# ORGANIZATION REGIONAL OFFICE FOR THE EASTERN MEDITERRANEAN

#### ORGANISATION MONDIALE DE LA SANTÉ

### BUREAU RÉGIONAL DE LA MÉDITERRANÉE ORIENTALE

### SECOND REGIONAL CONFERENCE ON MALARIA ERADICATION ADDIS ABABA, 16 - 21 NOVEMBER 1959

'M/ME-Tech, 2/40

16 November 1959 English Only

## PRELIMINARY INVESTIGATION ON THE ECOLOGY OF A. PHAROENSIS IN (EGYPT) U.A.R.

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Dr. A.P. Zahar, Senior VHO Adviser, T. Zaghloul, Assistant Entomologist & K. Thymakis, VHO Entomological Assistant Regional Malaria Eradication Training Centre, Cairo

The species of  $\Lambda$ , pharoensis was regarded by Kirkpatrick in 1925 as a potential malaria vector in Egypt but no evidence was given until 1936, then its infectivity was demonstrated simultaneously by Barber & Rice and by Hadwar on specimens brought from some localities in Egypt where a sporozoite rate of 0.33% was obtained by the former workers and 1.4% by the latter. regard to the habits of ... pharoensis, Barber & Rice remarked that it occured sundantly in areas of rice cultivation but was scarce in houses and stables while it showed marked preference to congregate in tents. Farid (1937-1942) observed ... pharoensis resting in rice fields. Concerning the Anthropophilic index, the precipitin tests made by Barber & Rice in 1937 showed that A. pharoensis collected from human habitations gave 97.5% positive for human blood; from stables this percentage fell to 10.8%. Vith the selection of the Field Training Area in the direct vicinity of Cairo, the writers were given an opportunity to investigate the habits of the species. Preliminary studies made in May and June 1959 showed that the Field Training area is a malarious area and that A. pharoensis is the only known vector present there. In a survey made in June 1959, density of A. pharoensis in houses, during daytime hand captures, was 0.23 mosquitos per man-hour and by flitting an average of 1.76 rosquitos per room was obtained. In most daytime collections there was a preponderance of blood fed and half gravid females while the gravid were either deanty or absent. The present investigation was intensified at selected farms in the T.T.A. with relatively large patches of rice cultivation. It started on 12 July 1959 with observations on outlet traps which proceeded regularly until about the end of October 1959. Various attempts were made to study the noctural activities of A. pharoensis and a few trials were also made in respect of determining the time and stages of its exodus from houses.

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The presence of A: pharoensis in natural outdoor shelters also occupied part of the present study. To determine the anthrophilic index, blood meal samples were taken from various resting places and sent to the Lister Institute for identification. The results which have been received so far are given later.

It should be emphasized that data presented in this paper is only preliminary and views given are tentative pending further confirmation. The whole work done within four months period may only be considered as an approach to the problems connected with the principal vector in the U.A.R., that may be useful for further studies to be inaugurated during the preparatory phase of the Halaria Eradication Campaign of Egypt.

#### Description of the area:

The field training area is a sector selected in the southern part of the Nile Delta, which is known as Ciza Province. More precisely, the area occurs at about 12 km. north-west of Cairo. About 25,000 inhabitants live in the area, distributed on four villages and 68 scattered farms. exception of the unlevelled edge of the western desert, the area is a flat land covering 57 sg. km. of arable land. Land is utilized in mixed crop cultivation such as cotton, rice, corn, vegetables and others. cultivation is patchy and may vary from one year to another. not received any house spraying or larviciding. The present entomological investigations were concentrated in a few farms which had rice cultivation in the summer of this year. In the background there is one village, Kombera, of about 2,522 inhabitants which was also subject to observations in the course of the general routine monthly malariometric and entomological surveys of the 🔖 whole area.

Malariometric surveys were carried out by the Training Centre personnel from pril 1959 until October 1959, as shown in Tables (1) and (2) on page 3.

The mean temperature and average relative humidity during the period of observation, according to data collected by the Egyptian Metereological Office for Giza Province, were as follows:

Date	Mean temperature oc	R.H. %
11-16/7	25.7	59.2
18-23/7	28.3	51.9
25-31/7	27.7	59•0
1 - 6/8	27:4	62.9
8 -13/8	27•5	63.4
15-20/8	26;3	64.1
22-27/8	26 <b>;</b> 4	63.9
30/8-3/9	26.4	66.1
5/9-10/9	26.3	65,1
12-17/9	22,5	57.1
19-24/9	23.5	65 <b>.1</b>
26-30/9	23.5	65,3

TABLE (1) Parasite Survey in localities of the T.T.A.

where the Entomological Investigations were carried out.

Place			Kon	bera	Vılla	age				8 Par	rms			
Month	Jun	e ·	July	•	Aug.		Oct.		Jul	7		Augu	st	Oct.
Age Group	Ľx,	Pos	űx.	Pos	Ex.	Pos	Ex.	Pos.	Цх.	Pos,	Ex.	Pos.	Ēx.	Pos.
Infants	91	0	51	0	99	0	81	11.1	2	-	3	0	<b>*</b>	_
1,- 2	2	100	30	12.3	-	_	41	14,6	2		1	0	-	-
2 - 9		-	150	8	-		121	15	21	4.7	10	20	8	0
10 +	-	-	409	2.8	-	_	3	33.3	63	7.9	27	7•4	19	21
Total	93	2	640	4.2	99	0	246	10,1	88	6.8	41	9,8	27	14.8

No survey was made in Kambera in September and no survey was made in the 8 Jarms in June or September

TABLE (2) Parasite Survey in all villages and farms of the Tield Training Area.

Place		Á11	vıl	lag	es C	EA				Λ	11 F	arms	FI	'A		
Month	Ju	ne	hug		Sep	t.	Oct	,	Jul	y 1	lug.	S	ept	•	0c1	t.
Age Group	Ľx.	Pos.	Ex.	Pos	'Ex	Pos	. Ex	Pos	Ex	Pos	Ex	Pos.	$\mathbb{E}\mathbf{x}$	Pos.	.Ex.	Pos
Infants	611	.16	498	•6	414	2.4	134	4.4	18	5.5	15	13.3	3	66.6	6 62	22.5
1 - 2	-	-	-	-	280	3.9	76	5.2	18	5.5	16	18.7	2	0	31	25.8
2 - 9	-	-	_		1127	5,7	932	3.8	119	13.	3 12	9 13	•9	9 22	262	2 21.3
10 +	-	_	_	-	112	7.1	46	4.3	283	4.2	332	5.4	15	0	619	.8
Total	611	.16	498	<b>,</b> 6	193	3 4.	8 11	88 4	439	6,8	492	8,3	29	13	974	13,8

No survey was made in villages during July No survey was made in farms during June The above data shows the melariousness of the Field Training Area, where the entomological investigation was carried out. It shows also that most infection is localized in farms. This may be explained by the fact that the close proximity of farms to rice fields renders the small human population more exposed to a higher biting rate, consequently to a higher risk.

However, transmission in the ITA on the whole seemed to have been of a low pace which was greatly enhanced in October. All positive cases were identified as P. vivax, the other species have not so far been encountered.

#### IETHODS

#### I) Outlet window traps.

The main objective of the use of window traps was to collect information on the exodus of  $\Lambda$ , pharoensis in unsprayed areas under natural conditions. Three traps were installed in five rooms selected in three farms, as defined in the following:

Trap 1 - was fixed onto a window facing north in a bedroom of a mud house. Observation with this trap started on 13 July 1959 and is still proceeding. The room has only one entrance which opens into a lobby.

Trap 2 - was fixed onto a bedroom window facing south, in the same house, opposite to the above. Operation of this trap started on 13 July as above. The room has only one entrance, a door opening into a lobby.

Trap 3 - This trap was installed in another farm, fixed to a bedroom window facing north. This room had only one door which opened also north connecting the room directly with the outside. It was found necessary to fix a screen of jute cloth on to the outside of the door to eliminate escape of mosquitos through small holes that occured between the door frame and the walk. The operation of this trap started on 24 August 1959.

The traps used in this investigation are of the portable type, which have wooden frames, the dimensions of which are 35 cm x 35 cm x 35 cm to which is attached a cone of mosquito netting allowing passage of mosquitos that fly out of the room to the confinement of a mosquito net cage-like trap around the cone. To avoid loss of time, arrangements were made to remove the traps when each had mosquitos and replacing it by another empty trap. Inhabitants were instructed to sleep inside and close the door at dawn, keeping it closed until time of collection which was usually between 8.30 and 9.30 am. Traps with mosquitos were transported to the laboratory for Although this was generally followed, some irregularities examination. The number of days of operation of the traps per could not be excluded. week varied, thus the average for the week is represented by the average mosquitos collected per trap per day. Collections from rooms of traps were made every second day.

In the initial stages of the investigation, determination of Solla stages was made on trap collections but later, after staff was trained, Christopher's stages also were simultaneously determined and later an attempt was made to determine the proportion of Mulliparous and parous females, but on a few numbers, thus it is not included here.

#### II NIGHT OBSERVATIONS:

Few night observations were planned during July and Lugust 1959, attn a view to determining the onset of activities of A. pharoensis, nightly influx, indoors and outdoors biting habits. In the first night observations, the first writer noted the presence of A. pharoensis population post feeding resting on cotton and okra plants (Hibiscus escolentus) and on the outside of walls outdoor, hence collections from such situations were made systematically through the hours of the night and in further night observations.

Samples of blood meals vere taken from mosquitos resting on cotton and okra plants and from outside walls and were sent to the Lister Institute for precipitin identification.

Collections of mosquitos during night capture were made on subjects sitting or lying outdoor near houses and subjects sitting or lying. Indoor. The collection at each situation was made by hand cipture by one collector for 15 minutes every hour of the night from the onset of biting activities until the time when the activities were fully depressed. Then a collection was obtained from two subjects, the figure was reduced to one. In all observations mosquitos were collected when they were on actual bite.

#### I OUTDOORS DAYTING RESTING PLACES:

The objective of this trial was to confirm the outdoor resting habits of part of the <u>n. pharoensis</u> population which was observed earlier by Farid, and to classify mosquitos obtained from outdoors shelters according to Sella and according to the results of the blood precipitin tests.

after preliminary searches in various types of vegetation, it was found that ... pharoensis populations favoured rice plants as a daytime resting place. Collection from rice plants by hand capture was strenuous, time consuming and yielded only a small number of mosquitos. Hence a cubical tent of 143 cm x 143 cm with an overall height of 155 cm. 15-20 cm of which are pushed into the soil, was devised by the first writer. The roof and entrance of the tent were made of mosquito netting but the walls were made of canvas. The tent, which can be installed within 10 minutes is later lifted and dragged in rice fields for further placements. In each placement, two collectors went to the inside and the door was tightly closed. The plants were shaken, mosquitos were seen on the canvas sides of the tent, whereby they were collected by suction tube. Collection of each placement was restricted to 15 minutes by two collectors. Most collections were identified by blood digestion stages of Sella (1920) and blood meal samples were sent to the laster Institute for precipitin determination.

#### IV TRIAL FOR DETERMINING TIME EXODUS:

In the course of study of the exodus of A. pharoensis, time only allowed for two trials which should only be taken as an approach to the question. During night observations, rooms of Traps 1 and 2 were utilized. The tent devised by the first writer for regular collection of mosquitos, was also introduced in this trial after being fitted with an outlet trap.

In the two trials made, the doors of the rooms and tents were kept opened until 1 am. after which they were kept closed and collection from traps was made at hourly intervals. The human subjects who slept in about 10 pm were allowed to go out at 5-6 am and the door of room No. 1 was kept closed until 9 am, only while that of No. 2 and the tent were kept closed continuously until 9 am, 48 hours later. During the 48 hours too, collections were made from each of the three traps at hourly intervals, except during night periods extending from 8 pm to 8 am.

More details on these trials are given later.

#### RUSULTS OF VARIOUS INVESTIGATIONS

#### II - NICHT OBSERVATIONS:

For convenience, results obtained from night cbservations are presented before proceeding to other observations. Might observations were conducted on 22 July 1959, 5, 19, 26 August Results of the 15 minute captures made at various sites every hour of the night are illustrated in Figs. 1, 2, 3 & 4. Initially it was difficult to find access to human subjects in the local mud houses but this could be solved later in observation of 26 August. Apart from the latter observations, captures were made not/regularly as inhabitants /ver Indoor counts taken from the tent device did not retire before 11 pm. were more controlled as the subject was a labourer temporarily employed.

It should be emphasized that observations were made at summer time which was one hour in advance of the local time. Local time being two hours in advance of Greenwich Mean Time. vations made at different dates were not identical. This might have been due partly to changes in climatic conditions and partly to individual variation caused by change of collectors in some nights. Nevertheless, the observations exhibited common characteristics.

First, all observations showed that A. pharoensis is an early biter. During the period of observations it started biting about 20 minutes after sunset. To illustrate, data of collections made at the very onset of biting activities up to 10 pm. is shown in the following:

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Site	Date	Number collected in 15 minutes per person by one collector	% of total nightly collection
From human outdoors	22 July 20 August 26 Aug.	42 27 27•5	30.2% 19.6% 39.5%
Tuman in tent device	20 Aug.	ı	2.1%
Turan in local mud house	26 Aug.	1.5	3. <i>3</i> %
From camel outdoors	22 July 20 Aug. 26 Aug.	308 35 16	49 <b>:3%</b> 18.7% 15.0%

The average number of bites received per person per 15 minute period throughout the night is given in Table (3). During the period of observations this average was fer human indeors 3.75 - 4.6 bites; for human outdoors 6.3-13.9; for human in tent 4.45 - 7; for human in rice fields 10.2 and for animals outdoors 11.3 - 62.5.

Second, biting activities in most observations were suppressed about half an hour before sunrise. Third, in observations of 5, 20 and 26 August, there was a tendency to renewed biting activities on animals outdoors, in the last hour before activities were finally depressed, while on humans activities gradually declined. Fourth, in all observations there was a tendency towards reduction of activities shortly after the initial peak, then mosquitos behaved differently later in various nights. For example, on 20 August, the level of biting activities remained much the same without a distinct peak for about 6 hours in the middle of the night, whereas on 5 August, activities fluctuated frequently.

Further the trend of biting activities on humans indoors seemed to have neen almost parallel to that of the outdoors, with some indication that biting outdoors was more intense on humans and even more exaggerated on animals. It is rather difficult, however, to draw a valid conclusion from a few observations in which many variables were involved.

#### III - TRIALS FOR DEPERMINING TIME AND STAGES OF EXODUS OF A. PHAROENSIS

Only two trials could be made in the course of this investigation, during night observations.

The first trial was started on Wednesday 19 August 1959 and comprised the bedroom trap (No. 2) facing south and trap (No. 2a) facing east, installed in the tent device near human habitation. Mosquitos were cleared off at the start from the bedroom and the tent. The door, which is the only entrance to the bedroom, was kept wide open for 5 hours for mosquito entry starting from the onset of biting activities, while that of the tent was also used for 15 minute night captures at hourly intervals. i.e. it was exposed to mosquito entry for about 10 hours.

Clearance of the mosquitos from traps was made hourly until midnight. The door of the room was clos ed on subjects, sleeping inside, at 1 am. of Thursday 20 August 1959. This door then had to be opened for a few minutes to evacuate subjects at 5-6 am. but was continuously sealed thereafter until 8 am. Saturday 22 August. The door of the tent was closed from 6 am. Thursday 20 August until the end of observation on Saturday 22 August, as above, i.e. closed for about 50 hours.

The second trial which started on Wednesday 26 August 1959 comprised the above bedroom and the tent operated in the same manner as in the first trial. In addition, two more traps were added, bedroom trap (No. 1) facing north and bedroom trap (No. 3) also facing north, installed in an adjacent farm. In this trial these two traps were operated in the same way as they were operated regularly in the prolonged investigation in order to assess the validity of results of observations made from 13 July to 31 October 1959. In both rooms human subjects slept and were evacuated about 5-6 am. of the 20th. Doors of the rooms with traps (1) and (3) were operated in the same manner up to 8 am. of the following day, Thursday 27 August. Then they were opened all day until 6 pm. when they were again closed until 8 pm. Thence, doors were reopened and inhabitants slept in and were instructed to close doors about 5-6 am. Results of the collections made are according to the following schedule:

	Time	Doors of T (2) & (2a)	raps Doors of Traps (1) & (3)	Collection from traps
'ed/Thur, 1st night	8pm-lam	opened	opened	hourly
Thursday, 1st day	1em-8pm	closed	closed	hourly
Thur/Fri, 2nd night	8pm=8am	closed	closed 5am-8am	8am Friday
Friday 2nd day	8am-8pm	closed	closed 6pm-8pm	hourly
Pri/Sat , 3rd night	8pm-8am	closed	closed 5am-8am	8am Sat.
Saturday, 3rd day	8am-9am	closed	closed	final observation

From the above schedule it is clear that 8 am. collections of the 2nd and 3rd days represented exodus throughout the previous night. - Mosquitos were removed from traps and immediately classified according to Sella's stages.

The results obtained from the two trials, Table (4), (5) and (6), indicated a considerable variation in the exodus of A. pharoensis population. In rooms that were allowed opened for mosquito entry the first half of the night and then sealed for about 56 hours, exodus of mosquites was not manifested until they became at the latter stage of the gonotrophic cycle. In the first trial (trap No. 2) about 35% of the total exodus of mosquitos was made by females in Sella stages V and VII after 31-32 hours and about 38.7% made exodus when they were full gravid after 41-42 hours. The loss of one observation at 19 hours did not allow drawing firm conclusion from the above observations. However, a week later the second trial gave a reading of exodus after 19 hours. Mesquitos came out after 19 hours in

the second trial, constituted 5.9% as Sella stage I; 9.8% as Sella stage V; 31.4% as Sella stage VI and 5.9% as full gravid in stage VII.

The presence of a proportion of gravid females after 19 hours from closing the door or 24 hours after the onset of biting activities, was also observed in mosquitos coming out of the tent trap at the same time.

It may be superfluous to assume in the present circumstances that such females were early biters which could secure a blood meal at the very onset of biting activities, as the doors were kept opened for about five hours in the first half of the initial night of observation. Also because in the two trials there was exodus at earlier stages in some traps, it may be possible that some females in Sella stages IV & V. on being interrupted from some other indoor resting places, may seek shelter indoor again. But again the question is further complicated if it is considered in the light of some other observations made by Zahar and Thymakis (1959) during their studies on the susceptibility of A. pharoensis to insecticides. In order to obtain an idea on the progress of blood digestion, batches of mosquitos were kept individually in tubes parallel to those which were under observation post exposure. These observations indicated that amongst mosquitos collected in the morning as Sella's stages II & III and confined in tubes the same morning, there were in some observations a few that could reach Sella's stage VI and an odd one reached Sella's stage VII. at the evening of the same day. The majority were in Sella's stage VI in the following morning, as shown in the following:

Date	Time of con-			Sella	s sta	ges		Remarks
	finment in tubes	II	III	IV	<b>V</b>		VII	
5 Sept.	9 am	20	_		_	_	<b>7**</b>	
#1	12 noon	16	4	-	_	-	<b>i</b> m.	
11	2 pm	2	18	-	-	_	244	10 were taken
Ħ	4 pm	-	2	8	_	-	-	for suscep-
11	6 pm	_		7	3	_	***	tibility test
11	8 pm	_	-	5	<b>3</b> 3.	2	- 🚙	
5 Sept.	li am	15	- 2		2			e e • •
ti	2 pm	1	2	14	0	2	-	
Ħ	3.30pm	-	3	14	O.	2	~	
11	4:30pm	~_	3	14	0	2	-	
Ħ	6.30pm	-	-	11	5	2	-	
41 .	8 pm	_		8	7	-2	1	
7 Sept.	11 sm	2	8	-	-	-		
11	1 - pm	-	10	<del></del>	-	-	_ ==	
स	3 pm	-	2	8	-		-	
41	5.30 pm	-	_	8	2	-	-	
Ħ	7 pm	-	-	8	2	-	-	
8 Sept.	8.30 am	-	-	•••	-	10		
8 Sept.	10 am	4	5	1	-	***	-	
<del>1</del> 1	1 pm		8	1	1	***	-	
11	3.30 pm	-	3	6	1	-	-,	
11	6 pm	_	1	7	1	1	-	
9 Sept.	9 am	-	-	-	1	7	1	

No conclusion can be drawn from the above observations in regard to the duration of the gonotrophic cycle as the exact time of feeding could not be known.

Until further trials are made which will be supported also by determination of the state of ovaries by Chritophers in the females that reach Sella's stages VI and VII, it may be reasonable to suggest that the conotrophic cycle in  $\frac{1}{100}$ , pharoensis may take about 48 hours at a mean daily temperature above  $26^{\circ}$ C.

In the two trials, A. pharoensis exodus exhibited two patterns, the first was that of the mosquito which did not leave the rooms except in later stages of development; the second was that of the exodus at early stages as was observed in trap (3) in the trial of 26 August and trap (2a) of the tent in both trials. By studying conditions at various resting sites. it became obvious that a. pharoensis tend to leave the indoor resting place under the influence of their unsuitability. In addition to microclimatic factors, disturbance may be considered as an additional factor influencing exodus of mosquitos. This was clearly demonstrated by the exodus of recently fed females in Sella stage II in the early hours of the morning starting at 3 am and ending with maximum exodus between 6-7 am (summer time) amounting to almost 30% of the total exodus of mosquitos into trap (2a). Another turn of exodus was observed in this trap after 24 hours had elapsed since the onset of biting activities, but mosquitos of this batch had made much advance towards completion of the gonotrophic cycle, as they were mostly in Sella's stages V and VI and a small minority in stages III, IV and VII. It may thus be postulated that, though a, pharoensis in other observations, showed marked preference to tent shelters, they are easily driven away partly by sharp changes in microclimatic conditions during the daytime and partly through disturbances that are likely to occur in a structure such as this.

Exodus of 1. pharoensis however was not restricted to tents but similarly almost the same phenomenon was observed in the trap (3) installed in a bedroom, but at a relatively reduced rate. Between 5-6 am, a number of mosquitos amounting to 5.6% of the total exodus left in Sella stage II

similar to what was observed in the tent, another exodus turn was observed but at a later date, 36 hours from the initial onset of biting activities. As the door of this room was not kept closed all the time as that of room of trap (2), there was a renewed entry in the following night and a proportion of freshly fed females appeared simultaneously with the gravid females of the initial entry of the first night of observations.

It is probable that again disturbances drive mosquitos to leave the indoor resting place, even in permanent structures. Observations made prior to installation of the traps showed that this room had widely varied density. In comparison with room of trap (2) this room was found liable to get more frequent disturbances. In rural areas in Egypt, the smoke generated from home stoves, in which dried stems are burnt, may be considered as a factor of disturbance to A. pharoensis.

#### RUSULTS OF REGULAR OBSERVATIONS BY OUTLET WINDOW TRAPS:

Identification of window trap collections made 8.30-9.30 am throughout the period 13 July-31 October 1959 by Sella's classification, provided some understanding on the problem of 1. pharoensis exodus, the results of which are illustrated in Figs. 5, 6 and 7. Combined Sella's/Christopher's classifications, which started 24 August 1959, gave better understanding. The total exodus during the above period as classified according to both classifications, is summarized in Table (7).

For comparison, collections from rooms of the traps, which were made every second day starting from 24 August, are also classified in the same table.

It should be pointed out that the present data for exodus at these stages does not well represent the actual happenings. In these observations mosquitos were collected from traps about 8.30-9.30 am and were not examined before 10am. In this respect, it should be emphasized that mosquitos classified as Sella stage II in the present investigation was based on the number of terga and sterna free of blood. It has already been shown from the special study of time of exodus (page 11) that in some traps a good proportion of recently fed mosquitos, Sella stage II, left indoor shelters between 3 am and 6 am, Accordingly, for the interpretation of the present data, a delay of 4-8 hours must be considered as caused by the retarded collection and examination of trapped mosquitos. Therefore the figures obtained for exodus made at the earlier stages of the gonotrophic cycle particularly those of Sella stages II & III, do not represent the real exodus made at this stage.

A. pharoensis exhibited exodus from mud rooms in various stages of the conotrophic cycle. If the total exodus of the traps in the whole period is considered, about 42.6% as Sella stage I, 3.6% left rooms at Sella stage II 4.8% at Sella stage III; 9.1% as Sella stage IV, 7.6% as Sella stage V, 23.5% as Sella stage VI and 8.2° as Sella stage VII. The unfed females were the most predominant while the proportion of full gravid was much smaller. The appearance of a large proportion of the unfed females in the traps at daytime did not necessarily mean that these were seeking outdoor shelter. They were probably trapped during the night when they were moved by hunger to leave the room in search of a host elsewhere. The small proportion of Lella stage VII indicates that a higher proportion of mosquitos leave the indoors before reaching this stage, as was almost constantly observed.

From data in Table (7) there was a low proportion of the unfed in room collections obviously due to large exodus made at this stage. The other stages were reasonably well represented in the rooms but again the small proportion of the gravid and pre-gravid Sella stages VI and VII is due also to their exodus. This indicates that in spite of A. pharoensis exodus observed, there was part of the population that seemed to have favoured the indoors resting places for completion of their genetrophic cycle.

The above observations have shown, however, that under the local conditions of the present investigation, part of A. pharoensis population remains indoor until it has almost completed its gonotrophic cycle, while the others tend to leave indoor shelters at earlier stages as blood fed and half ravid.

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Even this · should not be considered as valid until supported by further investigations by inlet traps for exploring the possibility of re-entry of such stages.

Related to this information, the study of trap components by Sella's classification and Christopher's classification provided some information on the process of development of the ovaries in relation to the stages of digestion of blood. The most important finding was the presence of certain proportion of females, a bout 0.7% of the total trap collection, with abdomen appearance often not completely distended, as in Sella's stages VI and VII and not resembling stage I of the unfed. A small amount of blood may be visible at the sterna of in some specimens was not visible but readily observed on dissection. On examination of the ovarian stage of development, it was found that ovaries were in stages I or II or III. As the original reference of Sella (1920) is not available, at the suggestion of the first writer, the above specimens were grouped as intermediate between Sella I and Sella II, designated as stage I-II. Pending further confirmation, this was based on the assumption that such females either had incomplete blood meal that could not produce complete development of the ovaries or were nulliparous, requiring more than one blood meal before they could complete the first gonotrophic cycle.

The study of other stages showed that mosquitos in Sella stage VI had ovaries in either Christopher's stage IV or V, but a small proportion appeared in Christopher's stage III and a negligible number in Christopher's stage II. Ith regard to mosquitos in Sella stage VII, they were mainly in Christopher's stage V and a smaller proportion in Christopher's stages IV but it was surprising to find a small number in Christopher's stages II & III. These should have been added to the newly designated stage I-II already defined above but, probably on account of fairly distended abdomens, were grouped with Sella stage VII. The presence of females with abdomen apparently resembling the later stages of the ronotrophic cycle, with delayed ovarian development which appears on dissection, provides support to the assumption that either incomplete blood meal was taken or that a further blood meal has to be taken by nulliparous females in order to complete development of the ovaries.

#### ... PHAROENSIS IN NATURAL OUTDOOR SHELTERS:

ns has already been advanced, \_\_\_ pharoeners was observed by Farid (1937-1942) resting on rice plants. The present investigation provided support to the above observation and provided also data on stages of the ronotrophic cycle that are exhibited by part of  $\Lambda$ . pharoeners population that favours this external natural resting shelter. From results obtained from various placements of the tent device described earlier, at different hours and dates, it is obvious that the recently fed Sella stage II and the subsequent stage Sella III were invariably encountered in collections made in the forenoon. Stage II was particularly obtained in appreciable numbers around 8 am. In a single observation, an odd female with conspicuous fresh blood, i.e. strictly stage II, was encountered amongst a collection made at 4.30 pm. The presence of such specimens in such a stage of fresh blood in

the afternoon in rice fields could not be explained except by the assumption that the female must have secured the blood a very short time before it was collected. Them precipitin identification which has been received recently, this blood meal specimen, have positive reaction for human. Perhaps the female could secure such a blood meal either from our workers or from farm labourers who slept in the afternoon in the shade of trees at the edge of rice fields. From an odd observation, it is superfluous to state whether biting at daytime is a characteristic that should be added to A. pharoensis piting nabits, as such.

The outcome of 116 tent placements in rice fields made in 22 days within the period 20 July - 21 October 1959 vas 122 ... pharoensis in differ ent stages of Sella, of which 8.1% were in Sella stage I and 12.9% were in Sella stages II & III. 45.70 were in Sella stages IV & V and 30.6% in Sella stages VI & VII (5 mosquitos were in Sella stages I, VI & VII and were grouped together). Blood meal samples were taken from ... pharoensis collected in rice fields Solla stage II, V and sent to the Lister Institute London for identification.

The results of 27 samples which have been so far received showed the following:

- 7 showed positive reaction for human
- 19 showed positive reaction for animals
- 5 negative

Purther results are awaited.

#### TITPOR RESTING OF A. PHIPOINSIS

During the first night observation, the first writer was able to detect about 3-4 am, ... pharoensis engaged females resting on the outside walls, doors and jute cloth screen of houses in a small farm. Even a few specimens were found resting in old iron machinery and on the wooden parts of a cart.

Later, in subsequent night observations, resting outside ralls, doors and windows was commonly mot with. The appearance of freshly fed females on the outside walls etc., occured usually from about midnight and increased steadily until dawn when mosquitos disappeared with the advent of the first glimpse of daylight. It was found that this resting occurs whenever a nost was around exposed to mosquito bites outdoors.

In the initial observation of 20 July a man was sleeping outside near the wall and other external objects on which resting was observed. Results of precipitin identification indicated that mosquitos of that batch collected from walls and various objects near the man sleeping outside showed that 14 gave positive reaction for human and only 2 gave positive reaction for bound.

lesults are awaited of samples collected from subsequent nights.

On 22 July, the first writer was also able to detect about 4-4.30 am, mosquitos resting on cotton and okra plants (Hibiscus esculentus) which occured around houses and the yard of the small farm in which the night observations were carried out. In subsequent night observations arrangements were made to obtain 15 minute captures at hourly intervals throughout the night.

Cotton and okra plants were subject to Sella's classification and blood meal collection for precipitin.

From data compiled for three night observations of 5, 19 and 26 august 1959 Table ( ) ... pharoensis appeared in few numbers on cotton and okra plants as early as the onset of biting activities about half an hour after sunset.

Specimens collected up to 9 pm. were mainly unfed and pre-gravid and gravid and there was an odd specimen in Sella stage  $V_{\bullet}$ 

The first freshly fed specimens were observed on cotton and okra plants about 11 pm., then the number steadily increased until about 5-5.30am in two observations and about 3-4 am in the last observation of 26 August.

The number of mosquitos in Sella stage III similarly increased, while the number of the unfed comparatively fell off. Posquitos in Sella stage IV appeared also on some plants during the latter half of the night but in much smaller numbers, but mosquitos in stage V appeared more abundantly thanthose of the preceding stage. Pre-gravid and gravid appeared also on cotton and okra plants but very scarcely.

Efforts spent in locating similar resting places on surrounding vegetation such as Salix trees, mango trees and maize plants etc., were fruitless.

Results obtained from the Lister Institute on the first batch of blood meal samples from mosquitos collected from cotton plants on 23 July 1959 showed that out of 48 blood meal specimens:

- gave positive reaction for human gave positive reaction for animals (bovid, camel & dog)
- 1 gave positive reaction for both man and bovid.

The above hosts were all represented in the farm yard adjacent to rice fields. On the light of this data it is possible to postulate that such observed temporary resting is correlated with the outdoor biting activities. However, the presence of other stages as well resting on cotton and okra plants and the dispersion of such population with the appearance of daylight, lead the first writer to sugrest that it is only a temporary stance during the nightly influx of \_\_\_\_ pharoensis to and from the preferential daytime resting place, the rice fields, which were at a distance of about 100-500 m from the site of biting activities.

The observation of the pharoensis resting on cotton plants during the night may be limked with the resistance of the species to heldrin and high tolerance to DDT, raised by chemical control of the cotton leaf worm, brodenia litura, as was already advanced by Tahar and Thymakis (1959).

#### LLCULTC OT PROCESTION IN INTERIOR :

About 250 blood meal samples which were collected from various indoor resting places in the Pield Fraining area, in June and July 1959 were sent to the laster Institute for identification. Results duly received showed the following:

#### From Human Habitation:

80% gave positive reaction for human

19.1% gave positive reaction for animals

0.83% gave positive reaction for both man and bovid.

#### From Inimal Habitation:

1.9% gave positive reaction for human 93.1% gave positive reaction for animals

#### From stores:

92.8% gave positive reaction for humans 7.1% gave positive reaction for animals

The presence of a certain proportion of ... pharoensis with animal blood resting in human nabitations and vice versa is not surprising since conditions in rural areas in Typt would allow for such mixing in resting. Inimal rooms are constructed within houses and sometimes have ill-defined separation from the main lobby thich is usually surrounded by bedrooms and other rooms, such as stores.

Disturbance may cause mosquitos to abandon their original resting places.

Disturbances in the animal rooms is sometimes much exaggerated.

The results of total precipitin identification of 250 samples may be compiled in the following:

Outdoor - 41.6 gave positive reaction for human 57.6% gave positive reaction for animal 1.11% gave positive reaction for mixed

Turther results of other batches are awaited.

#### SULCARY AND CONCLUSIONS

During the period of observation in July-August 1959, biting started about 20-30 minutes after sunset, with a sudden high peak in the early hours of the night. Thence biting activities tended to decline towards midnight but rose again later. Biting activities were completely suppressed about half an hour before sunrise. Biting outdoor seemed to have been more intensive. A. pharoensis from observations and from precipitin identification proved to be an indiscrimate biter. It bites man as well as a large variety of animals such as camels, buffalos, cows, horses, dogs and mammals. Even mixed blood meals of human and bovid were noted in 1.1% of the total of 250 samples identified by precipitin. Results of the batches which have been identified so far showed 41.6% positive for human and 57.6% positive for animals. The average number of bites received per person per 15 minutes throughout the night was as high as 4.6 in mud rooms, 7 in tents and 13.9 outdoor.

From the special trials made to study the time and stages of exodus of A. pharoensis and from prolonged regular trap observations, it was shown that part of the A. pharoensis population remained indoor until it has almost completed its gonotrophic cycle while the others tend to leave indoor shelters at earlier stages, as blood fed and half gravid. This should not be considered as valid until supported by further evidence from inlet traps for exploring the possibility of re-entry of such stages to indoor. Exodus of stages V, VI & VII was observed after 24 hours from the onset of biting activities and with some other observations the gravid and pre-gravid appeared after 31-42 hours. Suggestion is made in regard to the duration of the gonotrophic cycle being completed within 48 hours. The reasons for  $\Lambda$ . pharoensis to abandon the indoor shelters are thought to be unsuitability of the resting sites by changes in microclimatological conditions and by disturbance. The disturbances may be mainly raised by domestic activities and also by smoke commonly generated from home stoves into which wooden dry stems are burnt.

It was found that if Sella classification of the trap collections was coupled with determination of the ovarian development by Christopher's, more useful information could be obtained. An important finding was the presence of a small proportion of females in exodus of A. pharoensis with abdomen appearance often not completely distended as is in Sella stages VI and VII and not resembling stage I of the unfed. A small amount of blood may be visible at the sterna or in some specimens was not visible but readily observed on dissection. On examination of the ovarian development however, it was found that ovaries were in stages Christopher's either I, II or III. Pending further confirmation it was suggested that such females either had incomplete blood meal that could not produce complete development the ovaries or were nulliparous, requiring more than one blood meal before they can complete the first gonotrophic cycle. This might increase the frequency of biting.

The outdoor resting habits of 1. pharoensis were explored. Investigations showed that rice fields are the preferential outdoor resting places for this species.

Lesquites in various stages of the genetrophic cycle were found on rice plants, be freshly fed females were obtainable in appreciable numbers in the morning out later the half gravid and gravid predominated. Identification of the plood meal samples of specimens taken from rice fields showed that about 1/4 of the specimens gave positive reaction for human blood.

nother resting hapit was detected in the course of night observations, which was found to be of a temporary nature. A. pharoensis was found resting on cotton and okra plants throughout the night, and there was a preponderance of freshly fed females towards the early hours of the morning. In further peculiarity in temporary resting habits was found post feeding on outside walls, doors and other external objects proximal to the host. This again was found to be temporary as mosquitos dispersed with the approach of daylight. Of the blood meal samples taken from the mosquitos captured from cotton and okra plants, 1/5 gave positive reaction for human. Those which rested on walls showed type of blood meal comparable to the nearest host.

The identification of blood meal samples collected from mosquitos resting in various indoor shelters showed that a proportion as high as 80% was obtained from human habitation but a smaller proportion representing arimal blood was also found at the sole time. It was also in animal habitation, besides a high number of mosquitos with animal blood, 93.1,0, there were a small number of mosquitos that had human blood. This was explained by the fact that animal rooms are situated inside human habitations, not completely isolated, and there is every possibility of interchange of resting places when mosquitos are disturbed.

From the epidemiological standpoint, the importance of the partial expyrily exhibited by  $\Lambda$ . pharoensis could not be assessed in the absence of epidemiological backing and entomological evidence on the/portion of the population that feeds or rests outdoors.

Also, it would be desirable to know if those outdoor biters seek shalter indoors. However, there is ample proof that part of A. pharoensis poulation completes its conotrophic cycle indoors and therefore are apt to sucumb to residual spraying of houses. Even that portion which showed exodis at earlier stages of the conotrophic cycle, did not leave the indoors shelter immediately and probably they left only under the influence of disturances, hence the chances are also high with this group to be likewise affected by residual house spraying.

In virtue of the above considerations, residual spraying of houses should be performed with the utmost perfection and total coverage should be stresed.

is proposed by Dr. Farid, VHO Senior Remonal Malaria Adviser, further epidemiological and entomological investigations in the presence of total coverage in a fairly large area would be desirable.

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#### ACKNOTINDER INTS

The writers wish to express their thanks to the Pinistry of Health authorities for the facilities they provided in the Field Training free for this investigation.

Thanks are due also to HO ntomolorists, Dr. Emelin, In. Clarke and Hr. Nguy for their assistance during the initial stages of the invest-iration when they were inder training with the first rriter.

The writers also wish to extend their thanks to the staff assigned from the Insect Control Section for their assistance given in these investigations.

THE LYKELGE NUMBER OF BITHS RECEIVED BY ONE PERSON PUR NICHT... COLLECTION OF 15 MINUTES, EVERY HOTR, THROUGHOUT THE NICHT

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FIRST TRILL OF EXODUS OF ... PELROENSIS - 19-22 LUGUST 1959

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26 - 29 ...UGUST 1959

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TESTE (7) TOTAL COLLECTIONS FROM TRAPS AND ROOMS

24 AUGUST - 31 OCT BER

ell <b>a</b>	Christ	Traps		Rooms	
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_		152	14.5	38	26.7
٧	I III IV V	0 1 53 34 2	0 0.09 5.05 3.2 (.19	0 0 <b>34</b> <b>2</b> 0	0 0 23.9 1.4 0
		90	8,6	36	25.4
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TILE (7) continued:

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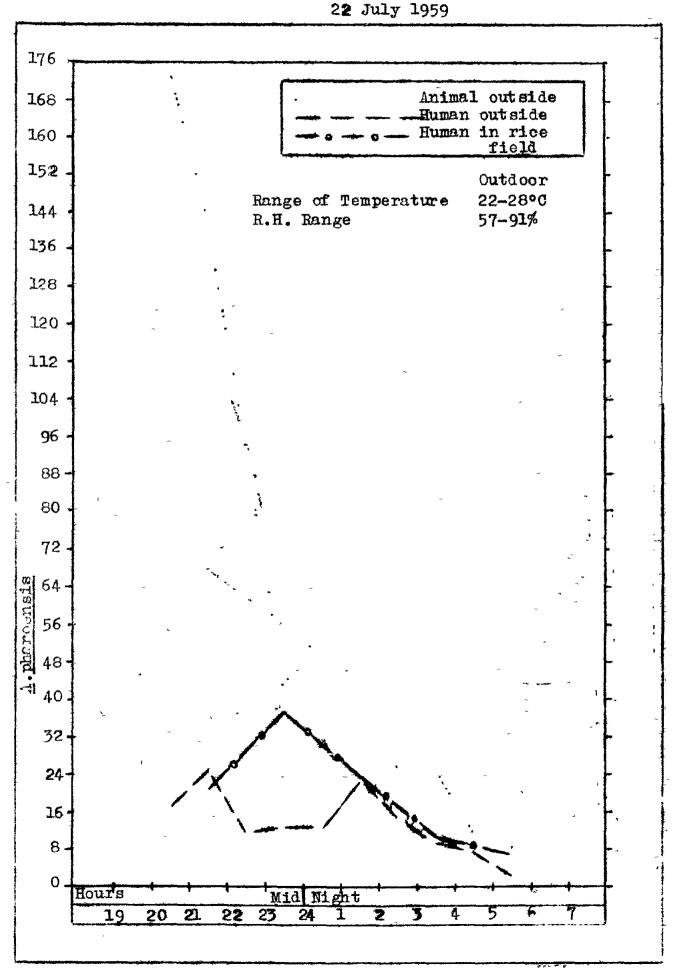
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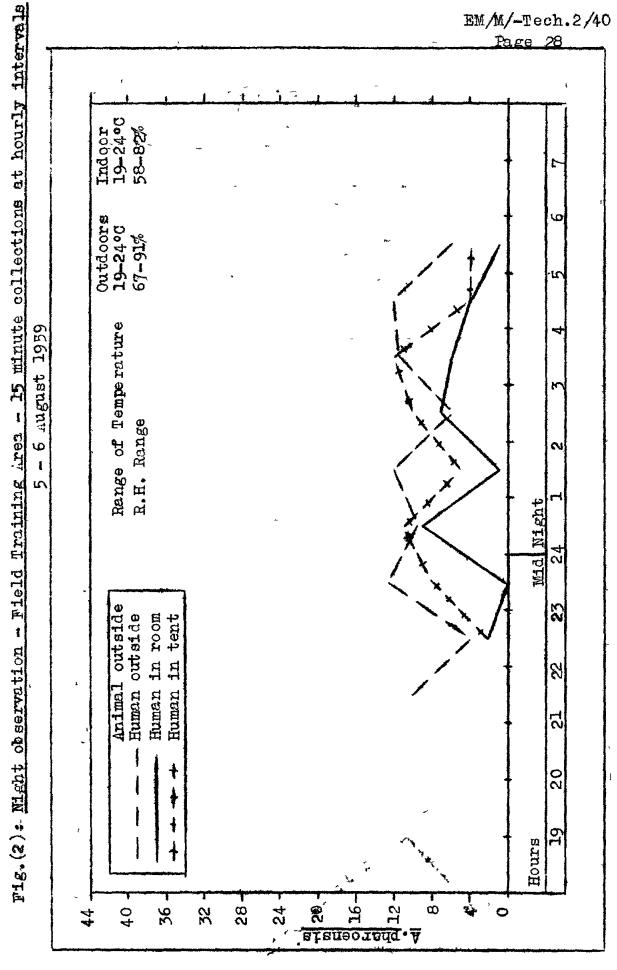
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.ugust 1959

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Fig.(1): Night Observations, F.T.A. 15 minute collections at hourly intervals.





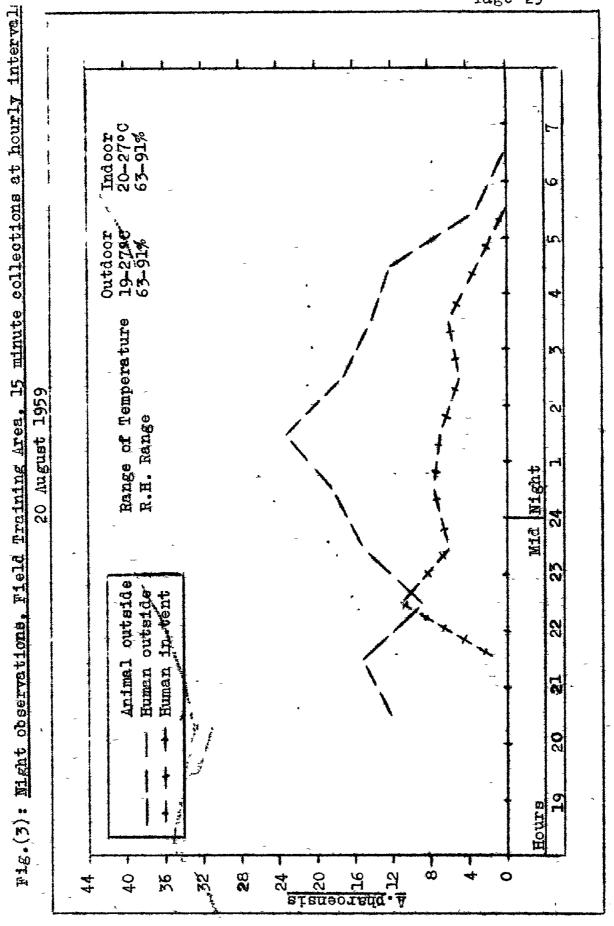


Fig. (4): Night observations, Field Training Area, 15 minute collections at hourly intervals 0utdoors 19-25°C 57-100% Range of Temperature R.H.Range 26 - 27 August 1959 Mid Night Animal outdoor Human outside Human in room S Hours 8 40 90 22 8 stensorend. A 44 0

