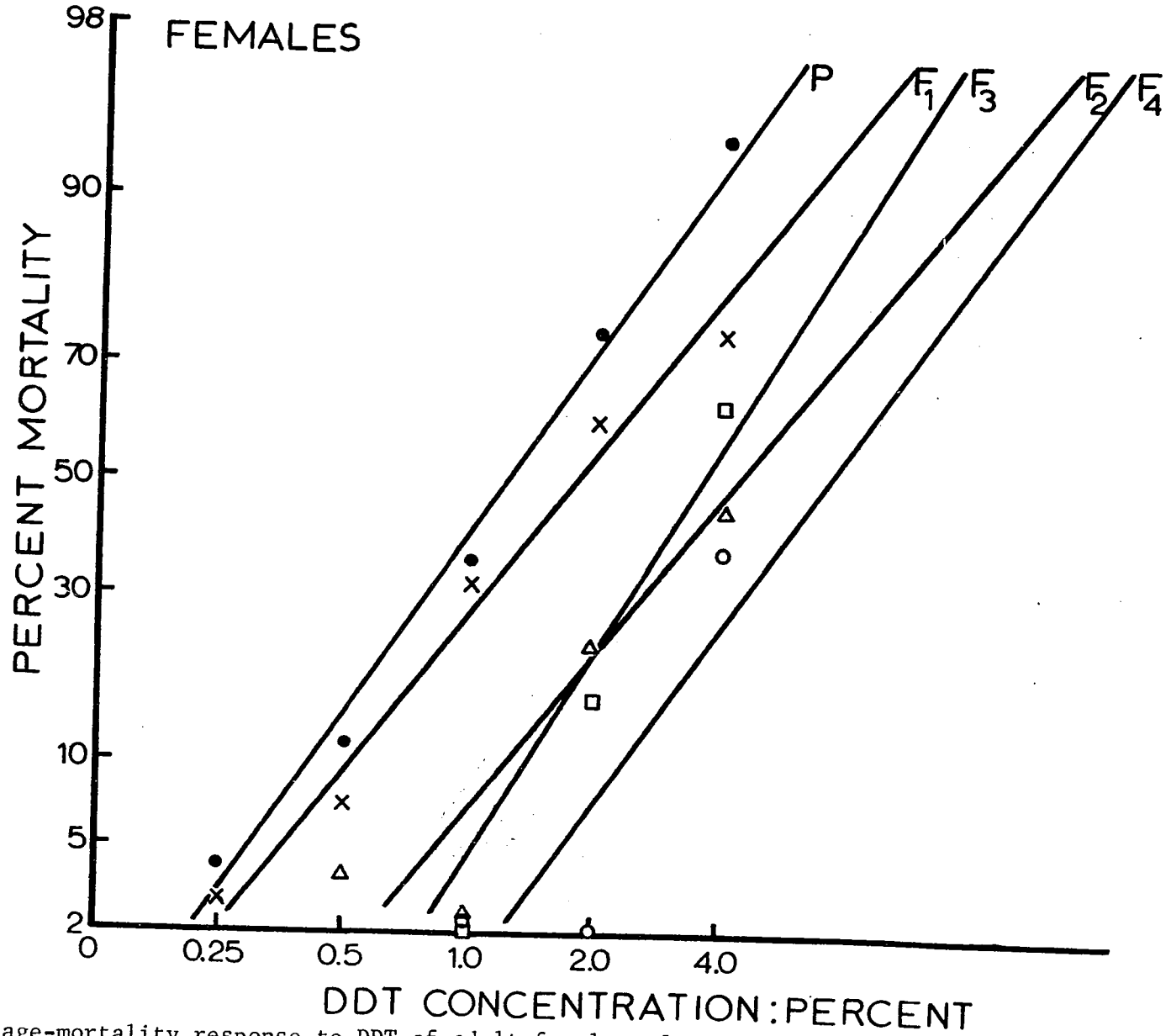


Figure 1. Dosage-mortality response to DDT of adult females of successive generations of the susceptible Guelph strain of C. pipiens exposed in each preceding generation to sublethal concentrations of DDT.

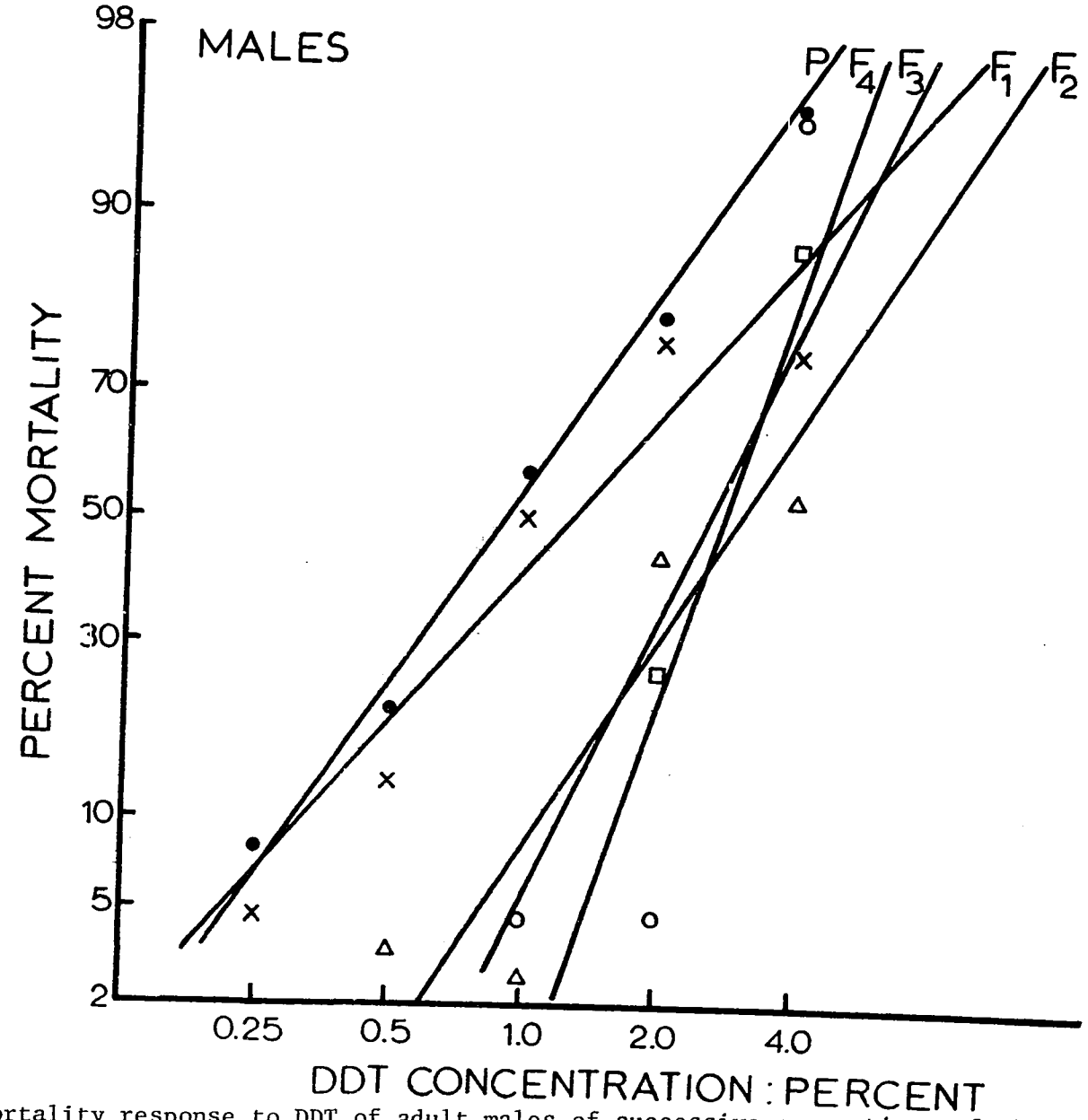


Figure 2. Dosage-mortality response to DDT of adult males of successive generations of the susceptible Guelph strain of C. pipiens exposed in each preceding generation to sublethal concentrations of DDT.

Unfortunately there are no figures for untreated females of the successive generations to compare with these females exposed to sublethal concentrations of DDT. It appears that the Guelph strain did not suffer from the repeated sublethal treatment until the F_3 was reached; at this stage the feeding success fell below 20 per cent, and thereafter increased to an intermediate value.

Follicular Development. Those females which had taken the blood meal in each generation were submitted to the second exposure of sublethal DDT concentration for a further 24 hours; these concentrations were 0.25% for the F_1 (6% mortality), 0.5% for the F_2 (4% mortality), 1.0% for the F_3 (zero mortality) and F_4 (4% mortality), 2% for the F_5 (zero mortality) and 4.0% for the F_6 (5% mortality). Samples of these re-exposed females from each successive generation were dissected, and the developing and degenerating follicles in their ovaries were counted. The results were as follows:

Generation	No. females tested	Develop. follicles per female	Degen. follicles per female
P	26	105	30
F_1	5	116	35
F_2	-	-	-
F_3	10	114	32
F_4	5	137	33
F_5	10	157	26

These results, restricted to the 1st gonotrophic cycle, show a 10 per cent increase in the number of developing follicles developed in the F_1 as compared to the previous generation. Succeeding

generations developed even more follicles successfully after the sublethal DDT treatment, so that the F_5 generation matured 50 per cent more follicles than the P generation. Moreover, there was a negligible decrease from one generation to the next in the number of degenerating follicles, so that there was a net increase in the total number of follicles as compared to the P generation. Thus the total follicles of either type on the DDT-treated F_5 actually came to exceed the total follicles for the untreated P adults (183 as compared to 170 per female).

Egg-Raft Production. The undissected remainder of those mated and fed females of each generation which had been subjected to the 2 successive DDT-exposures of 1 and 24 hours were put in cages with oviposition dishes, the egg-rafts obtained were counted, and the larvae which hatched from them were taken to the pupal stage which were then counted. The results (Table 4) show that the numbers of egg-rafts produced in the 1st gonotrophic cycle by successive DDT-exposed generations remained more or less constant, and the average number of pupal offspring steadily increased. A similar increase in the pupal offspring was observed for the progeny produced in the 2nd gonotrophic cycle during the first 3 generations studied.

Effect of Sublethal Exposure to DDT on a Slightly DDT-Tolerant Strain of C. p. fatigans

The Rangoon-LP strain of C. p. fatigans was taken as an example of a slightly DDT-tolerant strain, and its adult susceptibility levels were determined by the standard WHO method. The results (Table 5) show that exposure to 4.0% DDT papers for 2 hours had

Table 4. Effect of sublethal exposure of adults to DDT in each generation on the production of egg-rafts and pupal offspring in the Guelph strain of C. pipiens

Generation	No. females tested	First gonotrophic cycle			Second gonotrophic cycle		
		No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft	No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft
P	63	46	73.0	34	54	85.7	21
F_1	32	23	71.9	29	7	21.9	27
F_2	27	17	62.9	38	3	11.1	28
F_3	16	11	68.8	50			
F_4	29	20	68.9	46			
F_5	19	13	68.4	65			

Table 5. Susceptibility levels to DDT of adults of the Rangoon-LP strain of C. fatigans: 4% DDT papers

Exposure periods:-	2 hours		4 hours		8 hours		16 hours		32 hours		Control	
	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.
Males	475	5.5	200	15.0	250	18.4	100	61.0	100	87.0	1194	1.6
Females	625	2.1	200	6.0	200	7.5	150	25.3	200	92.5	939	0.9

caused no higher mortality than that to control papers; therefore a 2-hour exposure to 4.0% papers was chosen as the maximal sublethal exposure for this strain. A similar experimental procedure was applied as for the susceptible strain previously studied, and the following results were obtained.

1. Direct Effect on the Treated Parents

Feeding Proclivity. The sublethal exposure of this slightly tolerant strain to 4% DDT papers for 2 hours had the effect of decreasing the proportion of females that took the blood meal by 16%, as the following figures show:

Exposure	No. offered blood	No. taking blood	Per Cent Feeding	t*
Control papers	148	124	83.8	
4% DDT papers	421	284	67.5	4.1

* $t_{0.05} = 1.96$

This significant decrease after the sublethal DDT exposure stands in contrast to the increase obtained in the susceptible strain.

Follicular Development. The females that had taken the blood meal upon prompt re-exposure to 4% DDT papers for 24 hours showed only 6% mortality as compared with 7% mortality on papers impregnated with oil alone. A sample of these females when they were dissected (Table 6) showed that in the 1st gonotrophic cycle the number of developing follicles was reduced by about 23 per cent, and the number of degenerating follicles was doubled, in the DDT-treated females. These effects became even more evident in the 2nd gonotrophic cycle, while in both cycles the total of developing plus degenerating

Table 6. Effect of sublethal exposure of adults to DDT on the number of developing and degenerating follicles in the Rangoon-LP strain of C. fatigans

Type of exposure	First gonotrophic cycle			Second gonotrophic cycle		
	No. females tested	Developing follicles per female	Degenerating follicles per female	No. females tested	Developing follicles per female	Degenerating follicles per female
Control Papers	19	128	11	42	146	7
4% DDT Papers	30	99	26	6	85	33

follicles was decreased by the treatment.

Egg-Raft Production and F_1 Pupal Production. The figures for egg-raft production and the pupae eventually obtained (Table 7) reveal that the treatment increased the oviposition rate by only 3 per cent in the first gonotrophic cycle, and decreased it by 7 per cent in the second cycle as compared to the control. On the other hand, the average number of filial pupae obtained was decreased by 15 per cent in the 1st gonotrophic cycle and by 20 per cent in the 2nd cycle.

2. Effect of Treatment Applied in Successive Generations

Some of the F_1 adults obtained from the previous experiments were used to found a substrain treated with adult sublethal levels of DDT in each generation until the F_6, as before. The 2-hour period of exposure to 4% DDT papers was increased to 4 hours for the F_6 adults without incurring any mortality among females.

Susceptibility Level. Tests performed on the unfed adults 1 day after emergence (Table 8) showed that the F_1 generation had become much less tolerant than the P generation. From the plots of the mortalities on 4% DDT papers at different exposure periods (Figure 3), it is seen that the female LT_{50} abruptly decreased from 21 hours in the P generation to 11 hours in the F_1, and thereafter dropped to a figure of 8 hours in the F_5 generation. However, in the F_6 and F_7 generations the LT_{50} sharply increased to 25 hours, bringing its tolerance back to that of the P generation. The pattern of susceptibility was the same in the males as in the females.

Feeding Proclivity. The females of each generation taken after

Table 7. Effect of sublethal exposure of adults to DDT on egg-raft production and on the numbers of F_1 pupae eventually obtained in the Rangoon-LP strain of *C. fatigans*

Type of exposure	No. females tested	First gonotrophic cycle			Second gonotrophic cycle		
		No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft	No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft
Control Papers	79	38	48.1	111	10	12.7	106
4% DDT Papers	107	52	51.4	96	6	5.6	86

Table 8. Susceptibility levels to DDT of adults of the Rangoon-LP strain of C. fatigans exposed in all preceding generations to sublethal DDT concentrations: 4% DDT papers

Exposure periods:-		2 hours		4 hours		8 hours		16 hours		32 hours	
Generation	Sex	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.
P	Males	475	5.5	200	15.0	250	18.4	100	61.0	100	87.0
	Females	625	2.1	200	6.0	200	7.5	150	25.3	200	92.5
F_1	Males	200	5.5	180	17.2	100	30.0	300	94.3	175	98.3
	Females	380	2.9	620	15.8	300	25.7	325	88.0	175	93.5
F_2	Males	270	3.7	210	22.9	150	26.7	125	81.6	200	97.5
	Females	174	4.0	165	18.8	200	30.5	200	82.0	200	94.0
F_3	Males	200	5.5	167	16.8	100	60.0	200	72.5	150	96.0
	Females	200	1.0	242	29.8	70	61.4	200	73.5	150	83.3
F_4	Males	396	12.6	200	32.0	200	53.0	200	100.0	-	-
	Females	275	2.5	200	17.0	200	42.0	200	91.5	195	100.0
F_5	Males	175	6.3	175	22.9	200	44.5	200	82.0	250	86.8
	Females	175	2.9	175	25.1	200	50.5	200	65.0	275	84.4
F_6	Males	75	6.7	92	6.5	100	16.0	85	54.1	200	81.0
	Females	275	2.6	88	0	100	12.0	257	23.0	165	72.7
F_7	Males	175	4.0	150	26.0	120	50.0	130	83.8	120	98.3
	Females	130	1.5	150	2.0	125	34.4	135	42.9	115	95.7

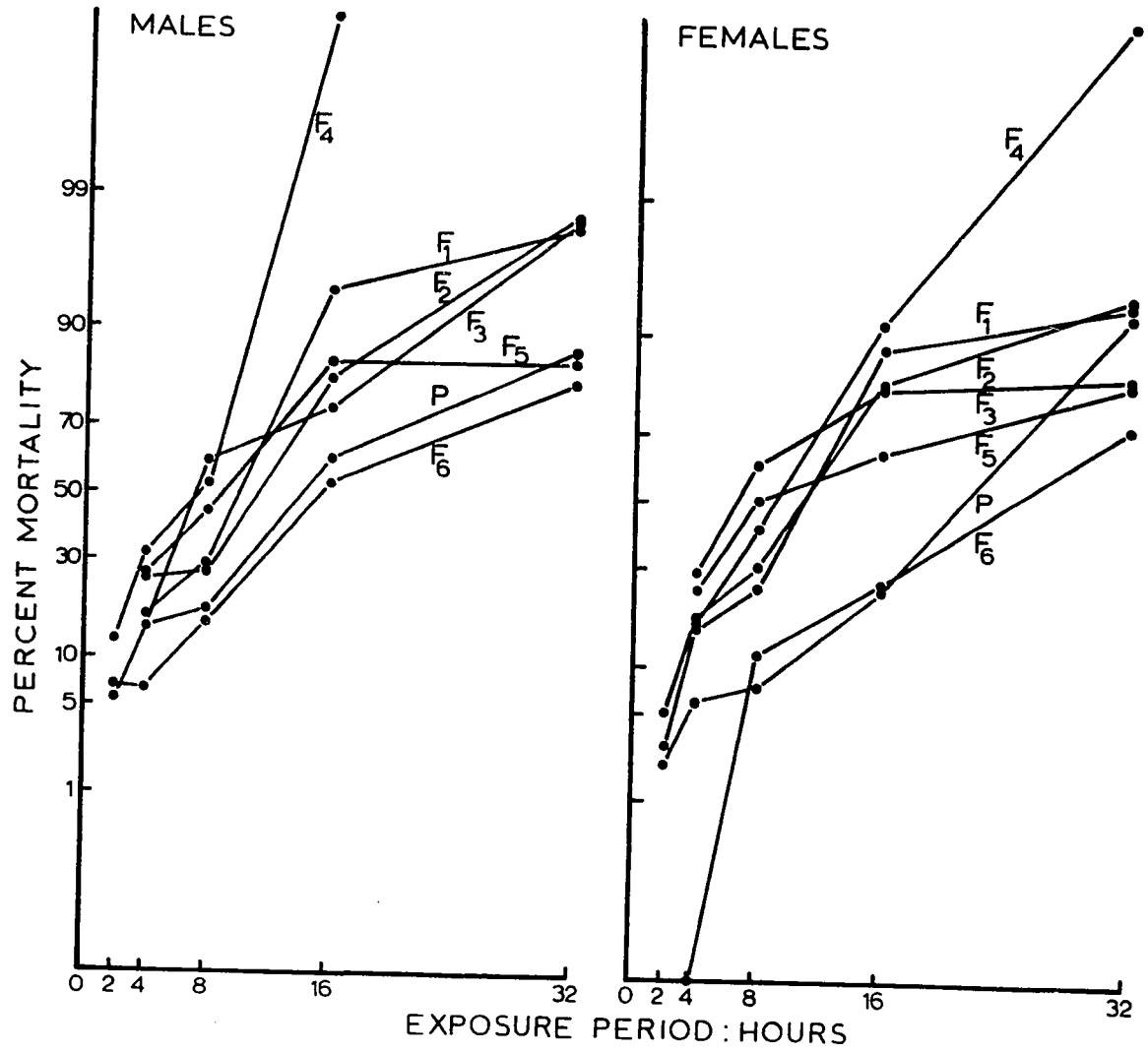


Figure 3. Dosage-mortality exposure to DDT of adults of successive generations of the slightly-tolerant Rangoon-LP strain of C. fatigans exposed in each preceding generation to sublethal concentrations of DDT.

their 2-hour exposure to the sublethal concentration showed the following proportions of success in taking blood:

Generation	Exposure period to 4% DDT	No. offered blood	No. taking blood	Per Cent Feeding
P	2 hours	421	284	67.5
F_1	2 hours	172	96	55.8
F_2	2 hours	127	86	67.7
F_3	2 hours	198	125	63.1
F_4	2 hours	219	130	59.4
F_5	2 hours	88	29	32.9
F_6	4 hours	100	19	19.0

It is seen that the Rangoon-LP strain suffered only a slight progressive reduction in the post-exposure feeding proclivity during the first 5 generations, but then the proportion taking the blood meal abruptly declined in the F_5 generation to a figure (33%) which was one-half of the initial feeding success. The success further declined to 19% in the F_6 generation despite the fact that the prolongation of the DDT exposure to 4 hours had not caused any mortality among the females.

Follicular Development. Re-exposure of the blood-fed females to 4% DDT papers for the further 24 hours involved further mortalities of only 5.8% in the F_1, 5.7% in the F_2, 9.0% in the F_3, 7.1% in the F_4, 6.9% in the F_5 and 3.2% in the F_6 generation. In each generation a sample of the re-exposed females was dissected, and the follicular counts of successive generations (Table 9) revealed that sublethal exposure to DDT had twice as much effect on the F_1 and subsequent

Table 9. Effect of sublethal exposure of adults to DDT in each generation on the number of developing and degenerating follicles in the Rangoon-LP strain of *C. fatigans*

Generation	First gonotrophic cycle			Second gonotrophic cycle		
	No. females tested	Developing follicles per female	Degenerating follicles per female	No. females tested	Developing follicles per female	Degenerating follicles per female
P	30	99	26	6	85	33
F_1	13	97	59	7	70	46
F_2	14	85	35	10	71	53
F_3	12	91	51	10	60	72
F_4	10	83	53			
F_5	12	60	49			
F_6	12	86	48			

generations as on the P generation with respect to the number of degenerating follicles in the 1st gonotrophic cycle. There was only a slight reduction in the number of developing follicles. These effects were even more pronounced in the 2nd gonotrophic cycle, as the tests on the first 3 filial generations demonstrate.

Egg-Raft Production and Pupal Production. When the undissected remainder of the re-exposed blood-fed females of each generation were tested for their fecundity (Table 10), the figures show that although there was no reduction in oviposition during the first 4 filial generations, the resulting pupal production gradually decreased. This reduction in hatch was particularly pronounced in the DDT-treated F_5 generation especially during the 2nd gonotrophic cycle, while in the F_6 the oviposition rate of DDT-treated females decreased abruptly in the 1st cycle, and not a single egg-raft was obtained in the 2nd cycle.

Effect of Sublethal Exposure to DDT on a DDT-Resistant Strain of C. p. fatigans

The maximal sublethal dose of DDT for males and females of the Rangoon-DDT strain of C. p. fatigans was determined by exposing 1-day-old unfed and unmated adults to the WHO papers impregnated with 4% DDT for exposure periods ranging between 2 and 32 hours. The results (Table 11) show that the period of exposure to 4% DDT papers could be increased to 8 hours without any effect greater than the control mortality; therefore an 8-hour exposure to 4% DDT was chosen as the maximal sublethal dose for this strain. Similar experimental procedure was applied as for the 2 strains previously

Table 10. Effect of sublethal exposure of adults to DDT in each generation on the production of egg-rafts and pupal offspring in the Rangoon-LP strain of C. fatigans

Generation	No. females tested	First gonotrophic cycle				Second gonotrophic cycle		
		No. Egg-Rafts	Per Cent Oviposition	No. Pupae	Pupae per Egg-Raft	No. Egg-Rafts	No. Pupae	Pupae per Egg-Raft
P	107	52	51.4	5002	96	6	516	86
F_1	63	32	49.2	3115	97	19	1594	83
F_2	60	39	65.0	2857	73	16	1057	66
F_3	65	41	63.1	3607	88	15	1059	71
F_4	73	64	87.7	4724	73	38	1642	43
F_5	38	35	92.1	2034	58	23	867	38
F_6	18	14	77.8	1193	85	0	0	0

Table 11. Susceptibility levels to DDT of adults of the Rangoon-DDT strain of C. fatigans: 4% DDT papers

Exposure periods:-	2 hours		4 hours		8 hours		16 hours		32 hours		Control	
	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.
Males	155	3.9	115	0.9	140	0.7	200	31.5	175	96.6	180	2.7
Females	150	3.3	150	0.7	207	3.9	173	27.7	155	80.6	177	1.1

studied, and the following results were obtained.

1. Direct Effect on Treated Parents

Feeding Proclivity. The results show that the sublethal exposure of adults to 4% DDT papers for 8 hours had caused an insignificant decrease in the proportion of females that succeeded in taking blood, as follows:

Exposure	No. offered blood	No. taking blood	Per Cent Feeding	t*
Control papers	175	126	72.0	
4% DDT papers	236	157	66.5	1.33

$*t_{0.05} = 1.96$

This insignificant decrease after the sublethal DDT exposure stands in contrast to the significant decrease obtained in the slightly tolerant strain.

Follicular Development. The females that had taken the blood meal were promptly re-exposed to 4% DDT papers for a further period of 72 hours, which caused a mortality of only 8.8% as compared with 4.8% mortality on papers impregnated with the oil solvent alone. A sample of these re-exposed females was taken for dissection, and the counts of developing and degenerating follicles obtained (Table 12) indicate that the sublethal treatment reduced the number of developing follicles in the 1st gonotrophic cycle by about 20 per cent, and increased the number of degenerating follicles by fully 4 times. These effects were less marked in the 2nd gonotrophic cycle, but were still conspicuous, and in both cycles the total of developing plus degenerating follicles was increased by the treatment.

Table 12. Effect of sublethal exposure of adults to DDT on the number of developing and degenerating follicles in the Rangoon-DDT strain of *C. fatigans*

Type of exposure	First gonotrophic cycle			Second gonotrophic cycle		
	No. females tested	Developing follicles per female	Degenerating follicles per female	No. females tested	Developing follicles per female	Degenerating follicles per female
Control Papers	16	105	7	10	97	22
4% DDT Papers	19	83	32	12	82	59

This increase in the number of total follicles is in contrast with the decrease obtained after a sublethal DDT exposure in the slightly tolerant strain.

Egg-Raft Production. The remainder of those fed females of each generation which had been subjected to the 2 successive exposure periods of 8 and 72 hours were put in cages with oviposition dishes. The figures for the egg-raft production and the pupae eventually obtained (Table 13) indicate that the treatment reduced the egg-production by about 30 per cent in the 1st gonotrophic cycle, but was without effect in the 2nd cycle. On the other hand, the average number of pupae eventually obtained was reduced by about 20 per cent in the 1st gonotrophic cycle and 17 per cent in the 2nd cycle.

2. Effect of Treatments Applied in Successive Generations

Some of the F_1 adults obtained from the previous experiments were used to found a substrain treated with adult sublethal levels of DDT in each generation until the F_5 as above. The 8-hour period of exposure to 4% DDT papers was increased to 16 hours for the F_3 and F_4, and to 32 hours for the F_5, without incurring any much higher mortality among the females.

Susceptibility Level. When 1-day-old adults of each generation were tested with 4% DDT papers (Table 14), and the percentage mortalities were plotted for the different exposure periods (Figure 4), it is seen that the F_1 generation was no less susceptible than the P generation. The F_2 however was much less susceptible, the female LT_{50} having risen from 23 hours in the P generation up to 58 hours in the F_2 generation. In subsequent generations the susceptibility

Table 13. Effect of sublethal exposure of adults to DDT on egg-raft production and on the numbers of F_1 pupae eventually obtained in the Rangoon-DDT strain of C. fatigans

Type of exposure	No. females tested	First gonotrophic cycle			Second gonotrophic cycle		
		No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft	No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft
Control Papers	27	18	66.7	77	14	51.9	71
4% DDT Papers	27	10	37.1	62	17	62.9	59

Table 14. Susceptibility levels to DDT of adults of the Rangoon-DDT strain of C. p. fatigans exposed in all preceding generations to sublethal DDT concentrations: 4% DDT papers

Exposure periods:-		4 hours		8 hours		16 hours		32 hours		64 hours	
Generation	Sex	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.	No. Tested	% Mortal.
P	Males	115	0.9	140	0.7	200	31.5	175	96.6	-	-
	Females	150	0.7	207	3.9	173	27.7	155	80.6	-	-
F_1	Males	95	3.2	75	33.3	120	39.0	150	59.3	-	-
	Females	75	5.3	90	26.7	115	32.0	125	47.2	-	-
F_2	Males	-	-	150	2.0	155	9.0	325	16.0	325	68.6
	Females	-	-	100	0	225	4.0	305	3.6	305	66.6
F_3	Males	-	-	-	-	148	2.7	200	20.5	220	95.4
	Females	-	-	-	-	143	0.7	148	22.3	217	83.4
F_4	Males	-	-	-	-	90	7.8	100	12.0	100	96.0
	Females	-	-	-	-	80	0	95	20.0	100	94.0
F_5	Males	-	-	-	-	-	-	275	7.6	120	95.8
	Females	-	-	-	-	-	-	226	4.9	100	80.0

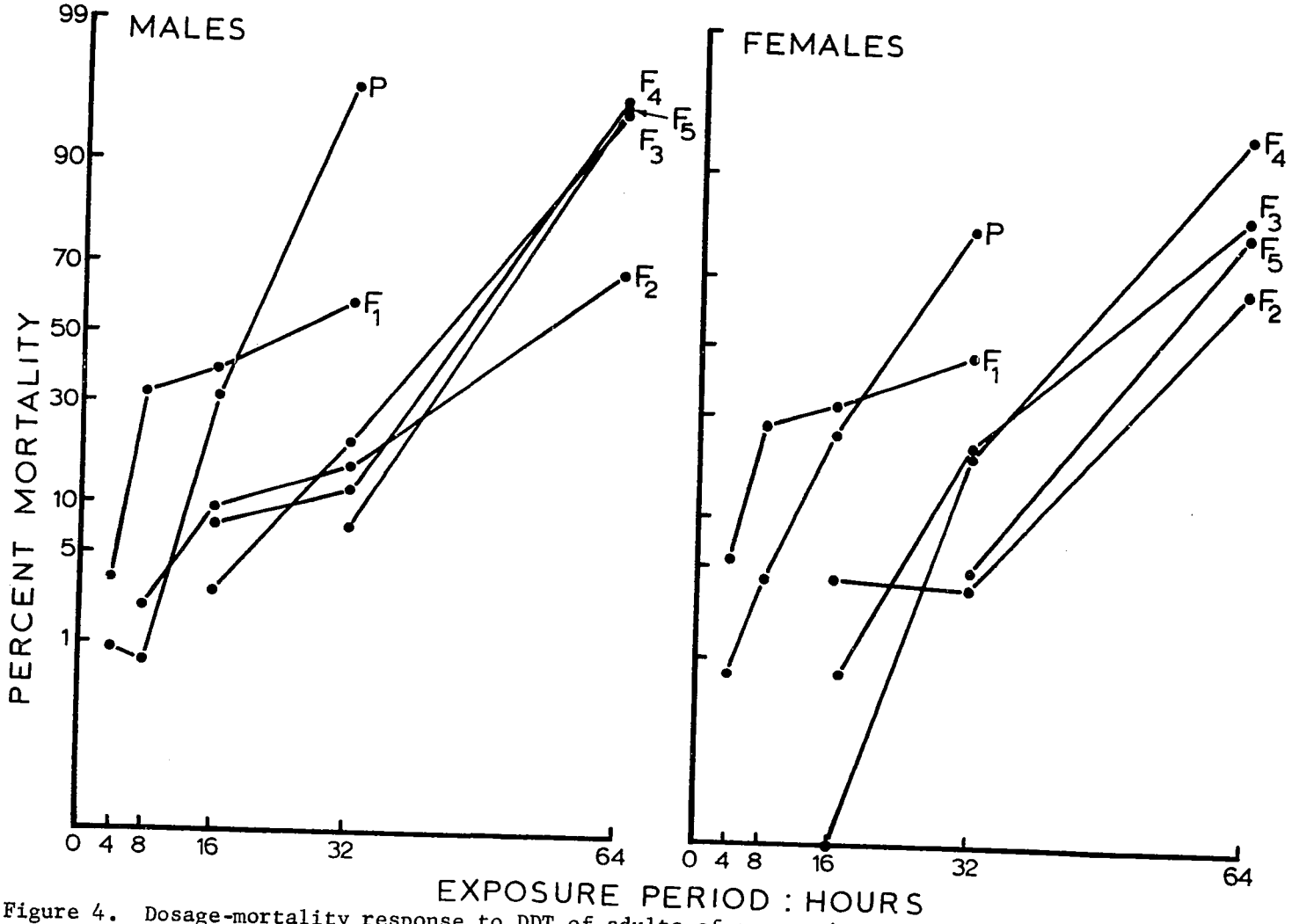


Figure 4. Dosage-mortality response to DDT of adults of successive generations of the resistant Rangoon-DDT strain of C. fatigans exposed in each preceding generation to the sublethal concentrations of DDT.

levels remained roughly at the same level of increased tolerance. The contrast between the P generation and the F_5 generation may be seen at the 32-hour period of exposure, for which the percentage mortalities were:

	Males	Females
P	97	81
F_5	8	5

Feeding Proclivity. Females in each generation were taken after their initial exposure to the sublethal concentration and tested for their proclivity to take blood. The results were as follows:

Generation	Exposure period to 4% DDT	No. offered blood	No. taking blood	Per Cent Feeding
P	8 hours	236	157	66.5
F_1	8 hours	171	82	47.9
F_2	8 hours	200	107	53.5
F_3	16 hours	186	61	32.8
F_4	16 hours	80	41	51.3
F_5	32 hours	215	29	13.5

It is seen from the above figures that the Rangoon-DDT strain suffered a slight progressive reduction in the feeding success after DDT treatment. Whereas in the P generation the treatment caused an insignificant decrease from 72% down to 66%, the feeding success shown by the F_5 after treatment had fallen to a 14% figure.

Follicular Development. When those females which took the blood meal were re-exposed to the maximal DDT sublethal concentration for a further 72 hours, the mortalities involved were only 5.1% in

the F_1, 11.9% in the F_2, 9.8% in the F_3, 2.4% in the F_4 and 12.1% in the F_5 generation. A sample of those females that survived the two exposure periods were dissected and the developing and degenerating follicles in their ovaries were counted. The counts were restricted to the 1st gonotrophic cycle and the first 3 generations in this strain. The results were as follows:

Generation	No. females tested	Developing follicles per female	Degenerating follicles per female
P	19	83	32
F_1	12	56	83
F_2	12	53	63

It is seen that in the F_1 and F_2 the DDT treatment had inhibited the development of the follicles so greatly that the number of degenerating follicles came to exceed the developing follicles.

Egg-Raft Production. The undissected remainder of those females that had been twice subjected to the sublethal concentration of DDT were tested for their fecundity (Table 15). The figures indicate that DDT treatment caused no greater reduction in oviposition in the first filial generations than in the P generation. However, the F_3 and the F_5 generations showed a marked decrease in oviposition after DDT treatment and completely failed to oviposit in the 2nd gonotrophic cycle. The number of pupae developing from each egg-raft in the 1st gonotrophic cycle steadily decreased in the successive generations.

Table 15. Effect of sublethal exposure of adults to DDT in each generation on the production of egg-rafts and pupal offspring in the Rangoon-DDT strain of C. fatigans

Generation	No. females tested	First gonotrophic cycle			Second gonotrophic cycle		
		No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft	No. Egg-Rafts	Per Cent Oviposition	Pupae per Egg-Raft
P	27	10	37.1	62	17	62.9	59
F_1	91	38	41.8	54	14	15.4	53
F_2	61	24	39.3	50	13	21.3	48
F_3	54	8	14.8	37	0	0	0
F_4	40	16	40.0	48	10	25.0	10
F_5	21	4	19.0	46	0	0	0

Resumé of Effects of Sublethal Exposure on the Three Strains

The sublethal treatments of DDT had been applied to the adults of successive generations from the P to the F_5 in the susceptible strain, from the P to the F_6 in the slightly-tolerant strain, and from the P to the F_4 in the resistant strain. In each generation both the males and females had been exposed to the sublethal dose, and the mated females that took a blood meal were given a second more lengthy exposure to a sublethal level of DDT.

The susceptibility levels of the adult offspring of each treated generation were tested 1 day after their emergence, and those of the males proved to be not significantly different from those of the females. The levels finally attained in the offspring of the last generation to be treated in each strain were as follows:

	Initial	Final
Susceptible Guelph (F_6 finally tested)		
Female LE_{50} (concentration x time)	1.2% for 1 hr.	10.5 hr. on 4%
Larval LC_{50}, p.p.m.	0.002	0.48
Slightly-Tolerant Rangoon-LP (F_7 finally tested)		
Female LT_{50} on 4% papers	21.0 hr.	17.5 hr.
Larval LC_{50}, p.p.m.	0.12	0.33
Resistant Rangoon-DDT (F_5 finally tested)		
Female LT_{50}	23 hr.	52 hr.
Larval LC_{50}, p.p.m.	6.9 hr.	-

The terminal F_6 of the susceptible strain was so much more DDT-tolerant than the P that 1-hour exposure was insufficient to kill them even on 4% papers; they were best compared by tests on the F_6 larvae, which showed them to have become 240 times more resistant

than originally. The terminal F_7 of the slightly-tolerant strain was only slightly less susceptible than the original level in the larval test rates between the two levels being 2.8; in fact this strain had been more susceptible than the original during most of its treated generations. The terminal F_5 of the resistant strain was slightly more than twice as DDT-resistant as the original stock.

The effect of the 2 successive exposures of the mated and fed females on their development of follicles, which was tested in each generation, allowed the F_5 of the susceptible strain and the slightly-tolerant strain to be compared with their P generations; the F_3 was the last generation of the resistant strain that could be so compared. The figures for the developing and degenerating follicles for the treated adults were as follows:

	Susceptible Guelph		Slightly-tolerant Rangoon-LP		Resistant Rangoon-DDT	
	Dev.	Deg.	Dev.	Deg.	Dev.	Deg.
Initial Untreated P	160	10	128	11	105	7
Initial Treated P	105	30	99	26	83	32
Final Treated	(F_5) 157	26	(F_5) 60	49	(F_3) 53	63

It is seen that the susceptible strain had become less susceptible to the follicle-degenerating effect of the DDT, even although the concentration had been progressively increased from 0.25% initially up to 2% for the F_5 females in order to match the increase in the tolerance level.

On the other hand, the slightly-tolerant strain had become more susceptible to the follicle-degenerating effect of the DDT, the exposure period not having been increased beyond the 2 plus 24 hours to 4% papers. The resistant strain had become very much more susceptible to the ovarian effect of the DDT, so that there were more degenerating follicles than developing ones, even although the exposure period had been merely doubled to match the increasing physiological resistance level.

From the percentage of singly-treated females that fed, the percentage of doubly-treated females that oviposited, and the number of F_1 pupae that their egg-raft finally produced (essentially an index of the number of eggs and their hatch rate), it is possible to derive the F_1 production per female for the 1st gonotrophic cycle (Table 16). It is seen that in the slightly-tolerant strain, in which the P generation was stimulated by the DDT to an increased oviposition which compensated for the slight reductions in feeding and in hatch, the F_6 generation was even more stimulated to oviposit although the hatch was further reduced. The percent feeding however was greatly reduced in the F_6, partly due to the increase of the first DDT exposure to 4 hours, but it had already trended towards a reduction in the F_5 exposed for the regular 2 hours (see Table 10). Thus this Rangoon-LP strain, which had not lost much physiological susceptibility, did eventually suffer a considerable reduction in biotic potential.

The susceptible strain had been characterized from the start by a low feeding proclivity and a meagre oviposition rate; this turned out to be increased by the DDT treatment, so that the biotic

Table 16. Net productivity of the treated strains as compared to their original productivity and response to sublethal doses of DDT

	Percent females that fed (1)	Percent fed females that oviposited (2)	Pupae obtained per egg-raft (3)	F_1 pupae per female (1) x (2) x (3)
Susceptible Guelph: 1 hr, then 24 hr on 0.25% DDT				
Untreated P	43.4	45.8	50	9.5
Treated P	60.3	73.0	34	14.9
Treated F_6	42.7	68.4	65	18.9
Slightly-tolerant Rangoon-LP: 2 hr, then 24 hr on 4.0% DDT				
Untreated P	83.8	38.0	111	35.5
Treated P	67.5	51.4	96	33.6
Treated F_6	19.0	77.8	85	12.8
Resistant Rangoon-DDT: 8 hr, then 72 hr on 4.0% DDT				
Untreated P	72.0	66.7	77	36.9
Treated P	66.5	37.1	62	15.5
Treated F_5	13.5	19.0	46	1.2

potential would have been doubled but for a reduction in the hatch rate. By the time the F_6 had been reached, with its increased physiological DDT-tolerance, the increased dose of DDT which it was given raised the biotic potential even more, an improved hatch rate more than compensating for a reduction in percent feeding. Thus this Guelph strain, which gained considerable DDT-tolerance, also steadily improved its biotic potential following the sublethal DDT treatments.

The resistant strain started with good feeding, oviposition and hatch rates, and the only considerable effect of DDT treatment on the P generation was to reduce the oviposition rate. By the time the F_5 had been reached, the first exposure period having been increased from 8 up to 32 hours, this generation suffered a drastic reduction in feeding success. This, coupled with a great loss in oviposition and a reduced hatch rate, resulted in the biotic potential falling to about 1 F_1 pupa per parental female. It was not surprising to find that this Rangoon-DDT strain died out after these high but sublethal treatments with DDT.

Fate of Susceptibility Levels when Sublethal Treatments were Discontinued

The stability of the change in the susceptibility levels induced by the successive sublethal treatments of the susceptible Guelph strain and the slightly-tolerant Rangoon-LP strain was investigated by rearing them for 2 further generations without any sublethal treatment. The resistant Rangoon-DDT strain had declined to so few numbers that its reversion could not be investigated. Egg-rafts

obtained from adults of the last sublethally treated generation (the F_6 or F_7) were set to hatch and a sample of the filial larvae (the F_7 or F_8) were tested for susceptibility levels in their 3rd instar; the rest of the untreated larvae were taken to the adult stage and a sample was tested for susceptibility levels 1 day after emergence. The egg-rafts obtained from the remainder of the untreated adults were hatched and reared to maturity, and the larvae and adults of this untreated generation (the F_8 or F_9) were similarly tested. The results were as follows:

Generation	Larval LC_{50}	Adult Females	
		LC_{50} for 1 hr	LT_{50} on 4% papers
Susceptible Guelph strain			
Original P	0.002 p.p.m.	1.2%	-
Treated F_6	0.48 p.p.m.	-	10.5 hours
Untreated F_7	0.14 p.p.m.	-	4.5 hours
Untreated F_8	0.48 p.p.m.	-	9.5 hours
Slightly-tolerant Rangoon-LP strain			
Original P	0.12 p.p.m.	-	21.0 hours
Treated F_7	0.33 p.p.m.	-	17.5 hours
Untreated F_8	0.12 p.p.m.	-	6.5 hours
Untreated F_9	0.13	-	6.0 hours

It is seen that the slight increase in larval tolerance induced in the slightly-tolerant Rangoon-LP strain completely disappeared in the first untreated generation; the susceptibility levels completely returned to the initial parental levels in the F_8, and remained constant at the original levels in the F_9, the second

untreated generation. On the other hand, the considerable increase in tolerance induced in the susceptible Guelph strain by the successive treatments taken as sublethal proved to persist to a considerable extent in the two untreated filial generations. The susceptibility levels did commence to revert in the first untreated generation, but in the second untreated generation it had returned to the level of increased tolerance.

Effect of Larval Treatment with Sublethal Concentrations of DDT

Initially the Culex larvae were taken 3-4 hours after hatching and exposed thereafter continuously to the sublethal concentration, according to the method used by Sutherland et al. (1967) with larvae of Aedes aegypti. Lots of 25 larvae of the resistant Rangoon-DDT strain were put in jars with 250 ml distilled water, and to them was added 1 ml of 0.00015 p.p.m. DDT, a concentration that gave no higher mortality than the untreated control over a 3-day period. A similar group of larvae was treated with the ethanol solvent alone as a control; and both groups were reared in the treated water until they started to pupate and the adults emerged. The following results were obtained:

Treatment	No. larvae treated	No. resulting pupae	No. emerging adults
Sublethal DDT	75	35	20
Ethanol Control	56	42	19

They indicate that the effect of the ethanol solvent for the DDT on the newly-hatched larvae was to reduce the pupation success and even further reduce the emergence rate. It also greatly delayed the larval development, since pupation did not start in either group until at least 32 days had elapsed, instead of the normal

4-8-day larval period.

Accordingly in order to investigate the effects of the sublethal treatment, 3rd-instar larvae of the slightly tolerant Rangoon-LP and the resistant Rangoon-DDT strains were tested for their DDT-susceptibility level according to the standard WHO method for mosquito larvae. Since the concentrations that gave insignificantly higher mortality than the untreated controls were 0.01 p.p.m. for the slightly-tolerant strain and 0.64 p.p.m. for the resistant strain, these concentrations were taken to constitute the sublethal exposure for each strain. Groups of larvae in the 3rd instar were treated with these sublethal concentrations, while groups in untreated water and in water treated with the ethanol solvent alone served as controls. Following the 24-hour exposure period, the treated larvae were rinsed with water and transferred to clean water in beakers so that the pupae obtained could be counted as well as the adults emerging from each group. The results obtained were as follows:

Treatment	Number Tested	% Larval Mortality	Per Cent Pupation	Per Cent Emergence
Slightly-tolerant Rangoon-LP				
Untreated Control	200	1.0	85.0	100.0
Ethanol Control	200	1.0	83.0	100.0
Sublethal DDT	400	2.0	73.9	93.1
Resistant Rangoon-DDT				
Untreated Control	200	1.0	95.0	82.5
Ethanol Control	200	1.5	96.9	80.6
Sublethal DDT	398	2.8	80.9	86.1

It is seen that these DDT concentrations that had not caused a significant 24-hour larval mortality did induce a reduction in emergence and pupation as compared to the untreated and the ethanol controls. Both DDT-treated strains showed a reduction of at least 10% in the pupation rate due to the after-effect of the DDT treatment. The slightly-tolerant strain also suffered a reduction in the emergence rate, but the resistant strain showed an apparent increase of 5% in the emergence rate from the treated larvae as compared to the ethanol-treated controls.

The adults that emerged from each treated group were put in a separate cage and fed on sugar solution only for 3 days; during this period the treated females mated with the treated males. Then samples of females were taken and dissected and the number of basal follicles in their ovaries was determined. The results were as follows:

Treatment	No. females dissected	No. basal follicles per female
Rangoon-LP		
Untreated Control	8	173
Ethanol Control	12	157
Sublethal DDT	9	201
Rangoon-DDT		
Untreated Control	8	214
Ethanol Control	8	206
Sublethal DDT	10	254

It is evident that the exposure of larvae to DDT added in ethanol

to the water induced the development of a considerably increased number of basal follicles in both of these strains. The increase was especially marked in the Rangoon-DDT strain, and it was reflected in the larger number of eggs in each raft; the average was 118 eggs in each raft from untreated control females, and was 162 per egg-raft from females sublethally treated with DDT when in the larval stage. It may be also noted that ethanol alone caused reductions of 4% and 9% in the number of basal follicles, in contrast to its lack of effect on the pupation and emergence rates. In these experiments the dissection was made before the adults were fed on blood and therefore their follicles could not be categorized into developing and degenerating, and the count of basal follicles at this time included those which never reached the stage where they could degenerate.

The undissected remainder of the groups of females were offered a blood meal on a mouse and those that succeeded in feeding were counted; the egg-rafts that they produced were also counted, and the larvae hatched from them were taken to the pupal stage and then counted. The results were as follows:

Treatment	Percent females that fed	Percent fed females that oviposited	Pupae obtained per egg-raft
Rangoon-LP			
Untreated Control	72.0	66.2	181
Ethanol Control	70.0	64.3	176
Sublethal DDT	63.8	55.7	97
Rangoon-DDT			
Untreated Control	83.0	59.2	88
Ethanol Control	80.8	57.9	81
Sublethal DDT	44.3	52.5	37

The figures show that the sublethal treatment of the larvae with DDT had resulted in considerable reduction in the number of F_1 pupae obtained from the eggs they have laid after emergence and mating with similarly-treated males. This reduction, due almost entirely to decreased hatch of larvae from the egg-rafts, amounted to 45% in the slightly-tolerant strain and 54% in the DDT-resistant strain. On the other hand, the reduction in per cent oviposition was less than 10% in either strain. The reduction in feeding proclivity was also modest in the slightly-tolerant, but it was considerable in the resistant strain. The ethanol controls caused little reductions as compared to the untreated controls.

Incorporation of DDT into the Ovaries from Sublethal Treatments

Samples of the strains before and after the period of treatments were taken after an exposure to the sublethal dose of DDT and 1 microlitre of the ovarian extracts was analysed in the Pestilyzer. The peak areas registered on the recorder charts at different attenuation periods were measured by triangulation and then compared with the peak shown by a similar known amount of DDT and DDE from standard solutions.

The results (Table 17) show that the DDT was incorporated in the ovaries whether the sublethal concentration had been applied to the adults or to the larvae. The amounts of DDT absorbed by the ovaries were very much the same in the 3 strains, only the uptake was twice as great when the larvae were treated.

On the other hand, the amounts of DDE found in the ovaries differed from one strain to the other; whereas in both the susceptible and the slightly-tolerant strains no traces were detected in the initial P generation, the DDE started to appear in the terminal treated generations, and as expected it was produced quite abundantly by the resistant strain. However the ovaries of the 3 strains did not show any traces of DDE when the DDT treatment had been applied to the larval stage.

Table 17. Content of DDT absorbed by the pair of ovaries from sublethal exposures of the females to DDT in the adult or in the larval stage; 1-microlitre samples, attenuation setting 16

Strain and Generation	Type of Sublethal Exposure	No. females per sample	Determination per sample		DDT per female picograms	DDE per female picograms
			Peak Area Units	DDT picograms		
-	Standard 0.1 p.p.m.	-	288	100	-	-
Susceptible P	Adult, 0.25% DDT 1, 24h.	13	150	52	4.0	0
Susceptible F_5	Adult, 2.0% DDT 1, 24h.	12	111	38	3.2	0.6
Sl.-Tolerant P	Adult, 4.0% DDT 4, 24h.	10	163	57	5.7	0
Sl.-Tolerant F_7	Adult, 4.0% DDT 4, 24h.	12	158	55	4.6	0.7
Resistant P	Adult, 4.0% DDT 8, 72h.	13	215	75	5.7	1.4
Suscep. larvae	Larval, 0.0006 p.p.m. DDT	10	273	95	9.5	0
Sl.-Tolerant "	Larval, 0.01 p.p.m. DDT	10	297	103	10.3	0
Resistant larvae	Larval, 0.64 p.p.m. DDT	10	178	62	6.2	0

DISCUSSION

The experience with the slightly-tolerant Rangoon-LP strain showed that successive sublethal exposures to DDT in each generation did not induce the development of further tolerance. Here the duration of exposure was maintained identical in each generation, and all the first 5 filial generations turned out to be considerably more susceptible than the treated P generation; only in the F_6 and F_7 did the LT_{50} levels rise above the starting level. This initial increase in susceptibility is consistent with the results of Lineva (1955) who observed a similar increase to occur over a number of generations of house flies reared under the effect of sublethal concentrations of DDT. Derbeneva-Uhova et al. (1966) recorded that a population of house flies in a village annually sprayed with DDT showed a slight increase in susceptibility during the initial 2 or 3 years, a period when disturbances in oogenesis were prevalent; and Lineva (1966) reported similar results in a field population of house flies annually sprayed with chlorophos (Dipterex).

This slightly-tolerant strain was the most susceptible of the 3 strains to the inhibition of feeding by the sublethal exposure to DDT; the reduction was 16% in the P generation and as much as 55% and 64% in the F_5 and the F_6 generations respectively.

The principal effect of the sublethal exposure to DDT was to increase the degeneration of follicles. When the first generation was submitted to DDT, the number of degenerating follicles was double the normal, and the number of developing follicles was reduced by 23 per cent. Subsequently, the treated filial generations did not show any further decrease in the number of developing follicles until there was a transitory decrease in the F_5 only, and this was reflected in a lower production of pupal offspring. In the F_6 however, which was the generation where the strain lost its transitory susceptibility; the developing follicles recovered their numbers. Lineva (1955) had observed that it was when successive generations of house flies became more resistant that the disturbances proceeded to disappear.

It is strange that the susceptible Guelph strain developed tolerance to DDT so steadily. It could be suspected that the initial dose applied to the P generation was not truly sublethal, since it caused 4-8% mortality as against 1-2% in the untreated controls; but this difference was not statistically significant, and the 2nd-exposure doses applied to the subsequent generations were accompanied by mortalities of 0-6%, as compared with 3-9% in the slightly-tolerant strain which did not lose its susceptibility.

After 7 successive generations, of sublethal treatment, this strain developed a tolerance comparable to that of the slightly-tolerant strain, which it did not lose when continued for 2 generations untreated by DDT. This result suggests that the change in the strain was not postadaptive; moreover in contrast to the finding of Beard (1965) that when susceptible house flies were fed sublethal concentrations

of DDT their progeny were 10 times as tolerant as those of untreated flies, the increase in tolerance in these mosquitoes was less abrupt and proceeded regularly from one generation to the next. By the time that the F_6 had been reached, the larval dosage-mortality results indicated that fully 44% of the individuals survived the concentration of 0.64 p.p.m. which is the diagnostic dosage separating the heterozygotes for the main DDT-resistance gene from the homozygotes for the susceptible allele. Evidently, some degree of selection has changed the genotype of the strain towards increased tolerance, but since the selection intensity was insignificant in terms of mortality of the treated adults, it seems doubtful that adult kills were the selective agent favouring the survival of the more tolerant individuals.

This strain, unusually susceptible for Culex, started with a very low biotic potential, about one-quarter that of the other 2 strains; presumably only the most susceptible genotypes were being reproduced. When treated with DDT, the biotic potential immediately increased in that very generation, although the low number of pupae obtained per egg-raft indicated much selection in the egg. Eddy et al. (1955) had found that sublethal doses of DDT that did not kill body lice but only reduced their egg production were selective and induced a high resistance eventually. As the generations proceeded in these mosquitoes, the follicle-degenerating effect of the DDT steadily decreased, suggesting that the follicles that were successful gave progeny that could achieve a higher degree of success. This trend was accompanied by an increase in the number of eggs per raft that successfully developed into pupae, so that the net biotic

potential picked up even more. This ovarian suppression therefore, which had amounted to a decrease in the pupae per egg-raft of 32% in the first gonotrophic cycle and 50% in the second cycle, may have been the selective power which had induced the increase in tolerance by eliminating the weaker genotypes. Indeed Milani (1963) had pointed out that selective pressure is that given by the proportion of insects in the population that will not contribute to the next generation, whether mortality is involved or not.

The resistant Rangoon-DDT strain, exposed to sublethal doses that caused no more than 5% mortality, more than doubled its adult LT_{50} in the first 2 filial generations, and thereafter remained slightly below this increased resistance level. It was quite susceptible to the follicle-degenerating effect of the DDT at the start, and this susceptibility increased so that by the F_3 the treated females developed fewer follicles than those that degenerated. Whereas at the start they showed a high feeding success, oviposition and successful hatch under DDT treatment, all of these characteristics diminished so much that the biotic potential of the F_5 was only about 1 pupal offspring per female. In this generation and in the F_3 the second gonotrophic cycle failed to produce a single egg-raft among the 401 females (186 F_3 and 215 F_5) originally tested.

The further increase in resistance when this resistant strain was initially exposed to the high but non-killing doses of DDT may be connected with the fact that the proportion of females that laid eggs was reduced to only 40%, a reduction in percent oviposition considerably greater than in the other two strains. This would introduce a selective effect in that the successful mothers had not

only to survive the DDT but also be sufficiently unaffected by the long exposure (72 hours) to DDT that they could still lay eggs. But eventually, the price paid for this resistance is the extensive degeneration of follicles in the ovaries and a negligible egg-hatch. It had been reported by Beard (1965) that a highly DDT-resistant strain of house flies which he successfully reared in media containing 200 p.p.m. DDT paid the price by laying only 13% as many eggs as the strain not reared in DDT.

The resistant strain after it had been treated for 6 successive generations laid almost as many eggs as the initial stock when not treated, as the following figures, taken from small numbers of egg-rafts, show:

	No. Rafts Examined	Eggs/Raft	Larvae/Raft
Susceptible Guelph			
Untreated P	4	134	87
Treated F_6	4	127	57
Slightly-tolerant Rangoon-LP			
Untreated P	9	142	125
Treated F_4	4	123	108
Resistant Rangoon-DDT			
Untreated P	3	118	101
Treated F_6	3	98	40

It may be noted that the slightly tolerant strain, treated for 5 successive generations, showed little suppression in hatch upon DDT treatment. On the other hand, the susceptible strain showed

an intermediate suppression in hatch when the mothers were submitted to DDT at a sublethal level.

The treatment of larvae of the 2 strains of Culex p. fatigans with sublethal dosages of DDT applied in the 3rd instar continued what Sutherland et al. (1967) had found with Aedes aegypti treated in the 1st instar, namely that there was an increase in the number of basal follicles developed in the emerging female. This increase was of the order of 20% in both strains of Culex, as compared to the average of 9% which these authors obtained with a susceptible strain of A. aegypti. Despite this increase in basal follicles, the number of eggs in each raft that successfully developed into pupae was reduced to about 50% of the normal untreated figure, this reduction being less than those previously observed on exposure of the adults instead of the larvae to the sublethal dose of DDT.

All the adult treatments on all 3 strains of Culex show that sublethal levels of DDT increase the number of degenerating follicles while decreasing the number of developing follicles. In the 2 strains of C. p. fatigans they also always reduce the feeding proclivity, oviposition success and pupal production per egg-raft, in other words the biotic potential in toto. The slightly-tolerant strain was not changed permanently in its susceptibility level by such successive treatments, and the progressive loss in biotic potential was due mainly to a reduction in the ability to take a blood meal after DDT exposure, as well as to a progressive increase in the number of degenerating follicles. The resistant strain, while increasing somewhat in resistance as a consequence of these

lengthy sublethal exposures to DDT, lost all its elements of biotic potential to such an extent that it became unable to maintain its numbers. On the other hand, the susceptible strain had its feeding and oviposition rate actually stimulated by the slight sublethal exposure, and steadily lost its unusually marked susceptibility; its liability to degenerate its follicles due to the DDT actually decreased over the generations, and its pupal production per egg-raft actually increased, so that after 6 generations of sublethal treatment it had become a reasonably tolerant strain with a greatly improved biotic potential.

This strain also, like the slightly-tolerant strain, eventually developed the ability to detoxify DDT and lay down DDE in the ovaries, a conversion which the resistant strain strongly effected right from the start. The results obtained with the electron-capture assay of DDT confirm the finding of Kimura et al. (1965) that DDE is the principal metabolite of DDT in Culex mosquitoes. They also demonstrate that the developing ova (follicles) are under DDT selection pressure in the ovaries. In houseflies, Beard (1965) was able to follow the radioactivity from C^{14}-DDT ingested by the adults into their eggs and the F_1 pupae. Our results with Culex suggest that the reason that the susceptible strain became less sensitive to the follicle-degenerating effect of DDT, and became physiologically more DDT-resistant, was the development of the ability to detoxify DDT to DDE.

SUMMARY AND CONCLUSIONS

The effect of exposure of 1-day-old Culex females to the maximal dose of DDT that is sublethal to all is a reduction in the proportion of follicles that develop fully, not in the total amount of follicles; this is due to the percentage of degenerating follicles in the ovary, which is normally 6-8% in the 3 strains studied, being increased to 21-28%. The number of progeny obtained from the egg-raft was therefore reduced by 14-32% in the 3 strains.

The sublethal exposure in the adult stage also decreased the percentage of females that took a blood meal in the resistant and slightly-tolerant strains. In the resistant strain but not in the tolerant strain it decreased the number of engorged females that oviposited. In the susceptible strain, in which the percentage of feeding and oviposition was characteristically low, the sublethal exposure increased the percentages of females that fed and that oviposited by so much that the net production of offspring (the biotic potential) was increased despite the reduction in progeny per individual egg-raft.

Exposure to sublethal doses in the 3rd-instar larval stage actually increased the number of basal follicles (ovarioles) that the female developed by 18-19% in the 2 strains (resistant and slightly-tolerant) studied. But from the eggs laid only 46-55%

as many offspring were produced as from eggs from females untreated in the larval stage. The sublethal larval exposure also slightly reduced the pupation, emergence and oviposition rates, and reduced the feeding rate moderately in the slightly-resistant strain and greatly in the resistant strain.

When the 3 strains had been exposed to truly sublethal adult doses for 6-7 successive generations, the percentage of follicles that degenerated following exposure had changed; in the slightly-tolerant and resistant strains it had risen to 45-54%, but in the susceptible strain it had fallen to only 14%. In the 2 former strains the progeny per egg-raft had been correspondingly reduced as also was the feeding rate; a rise in oviposition rate saved the biotic potential in the slightly-tolerant strain from the virtual extinction that it suffered in the resistant strain. In the susceptible strain after the sublethal exposures in each generation the progeny per egg-raft had come to be increased, in agreement with the fewer degenerated follicles observed; this was enough to ensure a net increase in biotic potential after a sublethal exposure over the original stock similarly exposed.

During the 7 generations of successive treatments, the susceptible strain became appreciably resistant, so that approximately half of their members were heterozygotes for the DDT-resistance gene. It is concluded that this genotypic change was due to hidden selection not in terms of mortality of the female mosquitoes, but in degeneration of their follicles and in non-hatch of the eggs laid, as well

as in the choice of those that fed and oviposited. The role of DDT in working on the egg-follicles was proved by the demonstration of this insecticide in the ovaries of the treated females, and the development of the ability to detoxify it to DDE as the susceptible strain gained a measure of resistance. On the other hand, in the slightly-tolerant strain the 7 generations of successive treatments did not make it any more DDT-tolerant until the last 2 generations, and the tolerance disappeared immediately after the treatment was discontinued. It is the experience with this strain which proved that post-adaptation, as opposed to selective mortality of pre-adaptations, is not the cause of insecticide resistance in this species.

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