

## HIBERNATION OF MOSQUITOES IN KOREA

C. H. WHANG, M.D.

Central Malaria Eradication Service, Ministry of Health and Social Affairs, Republic of Korea, and Department of Parasitology, Yonsei University, Medical School, Seoul, Korea

Korea is located between  $124^{\circ} 11''$  and  $131^{\circ} 52''$  East-longitude and between  $32^{\circ} 30''$  and  $43^{\circ} 00''$  North-latitude. It is in the temperate zone and has four distinct seasons.

The meteorological data for the period of the present study were obtained in Seoul by the Central Meteorological Office, as given in Table 1.

Mosquito-borne diseases, including malaria, filariasis, and Japanese B encephalitis, occur in Korea. *Anopheles sinensis* Wiedemann has been regarded as a malaria vector, and *Culex tritaeniorhynchus* Giles as an encephalitis vector. The vectors, or vector of filariasis are not yet definitely known.

Kobayashi (3) reported that *A. sinensis* passes the winter season in the adult stage.

The present investigations on mosquito hibernation and physiology were carried out systematically in the vicinity of Seoul during the period of November 1959–April 1960. Random survey was also done in Kimhae, near Pusan (southern part of Korea), in December 1959.

THE MOSQUITO SPECIES AND THEIR HIBERNATING PLACES. During the survey, 5,262 adult mosquitoes were collected. All were females except six male *Culex* (*Culex*) *pipiens* Linnaeus 1758. *C. pipiens* occupied about 97 percent of the total number caught; the rest were *Anopheles* (*Anopheles*) *sinensis* Wiedemann 1825, *Anopheles* (*Anopheles*) *sineroides* Yamada

1925, and *Culex* (*Culex*) *orientalis* Edwards 1921.

Searches were made in numerous localities for what appeared to be suitable hibernating places (4), such as basements, roofs, barns, air shelters, natural and man-made caves, cement tanks, cisterns, wells, rock cracks, tree holes, underneath bridges, animal stables, culverts, empty jars, piles of straws, etc. The majority of the hibernating mosquitoes were found inside caves and long culverts where temperatures and humidity were higher than outdoors. The results of the survey are given in Table 2.

The last fed *sinensis* was caught on 5 November 1959 and the first fed one on 10 March 1960. The first fed *sineroides* was collected on 24 March. All of these were found in the cow stables.

THE HIBERNATING CONDITION OF MOSQUITOES. It was observed that the mosquitoes in hibernating condition were resting on the walls or ceilings in the places where they passed the winter seasons. Those resting inside the caves, where temperatures and humidity were higher than outside, were sometimes very active; occasionally hovering and flying around. The mosquitoes became more active when they were stimulated by breathing and light.

On a very cold day of January, when it was  $-10^{\circ}$  C. outside and  $2-3^{\circ}$  C. inside the culvert, the mosquitoes in the long culvert were gathered closely along the

TABLE 1.—Meteorological data

		November	December	January	February	March	April
Temperature (C.)	Average	10.6	1.1	-3.3	1.7	6.7	12.4
	Maximum	19.5	14.3	10.8	13.7	18.1	25.3
	Minimum	-5.5	-12.9	-17.6	-8.6	-5.8	-0.9
Relative humidity (%)	Average	69	67	63	64	65	59
	Total	153.5	135.4	148.7	168.0	181.7	239.8
Sunshine hours	Total	27.9	34.1	4.6	4.0	113.6	19.0
Precipitation (mm)	Total	0	0	0	10.4	0	0
Snowfalls (mm)	Total	0	0	0	0	0	0

TABLE 2.—Hibernating places of mosquito adult females

Localities	Total No. females caught	<i>A. sinensis</i>		<i>A. sineroides</i>		<i>C. pipiens</i>	<i>C. orientalis</i>
		f.	uf.	f.	uf.		
Underneath bridges	49	..	6	..	1	39	3
Culverts	4,380	..	22	..	8	4,338	12
Caves	796	..	..	..	2	789	5
Air shelters	12	..	..	..	..	12	..
Cow stables	19	17	..	2	..	..	..
Total	5,256	17	28	2	11	*5,178	*20

\* All unfed.

crack of the wall or the ceiling and were not disturbed even by strong breathing and light.

It was also noticed that many dead mosquitoes were still attached to the wet walls of caves.

Hackett (1) and Swellengrebel and de Buck (9) have stated that some mosquito adults in hibernating condition show different types of behavior, e.g., *A. atroparvus* Van Thiel hibernating in warm stables and houses occasionally biting, *A. messeae* Fall going into a state of more complete inactivity, etc. The hibernating behavior of the *Anopheles* in Korea seems to belong to the first category, i.e. some hibernating in warm stables and occasionally biting.

Survival tests were made with 499 *C. pipiens* caught from caves at Kimhae-kun near Pusan. When these mosquitoes were brought back to Pusan by car and then to Seoul by train, they were exposed to outdoor temperature ( $-6^{\circ}$  to  $-9^{\circ}$  C.) for 5-10 minutes and became completely inactive just like dead mosquitoes. However, they resumed activity soon after being kept in the train or room ( $10^{\circ}$ - $20^{\circ}$  C.). Of 499 mosquitoes tested, 86 died in 24 hours and 55 in 48 hours after collection. Of 33 *A. sinensis* and *A. sineroides* tested, all died in 12 hours after collection. It seems that anophelines are less resistant to cold weather than *C. pipiens*.

MOISTURE AND FAT CONTENTS AND BODY WEIGHT OF ADULT *Culex pipiens* IN HIBERNATING CONDITION. It has been shown by several workers (Hackett (1), Roubaud and Colas-Belcour (7b), Swellengrebel (8), Swellengrebel and de Buck (9), etc.) that

fat production in hibernating mosquitoes resulted from any combination of factors that tended to restrict the activity of the mosquitoes, and that the release from hibernating condition was caused by factors that tended to stimulate activity. Thus, fat production in wild mosquitoes in the fall was associated not only with a drop in temperature, but with the shortest period of evening twilight, with an appreciable shortening of the daylight, and with the onset of the fall rains, all of which would tend to keep the mosquito quiescent.

In order to show the variation of mosquito moisture and fat contents and body weight of adult *Culex pipiens* caught in hibernating condition and just before the release from hibernation, 1,100 *C. pipiens* adult females caught on 22 February in hibernating condition and 251 on 24 March, just before the release from the hibernation in the same culvert (about 300 meters long) at Susack, Seoul, were tested. The Soxhlet extraction method with ether was applied for fat content, and the drying method at  $105^{\circ}$  C. was applied for the moisture content. The results are given in table 3.

There seems to be some difference between adult mosquitoes caught on 22 Feb. in hibernating condition and on 24 March just before release from hibernation. When mosquitoes came out of hibernating condition, fat and moisture contents and body weight decreased.

ATTEMPT TO ISOLATE JAPANESE B ENCEPHALITIS VIRUS FROM *C. pipiens* IN HIBERNATING CONDITION. The only vector from which Japanese B encephalitis virus

TABLE 3.—Moisture and fat content and body weight of *Culex pipiens*

Date	Number tested	Body weight (mg)*	Moisture content (%)	Fat content (%)	
				Wet base	Dry base
22 Feb. '60	1,100	4.76	69.35	6.43	20.87
24 Mar. '60	251	4.63	67.23	4.85	16.19

\* Per adult.

has been repeatedly isolated by the various workers is *C. tritaeniorhynchus*. The first virus isolation from the mosquitoes was accomplished by means of mice inoculation by Mitamura (5), in 1938. Subsequently, Hammon *et al.* (2), reported the isolation of the virus from *C. tritaeniorhynchus* collected in Japan and in Guam, in 1948. In other countries, some workers have reported the isolation of the virus from other *Culex* species. Russian workers (6) state that the disease in the Maritime District is spread by *C. pipiens* and *C. tritaeniorhynchus*, but that certain other mosquitoes are also said to be involved.

Experimental transmission in the laboratory has been proved or claimed for a wide range of mosquitoes including *Anopheles*, *Culex*, and *Aedes*. *C. pipiens* appears to be one of the most generally common mosquitoes in Korea. Attempt was made to isolate the virus from *Culex pipiens* in hibernating condition.

Tests were done with 2947 *C. pipiens* adults, of which the majority was collected in the caves and culverts at the vicinity of Seoul in January to March and 436 were caught from the cave at Kimhae in December 1959. Fifty-eight groups (each group with 50 mosquitoes) were pooled. The results show that neither Japanese B encephalitis was isolated from the mosquitoes nor haemagglutination-inhibition antibody was present in the inoculated mice. The laboratory method for virus isolation is given below for ready reference.

Pools of 50 mosquitoes each were put in a mortar and ground thoroughly. One ml of 10 percent normal rabbit serum saline, containing 1000 units of penicillin and streptomycin per ml, was added by increments and the mixture ground again.

Three ml of the same diluent were then added and mixed thoroughly by additional grinding. The suspension was then spun in a horizontal centrifuge at 1300 rpm for 20 minutes. The supernatant from this horizontal centrifugation was carefully removed and centrifuged at 15000 rpm for 20 minutes at a temperature of approximately 32° F. in the centrifuge chamber. This throws down large particulates including bacterial contamination and suspends fatty materials in a surface pellicle. The six suckling mice received the inoculation of 0.1 of the supernatant fluid by means of intracerebral route and after 10 days blind passage was carried out. After the second blind passage, all inoculated mice were bled and checked whether the haemagglutination-inhibition antibody was present or not.

DISSECTION OF MOSQUITOES. Salivary glands of 28 *A. sinensis* and 10 *A. sineroides* were dissected and all were negative for sporozoites. In January–March 1960 ovary dissection of 26 *A. sinensis*, 9 *A. sineroides*, 175 *C. pipiens* and 6 *C. orientalis* showed that all of them were nulliparous.

LARVAE IN HIBERNATING CONDITION. Some species of mosquitoes would pass the winter in the larval stage. During the hibernation, the phenomenon of cessation of growth in overwintering larvae is called "asthenobiosis." Roubaud and Colas-Belcour (7b) mentioned that the asthenobiosis was quite independent of external influences. Kobayashi (3) reported that most *Anopheles* larvae when found in November were on the point of dying and they almost all would die in the winter in Korea. And he stated that when mosquito larvae were found in early March or April they must have hatched out newly from eggs.

The present survey revealed that the last larvae found were 6 *A. sinensis* of the third instar in the paddies in Wondang, near Seoul on 5 November 1959. After the winter the first larvae of anophelines were collected from a ditch in the same area on April 10, 1960. During the winter season no mosquito larvae could be found in paddies, streams, pools, etc., which are usually their favorite breeding places. Also no larvae were discovered in the water or the mud under ice.

**SUMMARY.** *Anopheles sinensis*, *A. sineroides*, *Culex pipiens*, and *Culex orientalis* hibernate in the adult stage. These anophelines hibernating in cow stables were all fed, showing that they could feed in winter. The most suitable places for the hibernation of *Culex pipiens* are culverts, and the next, caves.

The anophelines appear to be less resistant to low temperatures than the culicines.

Fat and moisture contents and body weight decreased in *C. pipiens* just before their hibernation was over.

No Japanese B encephalitis virus was isolated from *Culex pipiens*.

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