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The Chief of the Malaria Section
has the honour to communicate hereunder a note on
THE SUSCEPTIBILITY OF GREEK MALARIA VECTORS TO DDT*

Laboratory Tests

by ✓

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The first signs of development of resistance by A. sacharovi appeared in Greece in 1951, that is, six years after the commencement of the nation-wide application of the above insecticide (Livadas (1)). In the next year, the development of this phenomenon in the adult stage of A. sacharovi was experimentally proved in the area of Scala-Peloponnese (Livadas and Georgopoulos (2)).

Recently, Garrett-Jones and Gramiccia (3) reported that A. sacharovi showed resistance also in two villages of Lebanon.

Cases of development of resistance by other anopheline species were reported for A. quadrimaculatus (aquatic stage) in the United States of America (Krusé and associates) (4), and quite recently for A. sudaicus in some areas of Java (personal communications of Van Goor and Issaris). From further epidemiological investigations made in numerous areas of Greece during the period of 1952-1954 (Livadas) (5,6,7,8), the following evidence was evolved:

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(1) It was ascertained epidemiologically that A. sacharovi developed resistance to DDT, not only in Scala (Peloponnese) but also in other areas of the country, where the extended residual spray applications with DDT had not resulted in the eradication of that species. In such cases, the responsible service of the Ministry of Health tried the repetition of the house spray twice or three times in the transmission season or the replacement of DDT by other chlorinated insecticides (Chlordane, Gammexane, Lindane, Dieldrin).

(2) The effect of these insecticides also, especially the first three, soon showed signs of weakening.

(3) The two other local vectors (A. superpictus and A. maculipennis) also showed, in some areas, indications of development of some degree of resistance to chlorinated hydrocarbons.

(4) In some areas the development of resistance by the local vector to residual insecticides was not followed by any marked increase of malaria cases, in spite of the observed obvious increase of vector density within the sprayed quarters, while in others, it resulted in an increase of cases, which, in some instances (1954) assumed epidemic proportions (Livadas (9) and (10)). The explanation of the first case is very probably to be found in the absence of important local sources of infection.

(5) The observed limited local epidemic outbreaks were due exclusively to P. vivax, although a few sporadic cases of P. falciparum were detected.

In view of the situation thus created, we worked out a programme of wider investigation aiming at a more accurate evaluation of the susceptibility of Greek anopheline species to chlorinated insecticides by using the method of the topical application of microdoses of these insecticides and the Busvine and Nash method. This investigation, started in the autumn of 1953 and continued during the 1954 period of transmission, included, initially, the measuring of the susceptibility of A. sacharovi and A. maculipennis to DDT. For this purpose, the following areas were chosen (see map):

(1) Scala area (villages of Elos and Asterion), where the development of resistance by A. sacharovi (2) was proved experimentally for the first time. Of the above villages, Elos was sprayed with DDT* continuously from 1946 to 1951. In 1952 it was sprayed with chlordane, in 1953 it remained unsprayed and in 1954 it was again sprayed with DDT. The other village of Asterion was sprayed without interruption from 1946 to 1954 with DDT. It should be noted that, besides house spray, airspray of breeding places with DDT was also carried out in Scala area from 1946 to 1951. In the years 1952 and 1954, for the airspray work, apart from DDT, chlordane was also used.

(2) Agoulinitza (Elia) area: In this area, as indicated by the epidemiological investigations made recently, A. sacharovi had developed clear resistance, not only to DDT but also to Chlordane and Gammexane. Agoulinitza had been sprayed with DDT for three successive years (1946-1948), in 1949 it remained unsprayed, and DDT house spray was resumed in it during the following three years (1950-1952). In 1953, Agoulinitza remained unsprayed and in 1954 it was sprayed with chlordane and gammexane. In the breeding places of Agoulinitza area airspray was also applied from 1946 to 1953 with DDT and in 1954 with DDT and chlordane.

(3) Georgioupolis (Crete) area: The prevailing species in that area is A. maculipennis. DDT house spray was done there from 1947 for 4 consecutive years. From 1951 to date Georgioupolis remained unsprayed. It should be noted however that in the said area also, just as in other areas of Greece where house spray was discontinued, the farmers, in order to get rid of the nuisance of domestic insects, are using, to a varying extent, the chlorinated insecticides furnished by the Agricultural Bank of Greece for the control of agriculturally significant insects.

TECHNIQUE USED IN INVESTIGATING THE SUSCEPTIBILITY OF MALARIA VECTORS TO DDT

Topical application to adults with different solutions of DDT under field conditions

By means of a common sucking tube, anopheline mosquitos were collected in their natural shelters. Immediately after their capture, they were carried in clean wire-mesh cages to the field laboratory, all necessary precautions being taken to

* This DDT house spraying was done once in a year with 1.80 - 2.0 g DDT per square metre of surface.

avoid mishandling (covering the cage with a wet cloth, etc.). In the laboratory, the mosquitos were taken out of the cages by a sucking tube and, after being immobilized with chloroform, they were placed by means of a pair of forceps in a suitable position to receive the solution of technical DDT arranged for the test.

As solvent ethylated alcohol was used. The preparation of DDT ablutions was made locally from a 1% stock solution.

Topical application was made on the back of the thorax of each mosquito of a particular DDT solution by means of a calibrated microloop 0.24 mm^3 .^{*} After each application, the microloop was cleaned by dipping it in acetone so as to remove any residue of the insecticide that might have remained on it.

The mosquitos treated by each solution were then transferred with forceps to clean wire-mesh cages fitted at the base with white paper, properly fastened with rubber bands, and on their upper surface with pads of damp cotton dipped in a sugar solution. The mosquitos used as controls were transferred to similar cages after applying only ethylated alcohol on their thoraxes with the microloop. Fifty mosquitos were placed in each cage.

After 24 hours the mortality of mosquitos treated was determined as well as that of controls. The above tests, at a 25-28°C temperature and 60% relative humidity, were continued until the finding of the LD90's of the insecticide, estimated in micrograms per mosquito, of a statistically significant number.

Application of Busvine and Nash method under field conditions

Anopheline mosquitos caught in their natural shelters were introduced immediately after their capture (5 - 6 per vial) into vials lined with filter paper uniformly impregnated with DDT solution of a determined strength, in accordance with the technique described by Busvine and Nash (11). Shell risella oil was used as solvent and the solution prepared was diluted before use with two parts of ether. The paper-lined vials were corked and placed vertically in the field laboratory dark-room at 25-27°C and 60% relative humidity. After exposure of the mosquitos to the insecticide for one hour they were transferred from the vials, by approximately 50,

^{*} The estimate of the capacity of the microloop used was made by Dr. Perry in the bio-chemistry section, Oak Island Laboratories, Savannah, Georgia, USA.

to clean mesh-wire cages supplied with pads of damp cotton dipped in a sugar solution and a white paper base as in the previous case. After 24 hours, mortality counts were made of the caged mosquitos.

The mosquitos used as controls were also introduced into vials lined with filter paper impregnated only with Shell risella oil and ether in the same proportion, the same procedure being followed in this case also.

Results

The results obtained from the topical application of different DDT solutions are summarized in tables I, II, III, IV and V and illustrated in the graphs 1 and 2. The results obtained with the Busvine and Nash method are summarized in tables VII and VIII and illustrated in graph 3.

As shown in table I, the LD90's of DDT found for A. sacharovi in the village of Elos (Scala) in 1953 is 0.36 γ per mosquito (average mortality 92.5% with a solution 0.15%).

In the same village, as shown in table II and graph 1, the LD90's of DDT for A. sacharovi in 1954 amounted to 0.48 γ per mosquito (average mortality 91% with a solution 0.20%). In the village of Asterion (Scala) located two kilometres from the former village, as shown in table III and graph 1, the LD90's DDT in two series of tests made at various periods was also 0.48 γ per mosquito (average mortality 91.1% with a 0.20% solution).

As shown in table IV and graph 1, the LD90's of DDT found in Agoulinitza (Elia) in 1954 for A. sacharovi is much smaller than in the two former villages, i.e. 0.19 γ per mosquito (average mortality 92.2% with a solution of 0.08%).

As shown in table V and graph 2, in Georgioupolis (Crete) the LD90's of DDT found for A. maculipennis is still smaller, i.e. 0.14 γ per mosquito (average mortality 90.4% with a solution of 0.06%).

Moreover, as shown in table VI and graph 3, the average mortality found in Agoulinitza in 1954 for A. sacharovi exposed for 24 hours to a 2% DDT solution in accordance with Busvine and Nash method was 71.2%. Unfortunately, for technical reasons, the continuation of tests in that particular case was rendered impossible.

In Asterion, as shown in table VII and graph 3, the average mortality of A. sacharovi exposed to the same solution in 1954 was 22.2% after exposure to stronger solutions, but increased progressively, reaching as high as 96.9% after exposure to 4% DDT solution.

Trapido (12), by using the former method in Panama on A. albimanus, observed after 24 hours, an approximate 90% mortality after a 12 exposure to a 0.5% DDT solution. The difference in the technique used, especially in regard to the time of exposure, does not permit a comparison between our figures with those of Trapido.

The high resistance, however, shown by A. sacharovi in Asterion to the solutions used, as well as the difference in the susceptibility of that species in Asterion and in Agoulinitza ascertained by both methods used, are quite indicative.

Discussion

The lack of data in regard to the degree of susceptibility shown to DDT by the Greek malaria vectors in the early stages of the application of that insecticide, does not permit an accurate evaluation of the results obtained. It is, however, possible on the basis of the observations made to draw certain relevant conclusions.

The observation made in the village of Elos on the increase of the LD90's of DDT from 0.36 in 1953 to 0.48 γ in 1954 shows a tendency to reduction of the susceptibility of the local vector to this insecticide as a result of the continued contact. It is noteworthy that in 1954 A. sacharovi presented the same degree of susceptibility (LD90's 0.48 γ per mosquito), in the village of Asterion also, where malaria conditions are practically the same as in the former village and house spray as well as air spray of breeding places were also applied over a long period of years.

The observation made in Agoulinitza area is also important. In that area, although the epidemiological observations show a clear development of resistance by A. sacharovi, not only to DDT but also to Gammexane and Chlordane, the susceptibility of this species to DDT was found to be considerably higher than that observed in the two former villages (LD90/0.19 γ per mosquito). The higher susceptibility of A. sacharovi to DDT in Agoulinitza was also confirmed by the

Busvine and Nash method applied in that area. It must therefore be admitted that the original LD90's of DDT for A. sacharovi in Greece was smaller than 0.19 per mosquito.

Finally, the LD90's of DDT found in 1954 for A. maculipennis (0.14) in Georgioupolis (Crete), where, in spite of the discontinuation of the house spray programme since 1951, some use was reportedly made of chlorinated insecticides by the population during the ensuing period, do not exclude the assumption that the original levels of LD90's of DDT for A. maculipennis in Greece were still lower than 0.14 per mosquito.

Conclusions

From the results of the investigation made during the period of 1953-54 to evaluate the degree of A. sacharovi and A. maculipennis susceptibility to DDT in three areas of Greece by topical application of microdoses of DDT and the Busvine and Nash method on a total of 21,723 A. sacharovi specimens and 1,148 A. maculipennis specimens, the following conclusions can be drawn:

- (1) A. sacharovi has developed clear physiological resistance in Greece.
- (2) The degree of resistance developed by that species seems to be fairly high, although, for lack of comparative data, it is not possible to evaluate it accurately.
- (3) The degree of resistance found to have been developed by A. sacharovi shows substantial differences in the investigated areas, and a clear tendency was at the same time observed of increase in resistance from year to year within the same area under the effect of the contact of the insect with the insecticides.
- (4) A. maculipennis showed in the investigated areas a higher susceptibility to DDT than A. sacharovi.
- (5) The continuation of these investigations in Greece and elsewhere will provide further information on the course of the resistance phenomenon, as well as on the susceptibility in general of the various anopheline species to chlorinated insecticides.

Table I. Twenty four hours mortality of A.sacharovi (adults) after topical application with different solutions of DDT

Elos, 1953

Period of observations	Treated adults					Controls			
	number of observations	percent. of solution of DDT	number of female mosquitos treated	number of dead mosquitos	mortality percent. after 24 hours	micro-grammes per mosquitos	number of female mosquitos tested by ethylated alcohol	number of dead mosquitos	percent. mortality after 24 hours
14.8.53	1	0.01	80	16	20.0	0.024	112	17	15.2
14.8.53	1	0.05	50	18	36.0	0.120	112	17	15.2
15-20.8.53	6	0.10	807	696	86.2	0.240	615	52	8.4
21-23.8.53	3	0.15	319	295	92.5	0.360	260	12	4.6
23.8.53	1	0.20	88	88	100.0	0.480	90	2	2.2

Table II. Twenty four hours mortality of A.sacharovi (adults) after topical application with different solutions of DDT

Elos, 1954

Period of observations	Treated adults					Controls			
	number of observations	percent. of solution of DDT	number of female mosquitos treated	number of dead mosquitos	mortality percent. after 24 hours	micro-grammes per mosquitos	number of female mosquitos tested by ethylated alcohol	number of dead mosquitos	percent. mortality after 24 hours
23.8.54	1	0.10	50	17	34.0	0.240	60	5	8.3
23.8.54	2	0.15	210	130	62.8	0.360	120	10	8.3
30.8.54	9	0.20	1,321	1,203	91.0	0.480	510	36	7.6
30.8.54	1	0.25	115	115	100.0	0.600	50	4	8.0

Table III. Twenty four hours mortality of *A.sacharovi* (adults) after topical application with different solutions of DDT

Asterion 1954

Period of observations	Treated adults						Controls		
	number of observations	percent. solution of DDT	number of female mosquitos treated	number of dead mosquitos	mortality percent. after 24 hours	micro-grammes per mosquitos	number of female mosquitos tested by ethylated alcohol	number of dead mosquitos	percent. mortality after 24 hours
8.8.54	1	0.10	106	44	41.5	0.240	60	4	6.6
17.6.54 & 8.8.54	2	0.15	185	128	69.2	0.360	130	14	10.8
17-25.6 & 8.20.8.54	21	0.20	2,186	1,993	91.1	0.480	1,197	78	6.5
20.8.54	1	0.25	60	60	100.0	0.600	71	6	8.4

Table IV. Twenty four hours mortality of *A.sacharovi* (adults) after topical application with different solutions of DDT

Agoulinitza, 1954

Period of observations	Treated adults						Controls		
	Number of observations	percent. solution of DDT	number of female mosquitos treated	number of dead mosquitos	mortality percent. after 24 hours	micro-grammes per mosquitos	number of female mosquitos tested by ethylated alcohol	number of dead mosquitos	percent. mortality after 24 hours
26.7.54	2	0.05	184	112	60.9	0.120	120	10	8.3
17-26.7.54	11	0.08	2,056	1,910	92.9	0.192	700	58	8.3
17.7.54	2	0.10	203	203	100.0	0.240	120	16	13.3
16-17.7.54	2	0.15	235	235	100.0	0.360	120	16	13.3
16.7.54	2	0.20	215	215	100.0	0.480	120	16	13.3

Table V. Twenty four hours mortality of A. maculipennis (adults) after topical application with different solutions of DDT

Georgioupolis 1954

Period of observations	Treated adults						Controls		
	number of observations	percent. solution of DDT	number of female mosquitos treated	number of dead mosquitos	mortality percent. after 24 hours	micro-grammes per mosquitos	number of female mosquitos tested by ethylated alcohol	number of dead mosquitos	percent. mortality after 24 hours
15.9.54	1	0.03	50	23	46.0	0.072	50	2	4.0
15.9.54	1	0.05	50	40	80.0	0.120	50	2	4.0
17-21.9.54	6	0.06	648	586	90.4	0.144	300	8	2.7
15-21.9.54	3	0.08	150	150	100.0	0.192	150	4	2.7

Table VI. Twenty four hours mortality of A. sacharovi (adults) after exposure of one hour to different solutions of DDT in paper-lined vials

Agoulinitza 1954

Period of observations	Adults exposed					Adults in controls
	number of observations	percent. solution of DDT	number of female mosquitos exposed	number of dead mosquitos	mortality percent.	Average mortality percentage
16,17.7.54	2	0.2	101	18	17.8	12.0
16,17.7.54	2	0.5	100	21	21.0	
16,17.7.54	2	1.0	100	40	40.0	
16,17.7.54	11	2.0	1,142	813	71.2	

Table VII. Twenty four hours mortality of A. sacharovi (adults) after exposure of one hour to different solutions of DDT in paper-lined vials

Asterion 1954

Period of observations	Adults exposed					Adults in controls
	number of observations	percent. solution of DDT	number of female mosquitos exposed	number of dead mosquitos	mortality percent.	Average mortality percentage
8,9.8.54	2	1.5	60	10	16.6	7.6
8-25.8.54	17	2.0	1,031	229	22.2	
8-25.8.54	17	2.5	1,008	414	41.1	
8-25.8.54	17	3.0	1,014	675	66.5	
8-25.8.54	17	3.5	1,053	882	83.7	
8-25.8.54	17	4.0	1,334	1,293	96.9	

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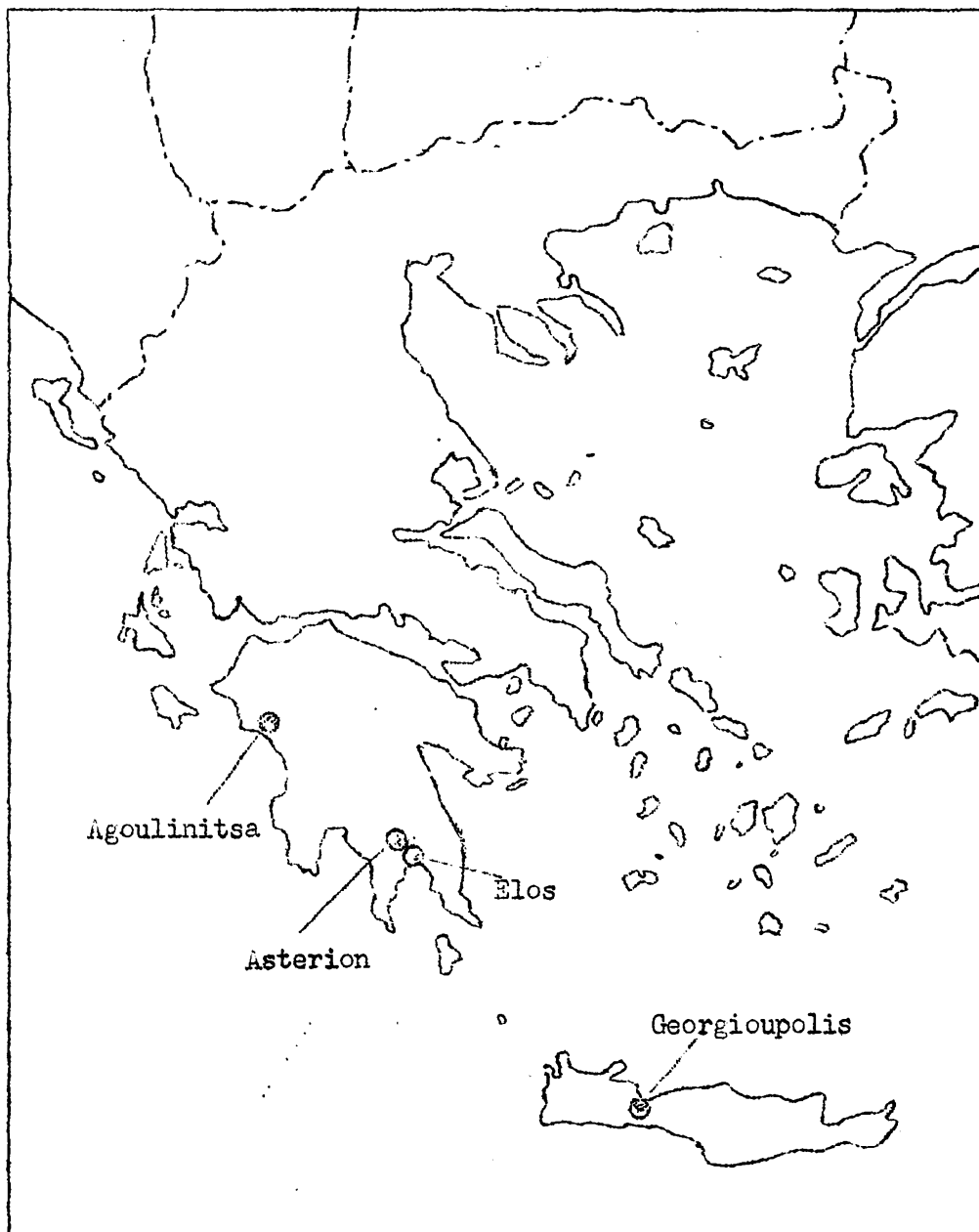


Figure I

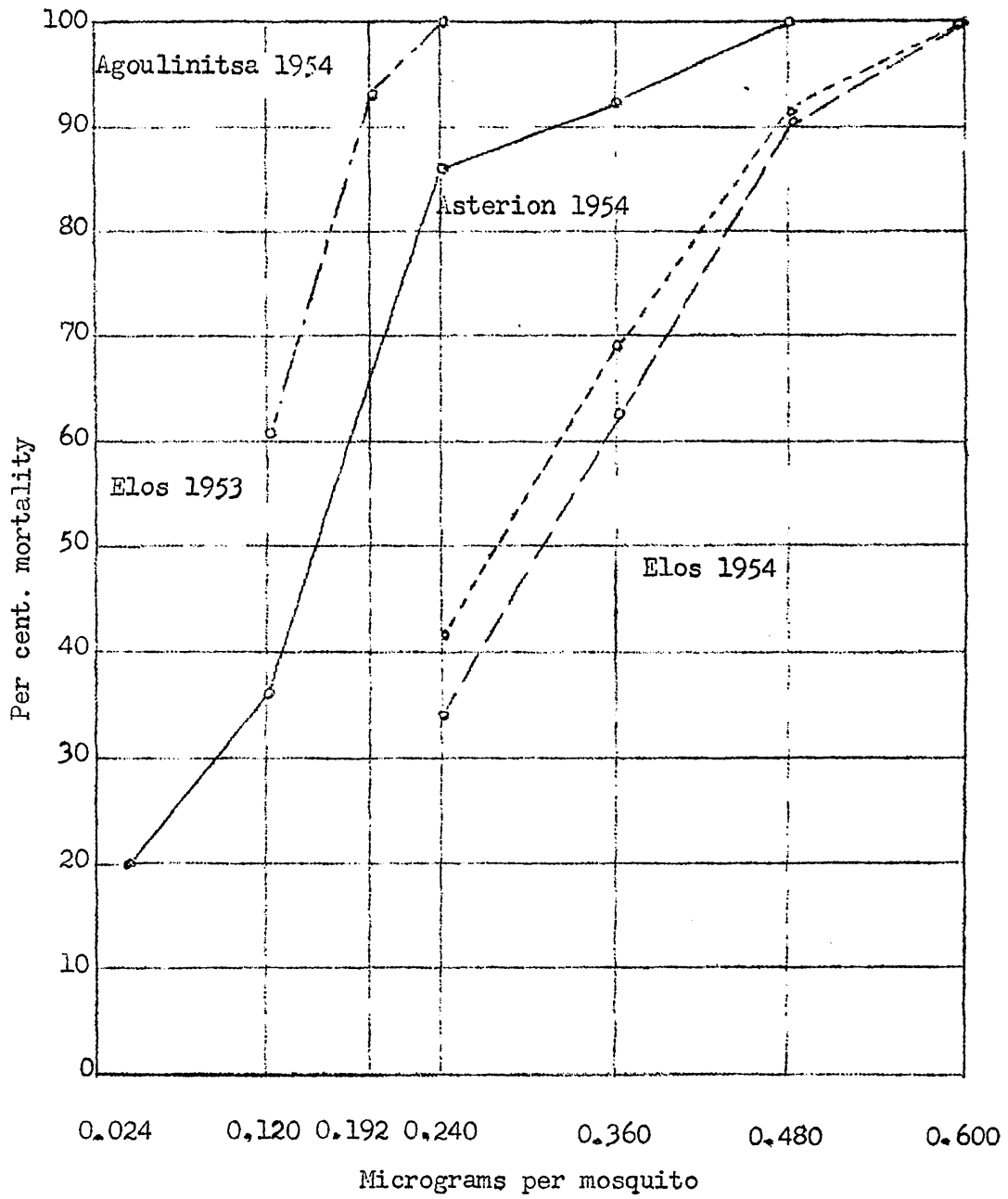


Figure 2

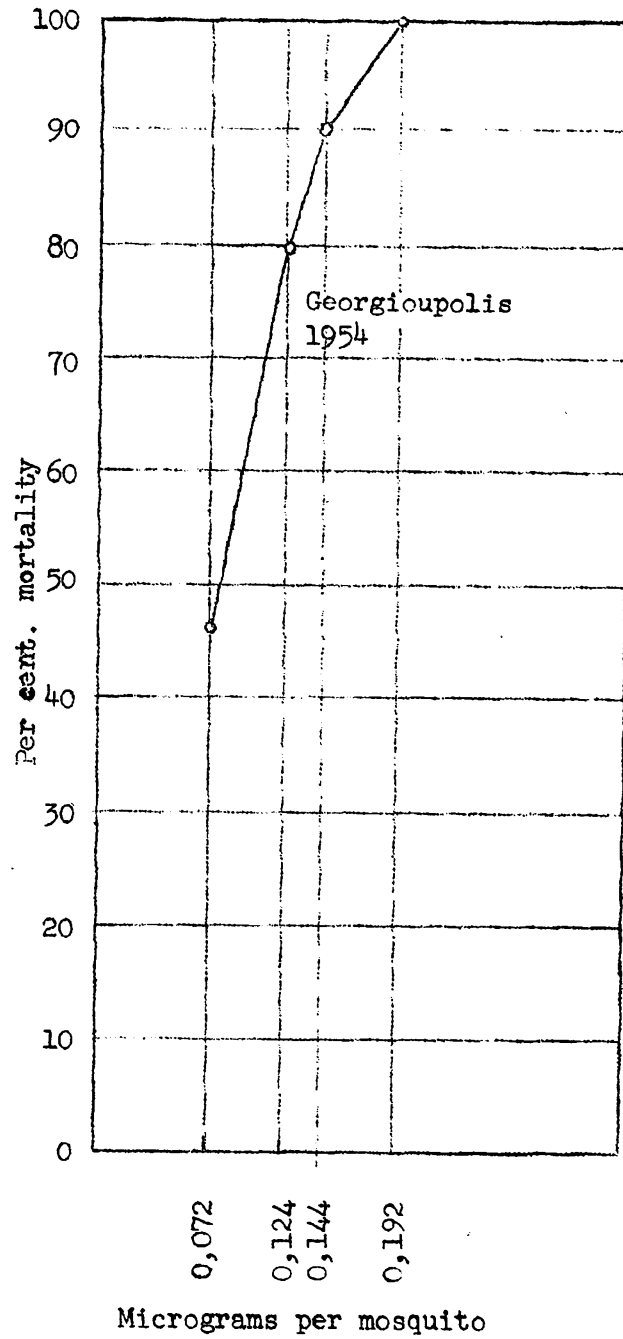


Figure 3

