# PHLEBOTOMUS AND RESIDUAL DDT IN GREECE AND ITALY<sup>1</sup>

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In a number of countries residual DDT applied in various ways has been shown to give consistently good results in the control of Phlebotomus. The treatment of the inner walls of living quarters in all reported instances (1, 2, 3) has given virtually complete protection from the bites of Phlebotomus, with usually no sandflies to be found in houses in daytime searches. A high degree of area control was secured in Peru by spraying outdoor stone walls as well as the interiors of houses (3). DDT was also used successfully against sandflies by various German investigators during the war (4). These various studies, however, did not determine the degree of area control which would be produced by house spraying alone. Furthermore there had been few long-term observations in the temperate zone where many of the most important sandfly-borne disease problems occur, and where theoretically a single annual pre-season treatment ought to yield a high degree of control. It has been obvious for some time that the now standard method of house spraying for malaria control is also and automatically a method for the control of Phlebotomus. It has so happened, however, that in the urgency of the malaria work only casual attention has been paid to the fate of Phlebotomus in various campaigns in regions where these two problems coexist.

In Greece for the past several years there has been carried out a very extensive malaria campaign. Houses in most of the villages of the country have received an annual treatment with DDT, followed by a spectacular decrease in malaria (5, 6). This project has been carried out by the Malaria Service of the Greek Government with the technical and financial assistance, first of UNRRA and later of the World Health Organization and the American Mission for Aid to Greece. It had not proved possible to make specific observations on *Phlebotomus* but general reports indicated that these normally abundant insects had greatly decreased or even "disappeared." This situation offered a favorable opportunity to evaluate the results of a house spraying campaign in terms of sandfly control, and perhaps also of disease control.

Arrangements were made for this study under the immediate auspices of World Health Organization-Interim Commission. The writer was in Greece from 6 June to 24 October 1948, with headquarters at the Athens office of the WHO-IC Mission in Greece. Laboratory facilities were generously provided by the Athens School of Hygiene.<sup>3</sup> The general plan was to make as many observations as

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possible on the mainland and to devote special attention to the District of Canea in Crete with its well known foci of leishmaniasis.

### THE SANDFLIES OF GREECE

There are listed below the eleven species of this subfamily which have been previously recorded from the Greek mainland and from Crete (7-15). The nomenclature of Theodor's (16) recent revision of Old-World Phlebotominae has been followed.

Phlebotomus (Phlebotomus) papatasi Scop. 1786.

Phlebotomus (Larroussius) major Ann. 1910.

11 perfiliewi Parrot 1930. (Macedonia only.)

tobbi Adl, & Theo. 1930. (Not reported from Crete.)

Phlebotomus (Adlerius) chinensis var. simici Nitz. 1931.

mascittii Grassi 1908 (syn. larroussei Lang. & Nitz. 1931). (Crete only.)

Phlebotomus (Paraphlebotomus) sergenti Parrot 1917.

alexandri Sinton 1928. (Not reported from Crete.)

Sergentomyia (Sergentomyia) minuta Rond. 1843 (syn. P. parroti var. italicus Adl. & Theo. 1931).

Sergentomyja (Sergentomyja) theodori Parrot 1942 (svn. P. minutus Adl. & Theo. 1926).

Sergentomyia (Sergentomyia) bruchoni Parrot 1935, (Island of Poros only.)

The application of residual DDT was begun in 1945 and got under way in earnest in 1946, when there were sprayed 700,000 houses and outbuildings, mostly in villages (5). It was estimated that 80 per cent of the population in malarious areas throughout continental Greece and the islands had been "protected," a proportion which has since been increased. The inner walls and ceilings of houses.

Dr. J. M. Vine, Chief of WHO-IC Mission in Greece, Colonel D. E. Wright and Mr. Paul Bierstein, Sanitary Engineers, and others of the Athens staff;

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We had the good fortune to secure the services, as interpreter and assistant, of Mr. Marios Balodimos, a sixth-year medical student, who quickly became an invaluable aid in both the

field and laboratory phases of the work.

The status of P. mascittii has been in doubt. It was considered by Adler and Theodor (7) as probably an aberrant form of perniciosus, while Sacch (17) and Parrot (18) have held that larroussei was probably identical with mascittii. That mascittii is a valid species is supported by the writer's examination of the only known cotype of Grassi's material found among Newstead's Phlebotomus slides in the British Museum. The results of the study of this specimen were forwarded to Dr. Sacca, but at his request will be presented in a separate note.

stables and other outbuildings have been treated at the rate of 2.0 grams of DDT per square meter. The solutions used at first, usually in kerosene, were largely replaced in 1947 and 1948 by water emulsions. Cylindrical, three- or four-gallon, hand-pressure sprayers have been standard equipment. Some swampy areas have been sprayed from the air, which is the only current antilarval measure.

The work of various investigators of sandfly-borne diseases has shown that sandflies, as in many regions about the Mediterranean, are normally abundant throughout most of Greece and Crete (7-15,5). Identified collections have totaled thousands of specimens and have furnished data as to the distribution and relative abundance of the different species. There were no precise data, however, regarding the abundance of sandflies immediately preceding the spraying. It was necessary, therefore, to make a rough reconstruction of the situation on the basis of reports by local residents. It has been our experience in various countries that where sandflies are abundant most of the people recognize Phlebotomus with considerable accuracy and distinguish them from mosquitoes. Greece proved to be no exception. Some know sandflies by sight, (The dominant Mediterranean sandfly, P. papatasi, may rest in great numbers in plain sight on smooth, lightcolored walls.) More commonly the people distinguish them by their silent flight, nocturnal habits and to a certain extent by the peculiarly irritating nature of the bite. In the various places where we worked a substantial majority of the people gave reports of sandflies formerly present in annoying abundance and usually added bits of corroborating circumstantial evidence. They also, almost without exception, volunteered the information that sandfly annoyance had ceased with the first DDT treatment. In regard to the latter point, somewhat more than ordinary weight may be given to popular reports since there is now a tendency to question the efficacy of DDT (or the integrity of those applying the material). Having experienced an undreamed-of absence of houseflies following the first treatment, the faith of the people was somewhat shaken by the reappearance of the now very abundant DDT-resistant flies (combined with the change from the earlier kerosene solution to the emulsion or wettable powder, thought by the people to be "adulterated" with water obtained locally).

An an index of normal sandfly abundance during the season of 1948 we made an effort to locate villages or groups of houses where DDT had never been used, or at least not recently. We met with success, as shown below, in certain villages near Athens, as well as in one of its suburbs. The very numerous sandflies found in some cases we took to indicate that 1948 was not an off-year for *Phlebotomus*.

We soon found that household sprays were in very common use, particularly in urban centers, and represented a factor very difficult to measure or estimate. For example, of about 70 households sampled in Athens, about 85 per cent used such sprays at least occasionally. Most of these sprays contained DDT. The crystals could be seen on the outside of the sprayer or bottle. Casual observations in other countries had shown that *Phlebotomus* was usually scarce in houses where

<sup>&</sup>lt;sup>5</sup> Professor Hadjinicolaou made rather extensive collections of sandflies in 1938-1939 in the Athens area. Our data as to abundance and distribution of species in unsprayed areas are comparable with his.

sprays with either pyrethrum or DDT had been used the previous night, and that this method provided good temporary protection from sandfly bites. In Athens the cost and relative scarcity of sprays made their regular, daily use uncommon. A scattering of sandflies and occasionally as many as forty could be found in houses which used sprays "sometimes." The principal target was houseflies and the spraying was done largely during the day, although in a few cases sandflies were primary objectives. In one case with before-and-after observations by us, regular space-spraying had reduced the sandflies from 200–300 to a dozen or so.

Technique. Searches for sandflies were made with the aid of tobacco smoke and flashlights. Smoke was used even on smooth white walls, since if sandflies are scarce their enforced movement will reveal them at once. Furthermore they are easier to catch after having reacted to the smoke. Specimens were caught in a suction tube and transferred immediately to 70-per cent alcohol.

No insect repellent was used at any time, although we had constantly available the Army repellent. In general, the sandflies were either very scarce or were not attacking persons actively moving about in the open. Even in the heavy flights of perfiliewi encountered in Italy, the exposure was relatively brief and it was decided that the slight disease hazard was outweighed by the desirability of not interfering with sandfly activity.

Oiled paper traps. Sandfly traps consisting of sheets of paper smeared with castor oil were used successfully by Kirk and Lewis (19). Late in the season we gave the method a trial. A comparative test was first made with sheets of white bond paper, typewriter size (21.5 x 28 cm.), and pieces of ordinary window screening. The latter would hold a tiny drop of oil at each intersection of the wires, and it was thought that it would be easily manipulated in the field. On the first trial, however, with paired paper and screening, the only sandflies caught were on the paper, and the screening was abandoned. A final, and apparently satisfactory, arrangement was to tack the paper onto wooden frames (Fig. 6). These were made of thin strips, 2.5 x 26.5 cm., nailed with their flat surfaces together to form a square. The paper was fastened with thumb-tacks in the mid-plane of the frames so that they could be stacked without the papers coming in contact with one another. The oil was applied with a brush before leaving the laboratory. The frames could be hung on walls like pictures or placed on articles of furniture without undue messiness or apprehension on the part of the householders. The frames were picked up the following morning and examined at leisure in the laboratory. The specimens, which were in good condition and at times still alive, could be picked off by "lifting" a small quantity of the surrounding oil with fine curved forceps, or by putting a needle under the specimen. By transferring directly to phenol the oil was got rid of and the specimens were cleared for identification. In the field they could be transferred to alcohol. One application of oil would last several days. If the paper became fouled with debris or too many other insects, it could be wiped clean with xylene and used repeatedly.

Permanent stained preparations were made by the phenol-copal method (20), the final mount being in copal. Most of the catches, however, were identified after clearing in pure phenol (with just enough water to keep it liquid) after which they could be returned to the alcohol. This method is rapid, does not injure the specimen and permits a high degree of accuracy once the species of a region are known. All the characters of taxonomic importance with the exception of the cibarium can be seen. The posterior part of the pharynx with its armature is rarely obscured by the eyes. We have found it convenient to arrange 6-12 specimens around the edge of the concavity of a hollow-ground slide, where they will be in contact with the cover-glass but not crushed when the liquid evaporates. and where they will be out of the way of the central bubble. A simple sketch diagram showing the orientation of the specimens in the circle, on which may be noted the identifications as they are made, makes it easy to be sure that all the specimens have been checked and, furthermore, makes it convenient to refer later to any of special interest. Such temporary preparations may be left indefinitely, adding a little phenol from time to time. Camera lucida drawings or photographs may be made with little danger that the specimen will move during the process. It is often the case that the spermathecae and their ducts, free of distortion, may be more clearly seen in such phenol preparations than at any subsequent stage of mounting. Advantage may be taken of this fact when new species or aberrant forms are encountered.

It may be remarked that after alcohol storage for a number of months, treatment with caustic may not clear up the tissue around the spermathecae. It is therefore desirable, although not practiced on this occasion, to avoid alcohol altogether with at least part of any given collection and store it as dried specimens.

#### OBSERVATIONS

Athens and suburbs. DDT history: The extensive metropolitan area of Athens, including Piraeus and the suburbs stretching along the sea, had never been included in the regular house spraying program, since the area was not malarious. However, a considerable amount of residual spraying had been done within this area from 1945 on. All military installations of the British, Greek and American forces had been treated. They included buildings in the center of Athens as well as in the outskirts. In addition a considerable number of both public and private buildings had been sprayed. It was apparently a common occurrence for those with official or personal contacts to have their houses sprayed. Among 70 private residences sampled at random, four were found to have had the residual spray in 1946, 1947 or 1948, and two of these houses had been sprayed twice. Colonel D. E. Wright informed us that about one-fourth of the personnel of UNRRA had sandfly fever in 1945. In 1946 their quarters were sprayed and no cases at all were reported among some 2000 employees of this organization.

During the summer of 1946 a large portion of the suburb of Nea Smyrni, between Athens and the sea, had been sprayed with DDT from the air. Residents of this quarter maintained that the sandflies, which had been very abundant, ceased to trouble them for the rest of that season and, indeed, had not been numerous in the following season. In the fall of 1947 practically all of Athens was sprayed from the air as a fly-control measure in connection with the Egyptian cholera epidemic. Since this was late in the season its effect on *Phlebotomus*, if any, would not be obvious.

Athens; house survey: A survey of houses in several districts of Athens was carried out, chiefly by Mr. Balodimos, at odd times during July and August. There were recorded on printed forms the names, addresses, number of persons, domestic animals, type of building and walls, history with regard to Phlebotomus, the use of household sprays, treatment with residual DDT. A search was also made for Phlebotomus. The districts were Ambelokipi and Gizi-Polygonon, in the northeast corner of Athens, and Pyritidopirion-Agios Sabas in the western part. In the two former districts 63 houses, with 292 persons, averaged a little over four sandflies per house, with 32 negative and three houses accounting for twothirds of the total collections (91 ♂, 190 ♀ papatasi). In the third district the incidence was greater, with eight houses averaging 17 sandflies (54 ♂, 82 ♀ papatasi). In this survey we learned of the widespread, albeit irregular and inefficient, use of household sprays. There was a general awareness of DDT on the part of the people and a number of them stated that the formerly numerous sandflies had decreased with the advent of DDT, even though their particular house had not been treated, and gave the year 1945 or 1946 as the beginning of this decrease.

A few groups of houses in the center of Athens were visited in the evening. An apartment building, the residence of a colleague, had had very few sandflies the past two years compared with formerly. There had been no special treatment with the exception of household sprays. Living quarters, corridors and courts were negative, as were also the walls surrounding a garden. A few blocks away a small house, one of several around an open court-yard, yielded 1 ? papatasi, with none on the outer walls. During June and July an occasional specimen of papatasi was taken biting in a fifth-floor room of a hotel in the center, and in September, in a residence just south of the Acropolis.

Athens suburbs; Nea Smyrni: This suburb between Athens and the sea, built up since 1922, consists of small separate houses, most of them with gardens. A friend, Mr. Harry A. Stephopoulo, of the Ministry of Health, who has been most helpful and to whom our best thanks are due, has been a resident of Nea Smyrni for a number of years. He informed us that sandflies were extremely annoying prior to the airspray of 1946, but that thereafter for the rest of that season, the annoyance had ceased, and that this had been the general experience of others in that suburb. His house was treated with residual DDT in the spring of 1947, with no subsequent treatment except a household spray used in a chicken-coop, and the airspray in the fall of 1947. His house was visited several times during the summer of 1948, both by day and in the evening. We found an occasional sandfly in the house and two or three in an unsprayed half-basement, all papatasi. No sandflies were seen outside nor were bites felt, although formerly there had been much annoyance when sitting outside in the evening. Three other houses a number of blocks distant, without previous residual spray, were negative in

daytime searches. A small dairy on the edge of this suburb had at least 50 sandflies in the house. Those caught consisted of 5 of 5 of papatasi. In the stable with
horses and cattle, two were seen on one occasion, while a storage building was
negative. The extreme scarcity of sandflies in stables, pigpens and chicken-coops
turned out to be a feature of all our observations throughout the season. This
was in constrast to the experience of Caminopetros (8) whose catches throughout
the summer of 1933 in stables on the outskirts of Athens averaged about 46
papatasi plus a scattering of major, tobbi and sergenti, with a slightly higher rate
in kennels. These catches were consistently much greater than his house collections. We have no explanation for the differences between his results and ours.

Elliniko: This suburb lying along the sea beyond the Hassani Airport is similar to Nea Smyrni in that the houses are separated by open ground and gardens. It has long been notorious for its sandflies. Mr. Stephopoulo informed us that the British had had numerous troops living in this area and in 1945 suffered severely from sandfly fever until DDT was applied. He accompanied the British Army authorities at the time the spraying was first done in buildings occupied by their men, and was our guide on our first visit. Many of the houses were badly damaged during the war and were still vacant. On 14 June one house had an estimated 200-300 sandflies, of which a number were caught (22 of 27 9 papatasi). A pigpen and chicken-coop were negative. The DDT history of the house was unknown. The people were new tenants and had not yet equipped themselves to cope with the extreme sandfly annoyance. On 5 July, however, the place had been more or less regularly treated with a spray containing DDT and only about a dozen sandflies were to be found. Four or five other occupied houses, most of them with household sprays in use, yielded a total of 9 ♂ 5 ♀ papatasi. The damaged vacant houses were consistently negative, even in those rooms still intact. Pigpens and chicken-coops were consistently negative.

A colleague living in a large residential suburb just north of Athens had formerly had severe sandfly annoyance, both indoors and out. The house was treated with residual DDT in the spring of 1947. Thereafter, throughout the seasons of 1947 and 1948, with limited use of household sprays, there had been no further difficulty. On two evening visits (13 June, 3 September) no sandflies were found. Colleagues, associates and others were queried throughout the summer for information about neighbors and friends who might be troubled with sandflies, without discovering any noteworthy foci.

Rock crevices and a limestone grotto at the base of the Acropolis above the Amphitheater of Dionysus were explored on several occasions. A single *Phle-botomus* was seen in a crevice, but not caught. The distance to the nearest house was about 150 meters.

It will thus be seen that sandflies in Athens and its suburbs were at a rather low level, with many individual accounts of great annoyance two or three years previously. That the sandfly incidence was potentially much higher in 1948 was shown by the numerous sandflies in several places, both in the city and in its suburbs, where no recent measures had been taken. Actually, if there are considered only those houses where we found any sandflies at all, our average

house catches in Athens proper (10.3 papatasi) nearly equalled those of Caminopetros (8) in 1931–1933 (11.6 papatasi plus a scattering of other species which we did not find at all). Our maximum house catch (one hundred twenty) was greater than his (73 papatasi). In assessing the current situation in Athens it is impossible to estimate the roles which various factors may have played,—the peripheral effect of the many buildings treated with residual DDT, the use of household sprays, and the treatment from the air. The only species in our Athens collections was papatasi.

Villages in Attica: The great majority of the villages in Attica had been sprayed in 1946, 1947 and 1948. A number were visited and, as shown in Table 1, were found to have very few sandflies, but we obtained consistent reports of much annoyance which stopped with the first spraying. It was fortunate for our purposes, however, that certain non-malarious places had been left unsprayed. Observations in the latter at various times throughout the summer we have used as an index of normal sandfly incidence in 1948.

Some of the village surveys were made by Mr. Balodimos, at times in the company of Professor Hadjinicolaou and Mr. Petritis, the members of the party being indicated by their initials. Data were recorded on the same printed forms used in the Athens survey. Village houses are small, usually of one or two rooms, plastered and whitewashed inside and out. The ceilings are usually finished. The cooperation of the people, who were very kind and hospitable, was excellent. Not only was admittance never refused, but we were usually cordially invited to enter and were aided whenever furniture had to be moved or ladders brought to reach the higher ceilings. Household sprays are rather less commonly used in villages than in Athens. Identified collections are summarized in the Table.

Sprayed villages: In sampling sprayed areas special effort was made to find any houses which had not been sprayed or which had been missed in the 1948 treatment.

The villages of *Liosati*, *A fidne* and *Kapandriti*, a group about 30 kilometers northeast of Athens, were sampled 26 August (MB). All had been thoroughly sprayed. Seven houses were negative, with practically no household sprays used.

Kato Souli, a village east of Marathon, had been very malarious and had been treated for four consecutive seasons. On 27 August (MB) six houses were negative, with reports in most cases that sandflies had been abundant before the first treatment and in one case that there were still a few.

Nea Penteli is a wind-swept locality on Mt. Penteli. Five houses were negative on 22 July (MB), two of them sprayed in 1947, the others untreated. Only two of the five reported moderate sandfly annoyance before the 1947 treatment. On 30 August (MB) three other houses which had been sprayed in 1948 were negative, two houses reporting a few sandflies previous to the treatment. As in certain other windy places, sandflies were apparently not normally abundant.

On the Sounion Peninsula, extending 45 kilometers southeast of Athens, most of the towns and villages had been sprayed. A few places left untreated are mentioned below.

In the town of Lavrion, 17 July (MH, JH, MB, IP), we learned of one un-

### TABLE 1

# Identified collections of Phlebotomus in Greece

Shows principally the distribution of species at different times and places. Relative abundance is indicated roughly by the figures following each location, which represent the total number of visits either to the same or different houses. Totals do not include some instances where sandflies were extremely abundant and were merely estimated, but where actual collections consisted almost entirely of papatasi.

Negative observations, which were the rule in sprayed houses and were common in

sprayed village areas, are included only where specifically noted.

DATE 1948	PLACE	NUMBER OF HOUSES OR VISITS	Phiedolomus papalari		P. chinensis		P. major		P. tobbi		P. sergents		P. alexandri		Sergentomyic	
			0	٥	on l	Q	3	9	7	0	3 9	2	0,	9	0"	0
JulAug. JunJul. 14 Jun. 5 Jul.	Athens, unsprayed houses  Ambelokipi	36 23 8 3 5 ca. 9	30 61 54 5 *31	79 111 82 5 7 *32												
Total, house catches			181	316		-										_
17, 23 Jul. 21 Aug. 21 Aug. 4, 18 Jul. 20 Aug.	Villages in Attica Sounion Peninsula Unsprayed houses in 4 villages Unsprayed areas; isolated houses Daskalio, unsprayed	8 6 5 ca. 5	8 1 8 8	8 5	3 1 2	2	1	1		1					1 2 1 5	
JulOct.	Unsprayed section House No. 11, inside	9	*6	*6			1							41		
8 Sep., 4, 8, Oct. JulOct.	OutsideOil-paper trapsOther houses, insideOutside	12	3 8 124	164		1	34 3	7			1	1	109 3 7 1	86 2 6 1		
27 Jun. 5 Aug.	Sprayed section  Before 1948 spray  Same 5 houses after spray	5	1	1	5	4		1/4								
8 Jul.	Inside, neg. Outside Sprayed house, inside Kinetta, unsprayed	1	1 1	1			10						6			Į.
25 Jul., 7, 20 Aug.	Houses, inside	1000 80	525	90			4	1						12		
19 Aug.	5 houses, outside	1 evening	25	-	1	-	-	_	2	1	4	1	_	1	9	-
Totals: House catches. Outside, night. Total, Attica villages.			682 37 719		3	7	48	18	2	1	4	1	119	90		

TABLE 1-(Continued).

DATE 1948	PLACE	NUMBER OF HOUSES OR VISITS	Phiebotomus papatasi		P. chinensis			F. Major	P. tobbi		P. sergensi		P. alexandri		Sereestoneria	Minuta
			3	9	3	9	ď	9	0	Q	3	0	en.	P	0	15
23 Jul., 13-27 Sep.	Crete City of Canea, unsprayed houses Agios Joannis Houses, inside	over 40	15	30		1					4	7			1	
	(12 sprayed, also neg.) Outside	9 evenings	1				١.	١.								ı
18 Sep.	Oil-paper traps, inside	1 night	6				1	4			1	1				П
18 Sep.	Oil-paper traps, outside Splanzia	I night	0	1			1				1				2	
6 Jul., 4 Oct.	Houses, inside(13 neg.)	17	1	4							1	1				1
i, 5 Oct.	Outside	2 evenings									1					l
8 Sep.	Houses, inside (6 neg.) Kounkapi	9		2		1										
Oct.	Houses, inside(11 neg.)  District of Canea, rural areas	12		1	-											
4 Jul., Sep.	Isolated unaprayed (?) bldgs	3		1	1	2						2				
0 Sep.	Unsprayed wall, outside	levening				1										
7 Sep., 7 Oct.	Farmyards, outside,															
, 10 Oct.	Oil-paper traps	2 nights 2 1 night			Post .	1	18	8								
Totals: House catches. Outside, night.			22	40	1		6	4		-	0.5	10 2	-	-	1 2	1
Cave ente	hes		23	41	2	6	19 25	8 12			7	12			3	
O 11 1 1 0000	sandflies		-			-	-	-			7		-	2.70	147	-

treated house. It was negative and we were informed that there had never been sandflies. However, three nearby houses reported former abundance, in one case extreme, which had ceased with the first spraying in 1946. We saw one sandfly in a sprayed living room, one of the rare instances of this phenomenon.

In Keratea and Sounion, 23 July (MB), seven houses, all but one or two sprayed in 1947, but not in 1948, yielded 1 ♀ papatasi, 1 ♂ minuta. Five of the seven gave histories of former sandfly annoyance, with no valid information in the other two.

The Sounion Peninsula was visited for the third time on 21 August (JH, MB, IP) principally to seek out houses in sprayed villages which had missed the treatment. Four such houses in *Liopessi* reported sandflies as formerly abundant and still present to a certain extent. Two of the houses, with almost daily use of household sprays, yielded five sandflies, while in the other two with infrequent spraying, 16 were caught. In *Koropi* four untreated houses with little or no household spraying harbored an estimated total of about 20 sandflies. This village had been sprayed from the air in 1946 with an immediate decrease of sandflies reported. Three houses in *Markopoulon* were negative, two sprayed in 1948, one last treated in 1946. In *Keratea* two houses sprayed in 1948 were negative

while three sandflies were caught in one last sprayed in 1947. Total catch in these four villages: 8♂ 13 ♀ papatasi, 3 ♂ 4♀ chinensis, 1 ♀ major, 1 ♀ tobbi, 1 ♂ minuta.

The data for the sprayed portion of the village of Melissia, where special ob-

servations were made, are reported below.

Unsprayed areas, Sounion Peninsula: Along the six-kilometer stretch of road at the tip of the peninsula between Lavrion and Sounion are a number of scattered houses, mostly summer residences, which had not been sprayed. Eight houses were visited 17 July (MH, JH, MB, IP). In only one house were sandflies even moderately abundant (20-30 seen, identified sample all papatasi), with a few seen or caught in three others. Sandfly annoyance was reported by all four. A new, unoccupied house was negative. Two other places were mere shacks with open fires and numerous openings at the eaves. The remaining house, of a type which could have furnished suitable sandfly shelter, reported no sandfly annoyance. Near the temple ruins at the extreme end of the peninsula, gun emplacements and dugouts were negative. This area is wind-swept and sandfly abundance is apparently not great. Identified catch: 1 ♂ 7 ♀ papatasi.

Daskalio, a small village on the coast east of Keratea, had never had residual DDT. On 21 August (JH, MB, IP) five houses reported "some" to "many" sandflies. One house was negative while in the other four were caught 16 sandflies, including two in a stable with goats (8 ♂ 5 ♀ papatasi, 1 ♀ chinensis,

2 & minuta).

South of Markopoulon two isolated unsprayed stone buildings were found (MH, JH, MB, IP). One, used at times as a stable, was negative. The other, a house apparently not regularly occupied, contained 1 9 papatasi, 1 3 2 9 chinensis, 1 3 major.

Ekali is a village-suburb 17 kilometers north of Athens on the Kifissia road. A colleague collected in his unsprayed room on 20 August, 3 ♂ major, 5 ♂ 4 ♀

minuta.

Louisa, a small village on the coast about 24 kilometers east of Athens, largely composed of scattered summer residences, had never been sprayed. On 4 July Dr. Belios caught during the daytime, mostly in one house, 1 or papatasi, 2 or 48 ♀ chinensis. The preponderance of chinensis was wholly exceptional in our experience. Two weeks later Mr. Balodimos in several other houses caught 7 3 5 9 papatasi, 1 9 chinensis.

Melissia is a village 12 kilometers northeast of Athens at the foot of Mt. Penteli (Fig. 1). It covers a large area with most of the houses 50 to 100 meters or more apart. The residents include a number of transients, summer residents and a number of permanent residents who are at least part-time farmers. Most of the village area is cultivated with vineyards and gardens. There are a few cattle, sheep and goats.

The regular treatment with residual DDT (22-27 June) was interrupted, leaving a group of about a dozen houses on the northeast edge of the village unsprayed. At our request the work in this non-malarious place was not resumed,

so that observations could be made throughout the season.

Sprayed sections of Melissia: On 27 June, with Dr. Belios, five houses were

searched just before being sprayed. Four of them, treated in 1947, yielded a total of  $1 \nearrow 1 ?$  papatasi,  $5 \nearrow 4 ?$  chinensis. The fifth, which had never been sprayed, was a small farm with a cow, donkey and chickens. The house contained a fair number of sandflies with one or two found in the stable ( $13 \nearrow 8 ?$  papatasi,  $1 \nearrow$  chinensis,  $1 \nearrow$  major,  $1 \nearrow$  alexandri). This same group of houses at an evening search 5 August (MH, GB, MB, IP) were all negative inside. Outside were caught a total of 20 sandflies, most of them at the small farm. At the latter,  $3 \nearrow 1$  freshly fed ? major were taken on the outside of the stable near a tethered donkey. No sandflies alighted on the animal during several minutes of observation, nor were any of our party bitten. Total evening catch:  $1 \nearrow 1$  papatasi,  $10 \nearrow 3 ?$  major,  $6 \nearrow 1$  alexandri.

In another part of the village on 8 July (MH, JH, MB, IP) nine sprayed houses were negative with the exception of one, in which 1 \$\sigma\$ papalasi was caught in an unsprayed closed cupboard and 1 \$\gamma\$ on the bedroom wall (one of the two instances during the summer when sandflies were found in a sprayed house). In another house a room built since the spraying (22 June) contained 2 \$\sigma\$ 7 \$\gamma\$ papatasi, while the sprayed part was negative.

On the evening of 7 August (MH, JH, MB, IP) a tour was made of a number of places in the sprayed area, most of which had not been previously visited. No sandflies were seen on the outer walls of houses, and the people reported no present annoyance, whereas there had been formerly. At an outdoor cafe with bright lights hung among the trees, we were informed that there were now no sandflies. One sandfly was seen on the wall of the cafe building. Several long low apartment buildings along the road near the village center reported no sandflies. None could be found on the outer walls nor in two or three unsprayed bedrooms.

Unsprayed section of Melissia: Immediately beyond the group of sprayed, negative houses visited 8 July, was the untreated area. Four houses were searched. In one, where household sprays were used, only two sandflies were seen, while in the other three they were very numerous, with estimates ranging from 100–150 (House 11) to 300–500 (House 10). In the latter, one corner near the ceiling was estimated to have 50–100 sandflies. Those caught were: 68 ♂ 75 ♀ papatasi, 1 ♀ chinensis, 1 ♂ sergenti, 2 ♂ alexandri, 2 ♂ minuta.

An evening visit 13 July (MH, JH, MB, IP) showed a striking difference between Houses 10 and 11, which were therefore chosen for subsequent observation during the season. Neither one had been sprayed in 1947 or 1948. In both there were many papatasi inside. About 50 alexandri were caught on the outer walls of H-11 during the evening, with only two seen outside H-10. These two houses were about 50 meters apart, H-10 set out in the open with no outbuildings, trees or high vegetation, while H-11 was on sloping ground with a succession of low, attached sheep-sheds on the uphill side, several trees and stone retaining and boundary walls. The latter house was definitely better protected from the wind, of which there seemed to be an unusual amount during the summer.

Beneath a cement porch at H-10 there was a shallow cellar with walls of loose stone, occupied by a man, a goat and stray chickens. There was also a lean-to chicken house with stone and adobe walls. In spite of the great numbers of sandflies in the house, these two outer structures yielded very few sandflies either by day or by night. Likewise the sheep-sheds and long stretches of loose stone retaining walls of H-11 yielded practically no sandflies. Our attention was therefore directed chiefly to the houses themselves. By early August the sandflies (chiefly papatasi) inside the two houses as well as in others nearby had decreased to a few stragglers. This may have been due in part to the very occasional use of household sprays instituted since our first visits, but it seems inadequate to account for the general decrease of papatasi which was still very abundant in another village, Kinetta, the latter part of August. Since these houses were within 100 to 200 meters of the sprayed section of Melissia we may have been encountering a peripheral effect of residual DDT.

On 5 August (MH, GB, MB, IP), one of the few very still evenings we happened to have, there was an extraordinary flight of alexandri at H-11. Between 9:45 and 10:15 we caught over a hundred of this species and a scattering of three others and could have taken many more. They were on the outer walls of the house, but especially on the ceiling of one room near an open window, on the sill of which a lamp had been set. The people, who had been sitting outside all evening, said they were being bitten somewhat, while our party of four, actively moving about, felt no bites. H-10 unfortunately was not visited. Total evening catch: 2 3 1 2 papatasi, 1 3 chinensis, 2 3 1 2 major, 53 3 60 2 alexandri.

No comparable flight of alexandri was seen again, even with the same arrangement of lights, and catches of this species declined after the end of August.

On 8 September (MH, JH, MB, IP) at H-11 a trial was made of the castor oil-paper traps. Several sheets caught a total of 8 sandflies during the night, There had been heavy rain early in the evening with considerable wind thereafter. On 20 September (JH, IP) about 30 sandflies were caught outside H-11, with chinensis and major forming a larger proportion of the catch than previously (6 ♀ chinensis, 1 ♂ 6 ♀ major, 5 ♂ 11 ♀ alexandri). During September H-10 never had more than five or six sandflies inside, with none seen outside. On 4 October (MH, JH, IP) the frames with castor oil-paper were set around the house and outbuildings at H-11. The evening was clear, still and cool. Only one or two sandflies were seen. During the night, of a total of eleven frames, two placed in the privy and one hung on a stone boundary wall caught 28 of the total of 33 sandflies, with major comprising all but five specimens of the total catch (1 ♂ 1 ♀ papatasi, 1 ♀ chinensis, 26 ♂ 2 ♀ major, 1 ♂ alexandri, 1 ♀ minuta). At the last visit to Melissia, 8 October (JH, IP) the frames were set out in approximately the same positions. P. major made up two-thirds of a somewhat reduced catch (5 of 1 9 papalasi, 7 of 5 9 major, 1 of alexandri).

It will be noted that the preponderance of major late in the season was revealed only by the use of the oiled paper traps. Most of them were caught 10–20 meters from the house. We found no point in the stone walls here or elsewhere at which this or any other species was emerging. It is clear that, along with the search for and study of outdoor resting and breeding places of Phlebotomus in general, the biology of major, the supposed vector of kala azar, is in need of thorough study.

Kinetta, a village consisting mostly of scattered summer residences along the

sea 55 kilometers west of Athens, was not malarious and had never been sprayed. On 25 July Dr. Belios collected about 100 sandflies in one or two houses which included specimens of tobbi, which we had not found in catches identified up to that time. On 7 August (MH, JH, GB, MB, IP) three summer residences along the road surrounded by nearly bare, sandy soil, had a great many sandflies, the catches averaging 171 per house (85 per cent papatasi, 11 per cent minuta). Sandflies were moderately abundant in the hotel and very scarce in a house with regular use of a spray containing DDT. In an open, unfinished house were caught about 30 sandflies, nearly all minuta. Two small farms 200 meters from the main road harbored only about one-fourth as many sandflies as the summer residences, with relatively fewer papatasi and more minuta. At one of the farms sandflies were nearly as numerous in the stable as in the house (24 and 33 respectively), an exception in our summer's observations. Those in the stable were nearly all males, in the house nearly all females. Total daytime catch in nine buildings: 287 ♂ 202 ♀ papatasi; 9 ♂ 1 ♀ chinensis, 2 ♂ 3 ♀ tobbi, 1 ♀ sergenti, 9 ♂ 7 Q alexandri, 73 3 28 Q minuta.

Evening observations were made 19 August (JH, MB, IP) from 6:30 to 10:30. At 6:30 papatasi was still abundant in the summer residences, though less so than on the morning of the previous visit. By 9:00 or 9:30 the sandflies inside houses had dropped by about two-thirds. While the evening had been fairly still it became very still at 9:30 and sandflies found on the outer walls increased somewhat, but had fallen again by 10:15. Of the 52 specimens caught outside, the proportions of papatasi and minuta were 63 and 17 per cent respectively, with species of the major and sergenti groups, of which there was no notable flight, comprising 19 per cent (25 & 8 & papatasi, 1 & chinensis, 2 & 1 & tobbi, 4 & 1 & sergenti, 1 & alexandri, 9 & minuta). The following morning 449 sandflies were caught in six houses, papatasi 78 per cent, minuta 14 per cent, the others 8 per cent (191 & 160 & papatasi, 11 & 2 & chinensis, 1 & 1 & major, 4 & 1 & tobbi, 1 & sergenti, 12 & 1 & alexandri, 39 & 25 & minuta).

Taking the group of villages in Attica as a whole, in those which had been treated with residual DDT sandflies were very scarce, even in those houses which had escaped the 1948 treatment but were in the midst of treated houses. Former sandfly annoyance had sharply decreased or ceased with the first spraying. Treated houses were consistently negative in daytime searches, with rare exceptions of an occasional sandfly. This virtual freedom from sandflies also applied to the village area out-of-doors where the spraying had been done in previous years as well as in 1948. In places first sprayed in 1948 a few sandflies could be found outdoors in the evening. In unsprayed places the sandfly incidence, where undisturbed by household sprays, was very high in a number of individual houses and moderate in most of the others. Sandfly incidence was apparently not very great in some wind-swept areas.

P. papatasi was by far the dominant species and was found almost entirely in occupied houses. The one catch of nearly pure chinensis in Loutsa was exceptional. Stables, pigpens and chicken-coops had very few sandflies of any species, even when papatasi was abundant in nearby houses. Stone retaining and boundary walls were likewise negative on direct examination either by day or by night. Indeed, we were unable to determine the outdoor resting or breeding places of any species. In night observations, the only heavy flights were those of alexandri at one house in Melissia. We never found papatasi on the outer walls in numbers corresponding to the house catches, which was also the experience of Adler et al. in Crete (10). It is known that certain species which do not rest in houses may have flight periods between midnight and dawn (e.g., wenyoni (21)) and if any such species were present it could have been missed. The variety of chinensis in the Middle East is found principally after midnight (21). However, the sandflies recorded for Greece are known to be abroad in the evening. Major, chinensis, tobbi and sergenti were slightly more numerous in evening catches than in houses during the day, but were seldom represented by more than a scattering of specimens, with the possible exception of the October night catches of major at Melissia.

### CRETE

Adler et al. (10) made a careful study of Phlebotomus and leishmaniasis in Canea, Crete, from July to November 1934. They paid particular attention to the sharply limited foci of leishmaniasis within the town itself,-kala azar, chiefly in the section known as Agios Ioannis (Figs. 4, 5) and oriental sore in the old Turkish quarter, Splanzia (Fig. 3). (See map of Canea with leishmaniasis foci (10, 22).) P. papalasi was abundant in both and formed the large majority of house catches. In evening collections out-of-doors in Agios Ioannis, sergenti was the commonest species the first half of July but later major predominated, with chinensis var. simici also present. In Splanzia sergenti was common with major extremely scarce and chinensis not taken at all. The abundance of major is indicated by their table showing the number of females which could be caught during several hours in the evening, which ranged from 40-50 per evening in July and August through a low of 20 the first half of September to the peak of 80-120 the first half of October, and declined to 20-30 the first half of November. Specific numbers or proportions of the other species in their total of 7000 female sandflies were not given. From their observations and experimental work they concluded that major and sergenti were the respective local vectors of kala azar and oriental sore. Mayer and Malamos (11) the following year also found Phlebotomus abundant. Hundreds of sandflies could often be found in living quarters. Their identified totals were: Agios Ioannis, 79 papatasi, 84 major, 9 sergenti; Splanzia, 88 papatasi, 99 sergenti.

Crete was visited with Professor Hadjinicolaou 21–31 July 1948, and again with Mr. Balodimos 12 September to 14 October. The latter period was chosen so as to include the peak of the sandfly season. Dr. E. Papantonakis, Medical Officer for the District of Canea, generously provided laboratory facilities at the Hygiene Center in Canea. He had similarly aided the previous investigators in 1934 and 1935 and was familiar at first hand with their work and the places where their observations had been made.

As in the case of Athens, the town of Canea had not been included in the

<sup>&</sup>lt;sup>6</sup> We are also indebted to Dr. Dimou and Mr. Fragoudakis, of the Malaria Service, and to Mr. L. G. Allbaugh, of the Rockefeller Foundation, for transportation and other aid.

malaria spraying campaign, but a number of public and private buildings had had the residual treatment regularly since 1946. In Agios Ioannis several groups of houses had been sprayed by UNRRA in 1946. Between 20 and 25 November 1947 the whole town, along with other cities in Crete, had been sprayed from the air as an anti-housefly-cholera measure. Practically all the villages in Crete had been treated for three consecutive seasons. In a few cases the 1948 spraying was delayed until July or August on account of bandits.

Observations on Phlebotomus: 21–31 July 1948. In Agios Ioannis (Figs. 4, 5) a few sandflies were seen in five unsprayed houses with casual use of household sprays (caught: 4 ♀ papatasi, 1 ♂ 1 ♀ sergenti, 1 ♀ minuta). There was one convalescent case of kala azar contracted probably in 1946. Four houses sprayed in 1946 and one in 1947 were negative. The occupants gave good circumstantial accounts of great sandfly annoyance which ceased completely with the treatment and had not reappeared. On one evening visit outdoor searches around the group of houses with the kala azar case yielded 3 ♂ 2 ♀ major, 1 ♀ sergenti. The sprayed houses were completely negative except for 1 ♀ papatasi caught on a garden wall.

A number of houses in *Splanzia* (Fig. 3) bordering the military post overlooking the harbor had been regularly treated with residual DDT. Only a few sandflies were seen in several unsprayed houses (3 9 papatasi, 1 3 sergenti). We found no house with complaints of sandfly abundance.

Various villages were visited: Charakies, 3–4 kilometers southwest of Canea; Kaseli and Spilia, 35 and 23 kilometers west of Canea; Brises and Georgopolis, about 20 kilometers east of Canea; Voukolies, Kandanos and others between Canea and the town of Paleochora on the south side of the Island. In no occupied house did we find any sandflies, whether sprayed in 1948 or not, with the sole exceptions of 1 ♀ papatasi in the guard house at the old Venetian fort in Paleochora, and 1 ♂ 2 ♀ chinensis in a deserted stone house near Kandanos, the previous DDT history of both of which was unknown. Weep-holes in retaining walls, outdoor privies, stables, pigpens, chicken-coops, hollow olive trees, were likewise negative. One sandfly was seen in a hillside rock crevice. In Paleochora five houses just before being sprayed were negative. An evening search was made around houses not yet sprayed without finding any sandflies. In all these places we were given consistent accounts of previous sandfly abundance. The use of household sprays in rural areas is rather uncommon.

12 September to 14 October 1948. In spite of this period being at the normal peak of the season, sandflies were quite as scarce as they had been in July. Eight evening searches in Agios Ioannis, with favorable weather, resulted in a total outdoor catch of seven sandflies (1 3 papatasi, 1 3 2 major, with three specimens lost and three more seen but not caught). On three evenings not a single sandfly was seen. Back of the two houses where we had caught most of our few specimens of major, there was a kennel with a litter of puppies and an open shelter with a tethered horse. No sandflies were seen in or near either one. Oiled paper traps were placed one night around these houses and inside bedrooms. Those outside caught 2 3 2 minuta; those inside, 6 3 2 9 papatasi, 1 3 major, 1 3 sergenti.

Daytime catches in  $Agios\ Ioannis$  in 46 houses, some of them visited several times, totaled 15  $\circlearrowleft$  26  $\circlearrowleft$  papatasi, 1  $\circlearrowleft$  chinensis, 3  $\circlearrowleft$  6  $\circlearrowleft$  sergenti, 1  $\circlearrowleft$  minuta. The greatest number of sandflies seen or estimated inside a house at any one time was 14. Twelve of the 46 houses had had the residual spray in 1946 and were negative, as were likewise 24 other houses not so treated. Two large caves which had been dug as airraid shelters had no sandflies, but various other Diptera were numerous. The DDT history was unknown although most of the houses on the same street had been sprayed in 1946.

Two other sections of Canea which had formerly been small foci of kala azar were also surveyed. Of ten houses in *Halepa*, at the eastern edge of the city, three yielded a total of 2 ♀ papatasi, 1 ♀ chinensis, 1 ♂ sergenti, 1 ♀ minuta. One of the negative houses, a police station, had been sprayed in 1948. Only 1 ♀ papatasi could be found in twelve houses in Kounkapi, the small section about 350 meters east of the old Turkish quarter. Evening searches, one in each of these two sections, were completely negative.

In Splanzia sandflies were found in only one of 14 houses (1  $\circlearrowleft$  1  $\circlearrowleft$  papatasi, 1  $\circlearrowleft$  sergenti caught, two more sandflies seen). One negative house had had residual DDT in 1946 and 1947. During two evenings, 1  $\circlearrowleft$  1  $\circlearrowleft$  sergenti were caught out-of-doors, with one other sandfly seen. Most of the residents stated that sandflies had been abundant up to the introduction of DDT.

In addition to the above observations, on various occasions when in outdoor restaurants during the evening, searches were made on walls both illuminated and in shadow, but no sandflies were found. It is clear that in the town of Canea sandflies were at a rather low level.

Villages near Canea: In a farmhouse in Charakies a child had developed kala azar during the winter of 1947–1948. There had been no other case in the village. This house and others forming a group of six or seven, had been sprayed in 1946, 1947 and 1948. The formerly abundant sandflies had "disappeared" with the first treatment. Since this was apparently an instance where kala azar had been contracted in a sprayed house, special attention was devoted to it. A sick dog had been destroyed during the summer of 1947. The family had lived there continuously for some time and the child in question had not slept elsewhere. Furthermore, the family had always slept indoors, whereas many sleep out-of-doors on hot summer nights.

At our first visit in July the house and several stables were negative, which was also true on several visits in September and October. On 17 September, 22 frames with castor oil-paper were put around the large farm courtyard, outside doors and windows of house and stables, in privies, near chicken-coops and on the courtyard wall. Search up to an hour after dark was negative, except for one male caught on a frame at 8:15. Two more were caught on frames during the night, the total for the 22 frames being 1 3 1 2 chinensis, 1 3 major. On 7 October a second trial of the frames was made, this time placing part of them around the other houses of the group. The results were completely negative. Culverts 200 meters from these houses at the edge of an olive grove were negative during the evening, and no bites were felt by a party of three.

Along the sea about 12 kilometers west of Canea is a village, Agios Marina,

largely dedicated to the manufacture of brick and tile. The clay is dug from exposed strata in nearby hills, leaving caves (Fig. 6). On 6 and 10 October were caught a total of 18 3 8 9 major in two caves within 100 meters of the village. No kala azar had occurred within the recollection of those interviewed. The village had been sprayed in 1946 and 1947 but not in 1948. While sandflies were reported to have been numerous before the spraying there were said to be very few during the current season. There were complaints, however, that fleas had returned along with houseflies. Searches in several houses were negative for *Phlebotomus*. On 9 October the frames were set out around several houses, with seven frames placed at the entrance or inside caves. The 22 frames caught just 1 3 major. This was on a frame inside a cave, where four more 3 major were caught free on the walls. This cave had yielded 12 sandflies on the first visit and a number had been seen the previous evening and left undisturbed

Several other villages, Daratzo (Fig. 8), Parigoria (Fig. 7), Bambokopoulo and Perivolia, within about eight kilometers west and southwest of Canea, had all been sprayed for three consecutive seasons and all gave histories of sandfly abundance before the first treatment, with little or no annoyance since. The first of these villages (see below) had had a great deal of oriental sore and well over half the people showed scars. However, no case was found which had developed within the past two years. In the other villages we learned of four cases of kala azar since 1941, the most recent one having developed during the summer of 1946.

Sandflies were also very scarce in the small villages and scattered groups of summer residences and restaurants (mostly sprayed) on the high hill between Canea and Suda Bay, and in the village of Khorafakia on the peninsula to the north. One of several evening searches in this region netted  $1 \ \$  chinensis outside a stable at the Convent in Korakies.  $2 \ \$  sergenti were caught at other times in this area. The village of Kalyves, south of Suda Bay, was negative.

The village of *Orthuni*, about 18 kilometers southwest of Canea, had been sprayed in previous years but the 1948 treatment had been delayed on account of bandits. On 22 September just before the village was sprayed, houses were negative, with the usual history of sandfly abundance before the first DDT treatment. A number of enormous hollow olive trees within 100 meters of houses harbored anophelines but no sandflies. A week after the houses were treated the same trees no longer harbored mosquitoes.

On a visit to certain oriental sore centers in the District of Rethimnon (see below) 15–18 October, Mr. Balodimos searched for sandflies both by day and in the evening. No sandflies were found in houses. An evening search for an hour after dark in the narrow streets of Archondiki was likewise negative. Of the seven villages visited, all had been sprayed in 1946, 1947 and 1948 except Maroulas, sprayed only in 1946. In the latter there were complaints that bedbugs and a few sandflies had made their reappearance, but no sandflies could be found on searching several houses and a stable. A suburb of Iraklion, Poros, had been sprayed in 1946 but not since. One sandfly (damaged in mounting) was caught in a house, while an early evening search was negative. A number of large

caves had been dug as airraid shelters and had been occupied by squatters ever since the war. The caves had never been sprayed but no sandflies were found.

While in eastern Crete, Professor Hadjinicolaou visited a village which had never been sprayed, Adiskari. Sandflies were moderately abundant along with normal household pests. A sample caught 6 October in one or two houses consisted of 8 ♀ papatasi, 1 ♂ 1 ♀ sergenti, 1 ♂ chinensis.

Phlebolomus in the District of Canea and also in other parts of Crete is clearly at a very reduced level of abundance compared with that prevailing in 1934 and 1935. The many solidly consistent reports of local residents leave no doubt that a sharp decrease occurred in 1946, and indeed began with the first actual spraying of the premises of the individuals concerned. This applies to all villages we visited and in part to the city of Canea. Even where the individuals' houses were not treated, a decline was reported as having occurred about that time and was associated by the people with the DDT which was being applied, usually somewhere in their neighborhood. The reports of the people must be considered as relating chiefly to papatasi, since this species was the dominant one.

Unlike Attica where we had several unsprayed areas for comparison, in Crete we were hampered by the lack of any available area, rural or urban, which had never had at least some DDT at one time or another. The closest approximation to the latter was a small group of houses in Agios Ioannis, where daytime searches consistently yielded a small number of sandflies. While these houses had never been treated, they lay between and within 100 meters of two groups of houses which had had residual DDT in 1946. The presence of a few or no sandflies during 1948 in places where no DDT had been applied since 1946 or 1947 was a phenomenon we encountered several times, as in Agios Ioannis, the town of Paleochora, and the villages of Orthuni, Agios Marina and Maroulas. Without attempting to draw any conclusion on this point, there may be mentioned the long-term effect observed in Pcru (3) where, however, outdoor resting and breeding places were sprayed as well as the houses.

In the villages sprayed for three consecutive seasons sandflies in all cases were extremely scarce. The complete absence of sandflies in daytime searches was to be expected on the basis of all previous work. Those evening observations which we were able to make also indicated the scarcity of sandflies out-of-doors within the treated area. The evening searches in villages are admittedly too few in number to clinch this point, particularly in view of the importance of major as the supposed vector of kala azar and the fact that this species rests in houses far less than papatasi. The true abundance of major has been revealed only when night observations have been included (10), if indeed we are entitled to speak of the true abundance of this species as ever having been measured. The process of catching sandflies "on the move" out-of-doors is hardly to be compared with finding a species in its favorite resting place, such as papatasi in houses. Whereas 80-120 major represent the highest catches in an evening's work (10), an equal number of papatasi can often be caught in a single room. However, in Agios Ioannis the evening observations amply showed that major had suffered along with other species.

The effect of the airspray of Canca in late November 1947 is problematical. Sandflies would have been scarce at that time in any case. The vast majority of the overwintering larvae would already have been in their breeding places and thus presumably beyond the reach of any such treatment.

#### LEISHMANIASIS

Kala azar. There has been a striking decrease in kala azar in Canea since 1934 and 1935. The one clear feature of the situation is that DDT had nothing to do with bringing about this decline. Dr. Papantonakis and other medical men attributed it to a decrease in dogs, and indeed there is good correlation of the two phenomena. The destruction of stray dogs on account of rabies was begun in 1933 with the result that the investigators of 1934 and 1935 had to deal almost exclusively with house dogs (in 1934 (10), 9 out of 50 infected with kala azar; 1935 (11), 42 positive out of 643). In 1938 Dr. Papantonakis, stimulated by the investigations of 1934 and 1935, undertook the examination of all dogs in Canea and vicinity by means of the formol-gel test (23), 229 dogs out of 1115 were found positive and were destroyed. We understand that the total of dogs examined in the entire campaign, which was continued after the published report, was about 3000. During the occupation, 1941-1945, the shortage of food reduced the city dogs nearly to the vanishing point, with a few "working" dogs surviving in rural areas. Dr. Papantonakis kindly made available to us his records since 1939, which he believes include practically all the cases of kala azar in the whole district (Nomos) of Canea (the western quarter of Crete), since the drugs for treatment were available chiefly through Government sources. For the ten years 1939 to 1948 inclusive the respective totals of kala azar cases per year were 10, 10, 13, 9, 10, 3, 2, 5, 4, 6. In our survey we learned of no case not on his list. The situation in Agios Ioannis (Figs. 4, 5) is perhaps the most striking of all. This small section of Canca, comprising about twenty city blocks, used to furnish most (about 35 cases annually) of the kala azar of the whole district. Only two cases are known from Agios Ioannis in the five years 1944 to 1948.

Since the war the dogs have increased and are apparently approaching normal, but no surveys have been made either as to their numbers or infection rate. As has been shown, *Phlebotomus* is rather scarce at present, the decrease dating from the introduction of DDT in 1946. The role which the sandfly reduction may have played in keeping kala azar at the reduced level which it reached before and during the war is difficult to estimate in view of the gaps in our knowledge of possible reservoirs other than dogs or man, and the absence of any information about local, long-term cycles of kala azar incidence.

The Ministry of Public Health furnished us a summary of the cases of kala azar reported from 49 districts or health centers, covering most of Greece for the years 1939 to 1947 inclusive, but at the same time pointed out that the reporting of kala azar is neither complete nor reliable. For what they may be worth, we cite the respective totals for these nine years: 294, 241, 137, 126, 93, 112, 159, 145, 121. The slight decline which these figures indicate for 1947, which would be the first year in which any effect of DDT could be expected, is obviously not

significant. The variations from year to year in the figures from the individual reporting centers are rather great. If it is assumed that the errors in this compilation would tend to be of the same order of magnitude for any given district, one gains the impression that the epidemiology of kala azar in Greece may resemble that of North China (24) where individual villages may have a number of cases over several years and then have none at all for some time. Adler and associates (10) cite as one of the main characteristics of Mediterranean kala azar its stability, with no annual fluctuation in the number of cases. While this may be true for well studied areas such as Sicily, Malta and North Africa, it has not been demonstrated for Greece. In the case of Canea, we are not convinced that the increase of diagnosed cases from about eight cases per year in 1923–1930 (10, 22) to 40 or 50 annually in the next few years was entirely due to the establishment of the Hygiene Center with facilities for diagnosis. In any case, the only available long-term data for Greece in which we have confidence are those for Canea gathered since 1931.

Oriental sore. We know of no exact data on the incidence of oriental sore in Greece, although several intense foci of the disease have long been known, notably those of Crete and Lakonia, in the southern part of the Peloponnesus (25). The common name for the disease in Crete, "Khaniotico," is derived from the name of the city of Canea (Khania) reflecting its local abundance. Most of the cases, however, have been concentrated in the old Turkish quarter, Splanzia (Fig. 3), although scattered cases have also occurred in other parts of the city and in nearby villages. Dr. Papantonakis (22) reported that between 1932 and 1934, 614 cases were treated at the Hygiene Center, but that this represented only a portion of the total since some were privately treated and many did not seek medical aid at all. Malamos (25) mentioned the occasionally epidemic nature of oriental sore in both Crete and Lakonia. Something approaching an epidemic occurred in Crete about 1938, according to information from both Dr. Papantonakis and Government medical officers in Rethimnon and Iraklion. It was apparently most marked in and near the two latter cities where the incidence, in contrast to Canea, is normally low. In Rethimnon we were informed that there had been a "great deal" of oriental sore (one estimate ran as high as 4000 active cases) about that time, but that since about 1941 there had been very little. The village of Archondiki was named as the only present center of the disease in that district. The District Medical Officer of Iraklion estimated that about 1938-1939 there had been a thousand active cases in the city but that the current level would be in the neighborhood of thirty. At the height of this "epidemic" a treatment campaign was initiated and pressed with considerable vigor in the western half of Crete, which was followed by a decline of the disease particularly in the Districts of Rethimnon and Iraklion. We know of no associated sandfly studies.

Splanzia was heavily bombed during the war. Many houses are vacant and there has been apparently considerable reshuffling of the reduced number of residents. However, among the people seen on the streets, the scars of oriental sore are relatively much more numerous in Splanzia than elsewhere in the city. This was particularly easy to demonstrate among the children who inevitably gather about foreigners engaged in a strange activity, and who would represent a more strictly local sample. Over half of such casual bystanders showed scars. Active lesions were scarce, particularly those which had developed in the last two years.

The records of the Dermatological Section of the Hygiene Center had been destroyed during the war and only those since April 1946 were available. The physician in charge estimated that he had been treating about 1200 cases per year prior to 1946, a number four or five times as great as those presenting themselves for treatment in 1932–1934 (22). His estimate was supported by the first few months for which he had records. The period 27 April to 31 July 1946 gave a monthly average of 120 cases, while the last five months of that year averaged 35. The cases dropped from 114 in June, 75 in July, to 41 in August, a figure never reached again. The monthly average was 26.5 in 1947, and 17 in the first eight months of 1948. In general the records did not distinguish between new and old cases, but in a few brief periods when this distinction was made, about two-thirds were new cases. It was the opinion of this physician and, indeed, of nearly all others we encountered in Crete, that oriental sore had declined sharply in the last two years. They attributed it to the introduction of DDT.

There are other examples of sharply restricted areas of intense oriental sore infection besides the classical one of Splanzia. There are certain "oriental sore villages." One, Daratzo (Fig. 8), lies about eight kilometers southwest of Canea, and is the only such village we learned of near Canea. Well over half the children show the scars. In other villages the disease is rather uncommon. From the frequency of the reports we received; two villages near Rethimnon have become notorious for their oriental sore, -Archondiki, about 20 kilometers west of Rethimnon, and Margarites, 25 kilometers east of that city. This region was visited in mid-October by Mr. Balodimos. Archondiki, an old Turkish village on top of a long narrow ridge, with a population of about 500, had had many cases about 1938-1939, with estimates of 100 active cases at one time. No active cases were known at present, with the exception of one of some ten years' standing. Scars were common among the children. Kufi, near Archondiki, with a population of 350, had had relatively little oriental sore, and it was said that there had been none before twenty years ago. Of 23 pupils in a school, only four had scars. One active case had developed in the past year. In Megali Episkopi, a village of 1200, about 15 kilometers west of Rethimnon, the doctor stated that there had been much oriental sore but that he had seen none in the last two years. School was not in session, but two teachers estimated the proportion of scars at thirty per cent, with no new cases among the pupils. In Maroulas, east of Rethimnon, population 450, scars were common, with no recent cases discovered. In one school in Perama, a large village 20 kilometers east of Rethimnon, 11 of 47 pupils had scars, with accounts of many cases ten years previously. Margarites, with a population of 1200, like Archondiki, is an old Turkish town situated on a long ridge. In two school rooms, 24 out of 35 and 45 out of 50 had scars. There were five active cases, one or two having developed within a year. The decrease in active cases was attributed both to the mass treatment of 1938-1939 and to DDT. In Orthes, a village of about 300, near Margarites, four out of 15 pupils showed scars. One active case had developed during the summer. The village doctor cited the decrease of new cases at the time of the mass treatment and had also noted a further decrease after DDT.

In seeking some correlation of intense oriental sore infection with the type of village and its topography, it may be noted that most of the above were old Turkish villages, located on a ridge or hillside, with stone houses larger and with more massive walls than those of recent construction, crowded along narrow streets. Of the villages near Canea, Daratzo (Fig. 8) is also of this type. It is an old Turkish village located on a low rocky ridge, in contrast with nearby villages (Figs. 2, 7) with little or no oriental sore which, whether of Turkish construction or not, are on more nearly level ground or are composed of houses scattered about among olive groves and cultivated fields. Much of Splanzia itself is on a rocky eminence overlooking the harbor.

Previous investigators have pointed out the contrast between Splanzia with its crowded masonry structures (Fig. 3) and few gardens, and the foci of kala azar in the newer parts of the city. The latter are composed of small houses, practically all with gardens (Figs. 4, 5). There is no obvious contrast, however, between the kala azar centers and adjoining non-kala azar sections. In the past few years the kala azar cases have been so few and so scattered both in the city and in villages, that it is impossible to speak of any present focus. Adler et al. (10) found good correlation between P. major and kala azar, P. sergenti and oriental sore, in the foci which they studied. There still remain to be determined, however, other factors underlying the differences between centers which are endemic for one or the other disease and those which are not, notably the types of available breeding places which favor certain species over others, and the possible reservoirs other than dogs or man.

### ITALY

A brief visit was made to Italy and Sardinia 14-30 August 1948.

Abruzzi. Dr. A. Corradetti, of the Istituto Superiore di Sanità, Rome, has in progress a large-scale experimental project for the control of oriental sore in the Province of Teramo in the Abruzzi. His survey in 1948 (26) showed that of over 28,000 persons nearly 3 per cent had active cases, with about 21 per cent showing the scars. In an area of about 200 square kilometers between the Tordino and Vomano Rivers and extending about 20 kilometers inland from the sea, practically every building has been sprayed inside, walls and ceilings, with 2.0 grams of DDT per square meter. The experimental area is surrounded on three sides by territory equally heavily infected. Spraying was begun 6 June 1948 and was about completed the middle of August. It was recognized that a pre-season application would have been desirable but causes beyond Dr. Corradetti's control had prevented it. At the expenditure of no little effort, Dr. Corradetti made arrangements for a visit to the region and was fortunately able to act as guide himself. The trip was most ably organized and in the four days 16–19 August

Our warmest thanks are due not only to Dr. Corradetti but also to Dr. Amalfitano, Medico Provinciale of Teramo, and to a number of other members of the Government and

we were able to see the work in progress and make sandfly observations both by day and by night in treated and untreated areas. Our notes and identifications were placed at Dr. Corradetti's disposition and are being included in his report, a typescript copy of which he has kindly forwarded. With his permission certain observations are repeated here.

The dominant sandfly of the endemic area in the Abruzzi has been known for some time to be P. perfiliewi (27). This species has been generally supposed to be the local vector of oriental sore (28).

Untreated area: South of the Vomano River daytime searches in several isolated farmhouses showed sandflies to be abundant in both houses and stables, but particularly in stables. Identified samples of house catches:  $1 \ \circ perfiliewi$ ,  $3 \ \circ papatasi$ ,  $1 \ \circ perniciosus$ ; stable catches:  $2 \ \circ 14 \ \circ perfiliewi$ ,  $1 \ \circ 4 \ \circ perfiliewi$ ,  $1 \ \circ 10 \ \circ$ 

The city of Ortona is apparently south of the endemic zone. Sandflies were not abundant. 5 9 papatasi were caught in one house. A sheep stable south of the city was negative.

Evening searches were made at two farmhouses on the road from Roseto to Atri, and inland several kilometers. At one, while still light at 8:30, a number were caught in the stable (3  $\circlearrowleft$  30  $\circlearrowleft$  perfiliewi) while a few were seen in the house (2  $\circlearrowleft$  perfiliewi). Most of the females had fed, probably the previous night. Between 9:30 and 10:00 it was windy, but in the lee of the stable sandflies were numerous and biting in the open (caught: 11  $\circlearrowleft$  17 unfed  $\circlearrowleft$  perfiliewi). Outside the second house near the village of Casole d'Atri, about 9:00 o'clock, when it was beginning to get dark, there suddenly appeared a considerable flight of sandflies, alighting on clothing and also biting freely. It was possible to take them three or four at a time about as fast as the suction collecting apparatus could be operated. In a few minutes 3  $\circlearrowleft$  23 unfed  $\circlearrowleft$  perfiliewi were caught.

Sprayed area: At the hotel in the town of Roseto where we spent three nights, no sandflies could be found inside or outside at night in the courtyard where there were pigs. Sandflies used to be abundant in this hotel. Various sprayed houses in towns and isolated farmhouses were negative, except for one sandfly seen in an unsprayed house in the midst of an otherwise thoroughly treated town, where they had been numerous before spraying. In one farmhouse just before the spray crew began the treatment, 3 or 7 9 papatasi were caught.

At the village of Castellalto on the evening of 18 August we witnessed an extraordinary demonstration of residual DDT "in action." This village had been sprayed 6 June. From 7:15 to 8:20, while it was still light, no sandflies were found out-of-doors either in the open or in weep-holes of retaining walls. At 8:20, as we paused in a narrow cobbled street, great numbers of sandflies settled on our party of six or eight, comparable in intensity to the flight of the previous evening in Casole d'Atri. A number were caught in a few minutes (14 unfed perfiliewi). This was near a house where we were guests of honor at dinner. At 8:30 just before being seated at the table, a sandfly was noted floating in a water

Provincial public health services and local medical men, who furnished transportation, gracious hospitality and were most helpful in many other ways.

pitcher, the first of what soon became a shower of Phlebotomus on the table. The sandflies were in evident distress. They were greatly agitated and fell frequently on alighting,—the typical reaction of sandflies after a few minutes' contact with DDT, observed experimentally (1). Those that fell on bare arms or hands sometimes succeeded in biting. Others made frantic but vain attempts, running about and lowering the proboscis repeatedly to the skin or even sleeve. About 9:00 a tour of the large room showed a number of sandflies on the floor and a few on the walls. About 9:45 it was noted that on the high ceiling above a cluster of bright lights suspended over the table, there were a great many sandflies, all in a state of agitation. It was estimated that 400-500 could be seen at one time in the circle of light. By about 10:30 there was only an occasional sandfly on the ceiling or on the table, with stragglers up to 11:30. A total of 102 sandflies (the females mostly unfed) were collected from the table, the assembled company picking them up with toothpicks dipped in wine and passing them to us. An identified sample consisted of 6 3 20 9 perfiliewi. The total number of sandflies which entered the room must have been well over a thousand.

Our host informed us that similar showers of sandflies had been observed on the table for a number of days after the spraying, but that there had been none in the ten days before our visit. Before the spraying the annoyance from sandfly bites while at table had been extreme. Villagers told us that they were now bitten out-of-doors but that the sandflies "didn't come into the houses any more."

Dr. Corradetti visited Castellalto again 1 September. At the same time and place in the street where we had encountered the outdoor attack, his party finally saw one sandfly after a wait of five or six minutes. In the dining room not a single sandfly was seen in 45 minutes. Our host informed him that there had been a few sandflies for about three days after our 18 August visit and none since. A possible explanation of the phenomenon we observed is that we just happened to encounter a flight representing a generation emerging from eggs laid before the spraying 6 June, 73 days previously. On the evening of 2 September, Dr. Corradetti visited the same place in the untreated area near Casole d'Atri where we had found the heavy flight. Sandflies were even more numerous and his party, after a few minutes of collecting, found it necessary to abandon the place on account of the number of bites they were suffering.

In our experience we have never encountered any early evening flights comparable in intensity to those of perfiliewi in the Abruzzi, with man being bitten freely in the open. This point is of immediate importance since it means that many are bitten at an hour before anyone has retired behind the barrier of residual DDT, bednets or other methods of protection. The net result would be the maximum exposure to the sandflies of the region. By the same token, the degree to which house spraying alone can eventually reduce the entire sandfly population within such an area becomes a matter of prime practical importance. Dr. Corradetti's results both in terms of sandfly and disease incidence will be awaited with much interest.

Pontine Marshes. Professor Missiroli informed us that in the Pontine Marshes sandflies had been very numerous before the DDT-malaria campaign begun in 1944-1945, and that they had been the cause of more popular complaint than the mosquitoes. They have ceased to be a problem.

An early evening search, 29 August 1948, in and around one group of farm

buildings near the Lago di Fondi, east of Terracina, was negative.

Sardinia, 23–27 August 1948. Through the kindness of Dr. John A. Logan, Director, and Dr. T. G. H. Aitken, Entomologist, of the ERLAAS anopheline eradication project, it was possible to visit a number of areas in the southern part of the Island. Beginning about the middle of November, every man-made structure in Sardinia had been sprayed during the winter of 1947–1948. While it had been impossible for the ERLAAS staff to pay much direct attention to Phlebotomus, it had been a matter of general experience that sandfly annoyance, formerly very great in many places, had ceased.

Dr. Aitken's staff had made house catches of sandflies in several villages north of Cagliari between 22 August and 12 September 1947, principally in Monastir. Four collections in this village totaled 27 ♂ papatasi and 55 ♀ of which about

half were examined and found to be all papatasi.

We made daytime searches in the villages of Muravero and San Vito, about 50 kilometers northeast of Cagliari, in Quarto, just east of the city, and in certain large occupied caves in the northern part of the city. No sandflies were found and most of the residents reported that sandflies had been abundant in former years but that there had been none in 1948. Monastir (sprayed 15 December 1947) was visited one evening about an hour after dark. Three or four court-yards with stables were negative.

Searches were made along the road in the hilly and sparsely inhabited region about 25 kilometers northeast of Cagliari. Culverts (all sprayed) were negative. Rocky crevices and recesses (unsprayed) within half a kilometer from the main road, were negative. These places were of a type which in Peru would probably contain *Phlebotomus*. No caves were found in the region. The lack of sandflies on these uninhabited hillsides is naturally not to be associated with any control measures. Along the road to Sanluri, running through a well cultivated region, recesses in exposed rocky surfaces were searched without success, with the exception of one small cave at the north edge of the village of Villagreca, 30 kilometers north of Cagliari. In this unsprayed cave, 60 meters from the nearest sprayed house, on two different days prolonged search netted a total of ten sandflies: 5 & 2 & perniciosus, 1 & perfiliewi (the first record, we believe, from Sardinia), and 1 & 1 & minuta group. These were the only live sandflies we saw in Sardinia.

These limited observations combined with the reports of the people indicated that sandflies were still at an extremely low level eight or nine months after the winter application of residual DDT.

# SUMMARY AND CONCLUSIONS

An evaluation in terms of *Phlebotomus* control has been made of the extensive DDT-malaria campaign which has been in progress in Greece since 1946.
 Observations were made in Attica and Crete throughout the sandfly season of 1948.

In unsprayed places the sandfly population was normal in abundance and distribution of species when compared with the reports of previous investigators. Nearly 3000 sandflies were caught and identified.

 Sprayed buildings were uniformly negative for Phlebotomus of any species. Night observations out-of-doors showed sandflies to be at a very low level within sprayed areas. The testimony of the people was solidly to the effect that sandflies had been annoyingly abundant but ceased to bother them indoors after the first treatment, and that there has been little or no annoyance outdoors at night.

4. The reduced sandfly abundance in the cities of Athens and Canea, Crete, which have not been sprayed in toto, may be due in part to the peripheral effect of the many treated buildings scattered through the urban areas, and in part to

the use of household sprays—factors which have not been measured. A modification of the oiled paper trap is described.

6. The present status of leishmaniasis in Canea is compared with that of

previous years.

 a. A very marked decline of kala azar had occurred prior to the use of DDT, and was associated with the destruction of infected dogs and the general reduction of the dog population. With the return of dogs to normal, it is difficult to assess the effect of the present low sandfly level because of lack of information about factors such as other reservoirs and normal kala azar cycles.

b. A sharp drop in oriental sore with the development of relatively few cases

in the last two years, coincided with the introduction of DDT in 1946.

c. Definite conclusions on leishmaniasis control must await long-term observations in endemic foci.

 A recently initiated project for the control of oriental sore in the Abruzzi, Italy, was visited. Observations on the dominant sandfly and supposed vector, P. perfiliewi, were made in treated and untreated areas. There was witnessed the actual destruction of great numbers of sandflies on encountering residual DDT, resulting apparently in the progressive reduction of the local sandfly population.

8. In Sardinia, where every man-made structure has been treated with DDT, houses were negative eight or nine months later, and sandfly incidence was at

an extremely low level.

9. On the basis of the present and previously published work, the following general conclusions seem warranted:

a. Treatment of interiors with residual DDT gives immediate and virtually

complete protection from sandflies indoors.

b. House spraying alone, in compact communities, with an annual, preferably pre-season, treatment, eventually reduces the Phlebotomus population within the sprayed areas to near the vanishing point.

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## PLATE I

Fig. 1. VILLAGE OF MELISSIA, NEAR ATHENS
Small houses widely separated in the midst of vineyards and cultivated fields.

## FIG. 2. VILLAGE IN CRETE

Houses of massive Turkish construction, crowded together, the surrounding rolling or hilly terrain with olive groves and cultivated fields.

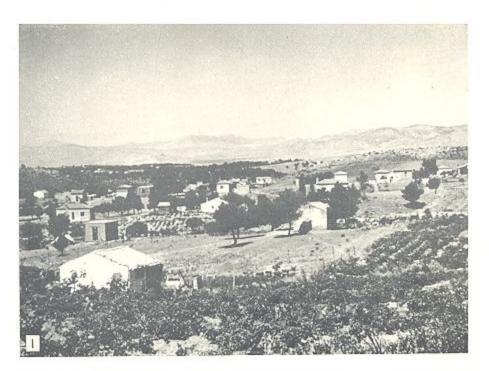




PLATE I

## PLATE II

Fig. 3. Canea, Crete

Narrow street in Splanzia, the old Turkish quarter; few gardens, little vegetation; an intense focus of oriental sore.

## Fig. J. Canea

Agios Ioannis, a small section at the edge of the city; practically all houses with gardens; formerly an intense focus of kala azar. Note contrast with Splanzia (Fig. 3).





PLATE II

# PLATE III

Fig. 5. Garden in Agios Ioannis

Fig. 6. Sandfly Trads, Made of Paper Tacked to Wooden Frames and Smeared with Castor Oil; Cave Near Canea



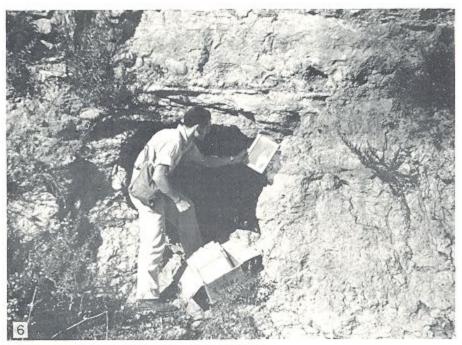


PLATE III

# PLATE IV

Fig. 7, Village of Parigoria, Near Canea, Where Kala Azar Has Occurred Sporadically

Small groups of houses scattered among vineyards and olive groves.

Fig. 8. Village of Daratzo, an Oriental Sore Focus Near Canea.

An old Turkish village on a low rocky ridge; road leading down from side of village to surrounding olive groves.





PLATE IV