DEVELOPMENT OF RESISTANCE TO DDT BY ANOPHELES SACHAROVI IN GREECE

by

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House spraying with DDT, as shown from its first experimental applications in Greece in 1945, as well as from its subsequent general application throughout the entire country between 1946 and 1950, is highly effective against the local malaria-vectors (Anopheles sacharovi and A. superpictus) and other domestic pests.

During these years, anopheline catches within sprayed quarters were, as a rule, negative from the day following the spraying and remained so during the entire breeding season of these insects (May to October). Occasionally, a few specimens were caught within sprayed quarters, especially during the early mornings; these anophelines showed obvious toxic symptoms (kinetic ataxia, convulsions, etc.) and died a few hours after being caught. On the other hand, it was noted that anopheline density in the few premises left unsprayed (control stations) in each treated village was usually low and that, not infrequently, - despite some fluctuations - complete annihilation of the
anophelines was attained. The immediate consequence was the rapid decline of malaria incidence in Greece to a point when malaria transmission was almost completely checked.

However, during the second year of the nationwide application of DDT by house spraying, the housefly (Musca domestica) — which, in the previous year, had completely disappeared from sprayed quarters — reappeared in high density, being entirely unaffected by the insecticide. During the following years the same phenomenon repeatedly appeared in the case of other domestic pests (Culex molestus, fleas, bed-bugs, cockroaches, etc.). However, until the end of the 1950 malaria season there was not the slightest indication of the presence of a similar situation with regard to anopheline mosquitoes. During the 1951 malaria season, signs of the lower efficacy of DDT against A. sacharovi were observed for the first time in some areas of the Peloponnese. This fact was reported by the senior writer in a brief report submitted by him in October 1951 to the Malaria Section of the World Health Organization. The present paper includes more recent observations made from July to September 1952 in certain areas, with particular reference to Skála, Lakonia, in the Peloponnese.

In various villages sprayed with DDT during 1952, a weakening of DDT efficacy on anophelines was observed. In the villages of the Mórnos area of Návpaktos, where systematic daily catches were made, it was observed that catches within treated quarters remained negative for 6 to 12 days after spraying. Subsequently, a few specimens of A. sacharovi appeared; and about four weeks after the spraying, the number of mosquitoes of this species caught daily within sprayed quarters ranged from 45 to 106 per ten catching-stations. It should be noted that this anopheline density was markedly lower than that observed in the same villages before spraying, or the density observed during the same period in unsprayed villages of the area.

Observations in Skála, Lakonia (Peloponnese)

The Skála area is located near the outlet of the river Evrótas and is approximately 45 kilometres (28 miles) from Sparta. It includes 13 villages with a total population of 7,182 (see fig. 1). The population is mainly engaged in rice cultivation, which has assumed great importance in that area during recent years, and in the cultivation of cotton.
Before the DDT-spraying operation malaria was widely prevalent in this area. Table I shows the fluctuations which occurred in the spleen- and parasite rates of schoolchildren during the years 1933-8; these figures can be considered as representative of conditions throughout the whole area.

After the application of DDT house-spraying in 1946, malaria rapidly declined and the parasite-rates of the infant and school age-groups in the village of Skála dropped to zero.

Early in 1951, difficulties involved in connexion with the procurement of the necessary quantity of insecticides made a reduction in the extended spraying-programme previously applied in Greece advisable. It was thus decided that two districts - namely, Peloponnese and Crete - should, in principle, be excluded from the house-spraying programme and that spraying should be carried out only in areas where careful epidemiological investigations had shown it necessary to take immediate suppressive measures. Accordingly, house spraying with DDT was not applied to the Skála area at first. A little later - about the middle of the 1951 malaria season - the high anopheline density observed in that area resulting from the too highly developed rice-cultivation suggested that house spraying should be resumed. Nevertheless, it was observed that, in spite of the spraying, the anopheline density in treated premises continued to be unusually high.

The Skála area therefore presented appropriate conditions for the carrying out of more detailed investigations during 1952. For this purpose, two observation stations with field laboratories were established - one in the village of Asterion and the second in the village of Souli. Of all the villages in the area only in Asterion was a systematic DDT house-spraying programme carried out in 1952. The personnel of the stations consisted of two laboratory assistants (a) and three insect-collectors who were provided with a jeep. The supervision of the work done in these two stations was made successively by the writers. It should be noted here that transport difficulties, the improvised nature of the installations, and the lack of various

(a) Special mention should be made here of the co-operation shown throughout the period of observation by the laboratory assistants, Mr. J. Petrides and Mr. F. Terzis.
facilities had some effect on the progress of the work and, to some extent, prevented its integration into the originally planned programme. Additional difficulties arose from the action of a service of the Ministry of Agriculture which, in compliance with outside pressure, resumed, at various intervals during the year, DDT air-spraying of the rice-fields in the Skala area. However, our observations do not seem to have been seriously affected by this activity.

Observations in the village of Asterion

During the period 2 – 4 July 1951, the houses in the village were sprayed with DDT emulsion. A dose of 2.0 g active ingredient per square metre of surface was used. Two premises were left unsprayed as control stations. The village had thus been sprayed for the seventh time since 1946.

1. Two days after spraying, catches made within the sprayed quarters proved positive for *A. sacharovi* and in the ten stations searched, 110 specimens of this species were found. Then followed a progressive rise of anopheline density in the sprayed catching-stations, about a month after the spraying, anopheline density in the catching centres approached the level of density observed in the unsprayed catching-stations (1,200 – 1,500 anopheline per 10 stations).

About the middle of September the natural seasonal decline of anopheline density was noted in both the sprayed and unsprayed stations.

2. During the catches made every morning in the sprayed quarters of the village for the collection of entomological material to be used for carrying out the proposed tests, some dead specimens of *A. sacharovi* were found on the floors and furniture. Of the living mosquitos, some presented advanced toxic symptoms, moving continually from place to place, while others (20% - 30%), were seen calmly resting on the sprayed surfaces with no sign whatever of poisoning.

3. The *A. sacharovi* caught\(^{(b)}\) in sucking tubes during the daily search in sprayed and unsprayed quarters were transferred into large wire cages, within which a high

\(^{(b)}\) Catching included all mosquitos found, irrespective of whether or not they showed signs of poisoning.
relative humidity was maintained by placing wet pieces of cloth on the cage walls. The time mosquitos stayed within these cages was 72 hours. At the end of each 24-hour period the dead specimens found in the cages were withdrawn and the number recorded on a card. The following observations are made on the results of this test which lasted from 6 July to 3 October 1952 (see table II):

(a) After the first 10-day observation period it was noted that a large number of the mosquitos caught in sprayed quarters continued to live longer than 24 hours. The survival-rate was still considerable after a third 24-hour period, and showed an obvious upward tendency as the date of spraying became more and more remote (see figs. 2, 3, and 4).

(b) The same remarks apply to the mosquitos caught in unsprayed quarters except that, in their case, the survival-rate, was on the whole higher (see figs. 2, 3, and 4).

Of the 12,158 A. sacharovi caught in sprayed quarters in Asterion, 9,857 (81.1%) survived after the first 24-hour period, 8,771 (72.1%) after the second 24-hour period, and 7,545 (62%) after the third 24-hour period.

4. A small container of water was placed in the cages into which A. sacharovi caught in sprayed places of the village had been introduced. The laying of eggs and the hatching of larvae from them was frequently observed but no follow-up was possible owing to lack of means and shortage of time.

5. A number of the A. sacharovi caught in sprayed quarters of Asterion village were placed in clean cages. After 72 hours, the surviving mosquitos were transferred, at intervals, to one or the other of two special contact-boxes. (c) One of these boxes had been sprayed with DDT emulsion at the rate of 2 g active ingredient per square metre of surface on 1 August 1952; the other was left unsprayed and was used as a

(c) These special contact-boxes were first used by Hadjinicolou for his experiments in the control of Dacus fly. These boxes are made of 5-mm-thick wood, and consist of six separate sides hinged together so that the box can be opened and closed very easily. Mosquitos introduced into this box come into contact with the sprayed interior surfaces. When the contact time has expired, the closed box is placed in a clean cage which has an opening large enough to allow the passage of the hand placing the contact box in the cage. The contact box is then opened inside the cage, the mosquitos enter it, and the contact box is withdrawn.
control. The mosquitoes were left in the boxes for 30 minutes and were then placed in two large clean cages.

It will be seen from table III, which summarizes the results of this test, that, of the 1,194 mosquitoes which had had renewed contact with the insecticide, 28.4% survived after 24 hours, 21.3% survived after 48 hours, and 18.3% survived after 72 hours. Of the 808 control mosquitoes, 81.5% survived after 24 hours, 63.9% after 48 hours, and 54.3% after 72 hours. It should be noted that the mosquitoes surviving this test remained without food for six 24-hour periods owing to the impossibility of feeding them.

6. A number of A. sacharovi, which had been hatched in the laboratory from larvae collected from breeding-places in the Skála area, were placed either in the contact box sprayed on 1 August or in the unsprayed control-box. The mosquitoes were left in the boxes for 30 minutes and were then transferred to large clean cages. As shown in table IV, of the 231 mosquitoes that came into contact with the insecticide, 52.4% survived after 24 hours, 41.1% after 48 hours, and 33.2% after 72 hours. Of the 199 control mosquitoes, 91.9% survived after 24 hours, 75.4% after 48 hours, and 68.3% after 72 hours. For the reason stated earlier, these mosquitoes were not fed after their hatching.

Observations in the village of Souli

This village had been sprayed annually since 1946, but was left unsprayed during 1952.

1. On 27 June 1952 five contact-boxes were sprayed in the Souli laboratory each with one of the following insecticides:

(a) DDT solution in kerosene, at the rate of 2 g active ingredient per square metre of surface;

(b) DDT emulsion concentrate containing 25% DDT, at the rate of 2 g active ingredient per square metre of surface;

(c) Gammaxane L.G. 140 (10%), at the rate of 250 mg active ingredient per sq. metre.
(d) Chlordane 74%, at the rate of 150 mg per square metre of surface;

(e) Dieldrin 24%, at the rate of 250 mg per square metre of surface.

The daily catches of *A. sacharovi* were placed in the contact boxes. After 30 minutes the mosquitoes were transferred to large clean cages. Twenty-four hours later the dead specimens found in each cage were counted. As in the tests described above, an unsprayed box was used as control. The results of each test were recorded on a special card.

Table V summarizes by 10-day periods the results of the test, which lasted from 1 July to 10 October 1952. The following conclusions are drawn:

(a) All the mosquitoes placed in the boxes sprayed with Chlordane and Dieldrin died 24 hours after being transferred to the cages.

(b) During the first 10-day period of the observations almost all of the mosquitoes that had been placed in the box sprayed with DDT solution died after 24 hours. However, as the experiment proceeded the number of mosquitoes surviving more than 24 hours increased considerably.

(c) A considerable number of the mosquitoes which had been placed in the box sprayed with DDT emulsion survived during the first 10-day observation period. The results of each succeeding 10-day period showed that the percentage of these mosquitoes surviving had an obvious upward tendency which increased steadily as the date of spraying the contact boxes became more and more remote. During the entire observation period this percentage was steadily higher than that observed in the mosquitoes which had had contact with DDT solution.

(d) The mosquitoes that had been placed in the box sprayed with Gammaxane had a higher survival-rate than the mosquitoes which had contact with either DDT solution or DDT emulsion and, from the fourth 10-day period, this percentage approached the survival-rate observed in the mosquitoes of the control box (see fig. 5). It should be noted that the area in which these observations were made had never been sprayed with Gammaxane.

2. For the sake of brevity, male and female mosquitoes are not shown separately in the
tables. It is noteworthy, however, that in all the tests the effect of the insecticides on male mosquitoes was decidedly greater than on the female mosquitoes.

CONCLUSIONS

The observations made this year in the Móros area (Návpaktos) indicate that the effect of DDT on the local malaria-vector, although presenting signs of weakening, is, however, still sufficiently strong. On the other hand, from the observations made in the Skála area, (Lakonia), it appears that the effect of DDT on the malaria vector of that area has considerably reduced.

The catching of a number of A. sacharovi within quarters sprayed with DDT two days after the spraying of Asterion village, the progressive increase in the numbers found during the following days, and the absence of toxic signs in a considerable number of these mosquitoes are all in complete contrast with observations made in Greece during the years 1946-50. The high survival-rate observed among the mosquitoes captured within sprayed quarters of Asterion village, as well as the results of other tests made in the Asterion laboratory, and in the village of Souli (which remained unsprayed during 1952) leave no doubt that the local malaria-vector has developed a considerable degree of resistance to DDT, and it seems even in a higher degree, to Gammexane, although this insecticide had never been used in the area.

The high survival-rate observed during the last phases of the test among mosquitoes that had had contact with Gammexane - which approached the rate observed among the mosquitoes in the control cage - is possibly associated with the shorter residual action of this insecticide.

The resistance developed by A. sacharovi in Skála should be mainly attributed to the systematically applied house-spraying in that area since 1946. However, the possibility cannot be excluded that antilarval airspraying applied concurrently with the house-spraying has also contributed to this effect. Airspraying, although irregularly applied in the area during the previous five-year period, probably accelerated in some measure the development of this phenomenon.
REFERENCES


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**Table I.** Spleen and parasite rates among schoolchildren in Skaļa, 1933-1938.

**Tableau I.** INDICE SPLENIQUE ET INDICE PARASITAIRE CHEZ LES ECOLIERS DE SKALIA, 1933-1938.
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**Notes:**
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<td>24 heures</td>
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FIG. 2

Asterion 2nd 24 hours period
Astérian : Deuxième période de 24 heures

Average % of surviving A. sacharovi

Unsprayed quarters
Secteurs non traités
Sprayed quarters
Secteurs traités

Days after spray
Nombre de jours écoulés depuis le traitement

July | August | September | October
0 10 20 30 40 50 60 70 80 90 100
FIG. 4

Asterion 3rd 24 hours period
Astérian : Troisième période de 24 heures

Average % of surviving A. Sacharovi

Days after spray
Nombre de jours écoulés depuis le traitement

Unsprayed quarters
Secteurs non traités
Sprayed quarters
Secteurs traités
FIG. 5

Average % of surviving A. sacharovi

Control box
Cage de contrôle

Gammexane 10% : 150 mg per sq m
Gammexane à 10% : 150 mg per m²

DDT emulsion 2.0 per sq m
Emulsion de DDT : 2.0 g per m²

DDT solution in Kerosene 2.0 g per sq m
Solution de DDT dans kérosène : 2.0 g per m²

Chlordan 74% 150 mg per sq m
Chlordan à 74% : 150 mg per m²

Dieldrin 24% 200 mg per sq m
Dieldrine à 24% : 200 mg per m²

July August September

Days after spray
Nombre de jours écoulés depuis le traitement