In six families there were two persons with microfilariae in the blood, and in five families, one person.

In evaluating these results it should be noted that the blood of six persons was not studied and, further, that since clinical examinations were often conducted in the presence of both the male and female members of the family, and sometimes in the presence of neighbors as well, it was not possible to examine the males for hydrocele or, as a rule, to interrogate them on the subject. Consequently the figures given for positive findings, whether clinical findings or microfilariae, are conservative, and probably lower than actual ones.

Similar observations were made at Puerta de Tierra in San Juan, and cases were studied from all sections. It became evident that in one particular area filarial infection was hyperendemic. This area was adjacent to the Bay of San Juan on swampy ground and included a group of houses or shacks in very poor condition. The relationship of these houses is shown on the accompanying diagram (Fig. 3).

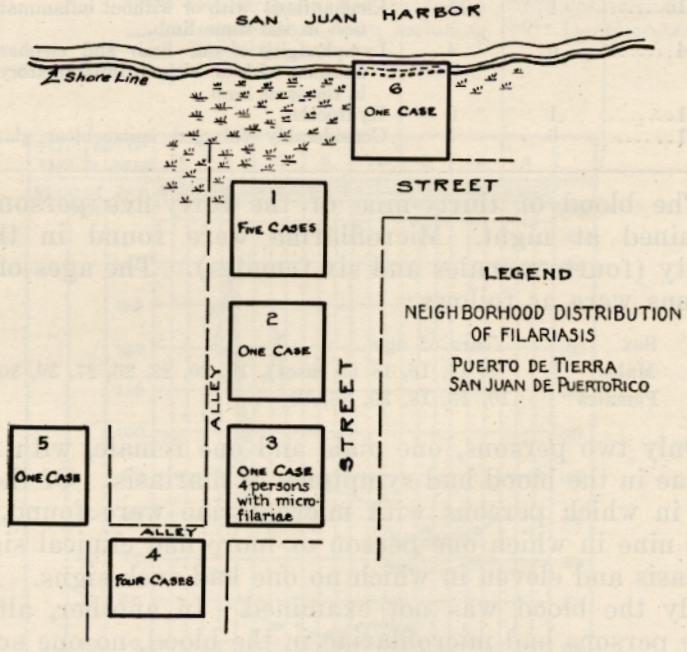

Fig. 3. Relationship of houses in Puerta de Tierra.
Grabado 3. Situación del Caserío en Puerta de Tierra

It is believed that sufficient evidence has been brought forward to show that in Puerto Rican towns there is not only definite evidence of positive family prevalence of filariasis, but also of neighborhood or environmental prevalence. While more females had symptoms than males, yet microfilariae were much more commonly found in the blood of males.

THE INSECT HOST OF FILARIASIS

Culex Fatigans

Prevalence:—The insect vector of filariasis in Puerto Rico, *C. fatigans,* is abundant, especially in the poorer sections of the towns. It is very numerous close to the sea front where there is protection from the prevailing winds, and troublesome even two thousand feet above the sea, as at Aibonito.

Breeding:—Its breeding places are extensive and are mainly in:

a. Uncleaned ditches in the vicinity of towns.
b. Borders of lagoons extending to the edges of populous towns.
c. Drainage from wells.
d. Water stored in barrels and other containers.
e. Various receptacles in the extensive dumping heaps near towns.

In existing circumstances the control of many of the breeding places is impossible.

The length of time required for the development of the adult mosquito shows some variation at different seasons, as may be seen from the tabulation below:

March, 1929	September, 1930
4th–5th day Egg rafts laid.	9th day Egg rafts laid.
6th day Eggs hatched.	11th day Eggs hatched
19th day Pupation.	18th day Pupation.
21st day Imagines emerged.	20th day Imagines emerged.

Nevertheless slight temporary changes in weather conditions delay or accelerate development at any season.

Resting and Feeding Habits:—For the adult mosquito, conditions are ideal in the dark, hot, steamy and overcrowded houses or shacks of the poor, where the inmates take little or no precaution against attacks by the insects. In Puerta de

Tierra, in a single room, several hundred culex, mostly engorged, may be collected at any time from behind furniture or clothing and under beds. A favorite resting place is beneath the houses, especially those in damp sections or in the vicinity of the breeding places.

At all seasons *C. fatigans* seem to feed most vigorously in the evening and the early part of the night, after which they are usually found resting on the walls or elsewhere in buildings. Amongst mosquitoes used for transmission studies it was noticed that those which had not fed by 10 p.m. rarely did so that night. If, however, imagines were starved sufficiently and not brought into contact with man until later, they promptly proceeded to feed when opportunity offered. These facts may explain the small number of filariae found in most mosquitoes and the occasional heavy infections that are encountered. The tendency of *C. fatigans* to bite early at night is important from the standpoint of filarial infection in Puerto Rico, inasmuch as the Puerto Ricans usually sit in their living rooms, on their porches, or in the vicinity of their houses until about nine o'clock in the evening and are thus exposed to the attack of the mosquitoes during the period of their greatest activity.

C. fatigans is a shy feeder and is easily disturbed until such time as blood has begun to enter the abdomen, after which she can be captured without difficulty. When attacking persons who are sitting, she usually lights upon the ankle. If disturbed, she will hover around and return again generally to this part of the body. Persons lying in the dark in a recumbent position, however, are liable to be bitten on any exposed surface. One mosquito which was observed biting below the right internal malleolus, was disturbed; she flew away, but returned and fed below the external malleolus on the same leg. Repeatedly disturbed thereafter, she returned on each occasion, piercing the skin of the inner side of the left ankle, the dorsum of the foot, and behind the right internal malleolus. These variations in feeding areas may play some part in the dissemination of larval filariae over extensive areas of skin.

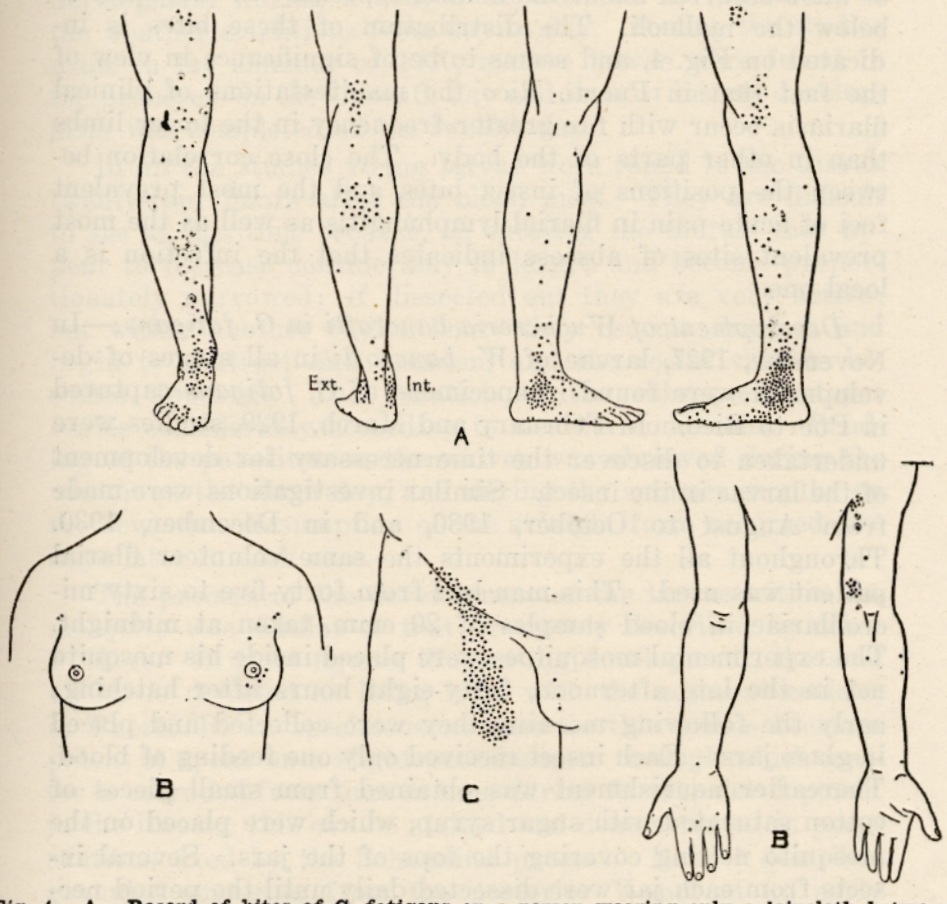

Fig. 4. A. Record of bites of C. fatigans on a person wearing only a loincloth between 7 p. m. and 10 p. m.

B. Foci of primary and most severe pain in cases of lymphangitis of the breasts and upper limbs.

C. Foci of primary and most severe pain in cases of lymphangitis of the scrotum and lower limbs.

Grabado 4. A. Número de picadas que produce el mosquito de 7 p. m. a 10 p. m. a una persona cubierta solamente con un lienzo sobre las caderas.

B. Focos primarios y álgicos en varios casos de linfangitis de las mamas y de los miembros superiores.

C. Focos primarios y álgicos en varios casos de linfangitis del escroto y de los miembros inferiores.

Our observations showed that by far the greater number of bites occurred about the ankles, especially in the shadows below the malleoli. The distribution of these bites is indicated on Fig. 4, and seems to be of significance in view of the fact that in Puerto Rico the manifestations of clinical filariasis occur with far greater frequency in the lower limbs than in other parts of the body. The close correlation between the positions of insect bites and the most prevalent foci of acute pain in filarial lymphangitis as well as the most prevalent sites of abscess indicates that the infection is a local one.

Development of Wuchereria bancrofti in C. fatigans:—In November, 1927, larvae of *W. bancrofti* in all stages of development were found in specimens of *C. fatigans* captured in Puerto Rico. In February and March, 1929, studies were undertaken to discover the time necessary for development of the larvae in the insect. Similar investigations were made from August to October, 1930, and in December, 1930. Throughout all the experiments the same volunteer filarial patient was used. This man had from forty-five to sixty microfilariae in blood samples of 20 cmm. taken at midnight. The experimental mosquitoes were placed inside his mosquito net in the late afternoon, forty-eight hours after hatching; early the following morning they were collected and placed in glass jars. Each insect received only one feeding of blood. Thereafter nourishment was obtained from small pieces of cotton saturated with sugar syrup, which were placed on the mosquito netting covering the tops of the jars. Several insects from each jar were dissected daily until the period necessary for development was determined. At the same time a number of the insects were fixed in Bouin Medium and later preserved in 75 per cent alcohol.

In general it was found, as Rao and Iyengar [11] have shown, that development of the parasite in the mosquito was more rapid with high humidity and high temperature, and in the first studies the average time required for this development was from 19 to 21 days. That this period is not constant for the winter months of every year is shown by the fact that in the winter of 1930–1931, fully developed larvae were found in the head and proboscis on the twelfth day after the infecting meal, and fairly frequently from then

on. It would seem, therefore, that the period of arthropod development during a season depends on the variation in the weather conditions from day to day. During the studies made in the summer of 1930, mature larvae were found in the proboscis on the eleventh day and in most insects development was completed on the twelfth day.

In all the studies young larvae were found in the thorax twenty-four hours after the blood meal. They are difficult to see at this time because, on entering the thorax, they appear to increase considerably in length and become proportionately narrowed; if dissected out they are very active; but within another twelve hours they become sluggish and begin to contract and broaden, and the rectal vacuole becomes apparent. The duration of the subsequent stages varies considerably according to weather conditions. Bending of the parasites on themselves was observed during the winter on the ninth day, and during the summer months on the seventh. Resumption of the straight form occurred two days later in each case.

The process of the development of the larvae within the mosquito was similar to that previously described by other workers. In most figures illustrating development, the anal orifice is depicted as being level with the adjacent cuticle; in Puerto Rican specimens as soon as this orifice becomes patent it opens on a slight protuberance through which matter is extruded from time to time during further development in the insect; this can be stained and demonstrated in preparations fixed by the Schaudinn process. Bahr [12] describes granules emerging from the posterior V-spot at an early stage and throughout the whole cycle of development. In the vicinity of the parasite, especially at the posterior end, in stained sections, a clear and slightly granular area can usually be made out in the muscles of the mosquito. Whether this represents material excreted from the parasite or rarefaction of the muscles themselves has not been determined.

When the parasites are fully developed and reach the head they show a proclivity for the tissues supporting the eyes, and in most specimens can be demonstrated in this situation. In the proboscis they are commonly observed in pairs, but single specimens are frequent, and more rarely several are found in this situation. In the thorax and ab-

domen the fully developed larvae are usually found in the loose connective tissue between the vital organs and the skeletal structures, very commonly in the connective tissue of the dorsum of the thorax overhanging the neck.

During the earlier stages of development after the larvae leave the stomach they are observed only in the thorax. When fully developed, however, they frequently indulge in apparently aimless wanderings. The distribution of the fully developed parasites in seventy mosquitoes may be of interest.

Part of Mosquito in which Fully Developed Larvae Were Found	*Number of Mosquitoes*
Proboscis only	19
Proboscis and head	10
Proboscis and thorax	1
Proboscis, head, thorax	9
*Proboscis, head, thorax, abdomen	4
*Proboscis, head, abdomen	4
*Proboscis, thorax, abdomen	1
*Proboscis, head, thorax, abdomen, leg	1
*Proboscis, head, leg	1
Head only	3
Head, thorax	1
Thorax only	3
*Thorax, abdomen	3
*Thorax, leg	2
*Abdomen only	8
Total	70

Thus in twenty-four * of the seventy mosquitoes larvae had made migrations, the object of which was not clear. The specimens in the abdomen were as commonly found posteriorly as anteriorly. In three instances the parasites seen in sections were at the posterior extremity of the insect.

Wild specimens of *C. fatigans* were dissected for the purpose of observing the general distribution of parasites amongst the mosquito population and the incidence of parasitized insects in hyperendemic localities. No parasites were found in twelve mosquitoes from the Municipal Hospital in Santurce, in eight from the District Hospital in San Juan, in nine from the Presbyterian Hospital in Santurce. At the Municipal Hospital in Guayama twelve engorged mosquitoes were studied, but contained no parasites; all of these buildings are well lighted and situated in spacious grounds

well exposed to wind. Nine mosquitoes collected in a house in Puerta de Tierra where there were no persons with filariasis and none harboring microfilariae in the blood, were negative. No parasites were found in numerous insects collected on many occasions from a private residence in Santurce.

On the other hand, the following results were obtained in the dissection of *C. fatigans* mosquitoes collected under the house of J. R. in Puerta de Tierra, which is next door to the house of B. C., in which five occupants had microfilariae in the blood and two others had symptoms of filariasis:

Date	Mosquitoes Dissected	Mosquitoes Parasitized
December 19, 1930	11	7
December 22, 1930	14	10

Two of these mosquitoes were found to have fully developed larvae; one contained 4, the other 6. In the latter there were also 4 half-developed forms. In another insect there were 16 short, fat parasites.

December 26, 1930	12	3
December 31, 1930	13	2
January 1, 1931	2	1
January 7, 1931	14	3

Dissection of a mosquito captured in the house of B. C. showed eighty parasites in early stages of development in the stomach blood and the thorax.

Amongst the mosquitoes of this species collected inside a house which sheltered three filariasis patients, eight of eleven mosquitoes were parasitized.

In collections made in and under the house of S. M. (Puerta de Tierra), who had 590 microfilariae in 20 cmm. of blood taken at midnight, 20 per cent of ninety-seven culex taken on six occasions in December and January were infected, the highest daily percentage being fifty.

In a house (Santurce) in which lived Mrs. H., who had elephantiasis, a son who had lymphangitis and five other children who had microfilariae in the blood, the percentage of parasitized mosquitoes was forty.

Although large numbers of parasites were never found in experimentally infected mosquitoes, this was not the case among mosquitoes infected in nature. Sixty-three parasites

were found in one mosquito in the foregoing series; only eight of these were fully matured, but the other fifty-five appeared to be developing satisfactorily.

In Aguadilla three recently fed mosquitoes were collected from inside the mosquito net of a man with microfilariae in the blood. In two, no parasites were found; in the third, sixty-three unsheathed or young larvae were discovered, fifty-five in the stomach blood and fifteen in the thorax.

This affords no evidence that most larvae reach the infection stage or that the mosquito can long survive such massive infections.

Fate of Larvae of W. bancrofti in Water:—On September 8, 1930, two experimental mosquitoes were dissected in water in a watch glass at 3:40 p. m. Three fully developed larvae emerged from the proboscis and head of one, and one larva from the proboscis of the other. The four larvae were undamaged and very active in the abundant supply of water. At 4:30 p. m. the movements of all were sluggish; at 7:30 p. m. three were motionless; and at 8 p. m. all were motionless and apparently dead. Several of the experimental mosquitoes with fully developed larvae died in water during the night. When these were dissected the larvae were usually dead. On one occasion, however, February 26, 1929, an extremely active larva was found in the proboscis of a mosquito which had so died.

Other Insects as Hosts of W. bancrofti:—The ideal host for *W. bancrofti* is not *C. fatigans* but *A. scutellaris (A. variegatus).* Manson-Bahr, working on the development of these larvae in *A. scutellaris* in Fiji, pointed out that mature forms were often found in the abdomen and legs of the mosquito. O'Connor's experience in Samoa and the Ellice Islands confirmed these findings, but his work with *C. fatigans* in Puerto Rico suggests that this insect is not nearly so efficient a host for the periodic microfilaria as is *A. scutellaris* for the non-periodic type. In *C. fatigans* the larvae did not tend to develop at the same rate as in *A. scutellaris.* Not uncommonly, fully developed larvae were found in the proboscis of *C. fatigans* while only half-developed forms were present in the thorax. Degeneration, either by calcification or by granulation, of the parasites in the thorax of the mosquito seemed to be much more common in this species than in *A. scutellaris.*

It has been shown that *C. fatigans* feeds mainly in the evening and the early part of the night at which times microfilariae are not numerous in the blood. It is not surprising then that the mosquito usually does not become heavily parasitized. The volunteer patient used for the feeding experiment had, as has been said, from forty-five to sixty larvae in 20 cmm. of blood. On the average, the experimental culecines rarely harbored more than four or five parasites in the thorax. However from the massive infections reported as occurring in nature, in which various stages of larval development were observed in the same mosquito, it is clear that these are frequently reinfected.

Repeated attempts were made to infect *A. aegypti* with *W. bancrofti,* but without success. In a few instances the larvae reached the thorax, but began to degenerate after two or three days.

On several occasions bed bugs were collected from the beds of persons with microfilariae in the blood. In every instance microfilariae were found in the stomach of these insects, but invariably they were dead in their sheaths.

STUDIES OF FILARIAL INCIDENCE IN PUERTO RICO

The usual method of determining the incidence of filariasis in any area is to add to the percentage of the inhabitants showing clinical manifestations of the disease the percentage of other persons found to have microfilariae in the blood. The sum of these percentages constitutes the total incidence. In 1927 and 1928 Hoffman, Marín and Burke[1] made a comprehensive survey of the incidence of filariasis in various communities throughout the Island. Amongst 4,590 persons whom they examined, 483 showed some evidence of infection.

Microfilariae were found in the blood of 275 or 5.5 per cent of these, while in 208 there were clinical manifestations of infection. Fourteen persons had embryos in the blood at night as well as clinical signs of infection. The highest incidences, on all counts, were observed in Puerta de Tierra, Aguadilla, Caguas and Ponce. These authors believe that microfilariae are found most frequently in immature persons, but they point out that the parasites are by no means absent in persons between the ages of forty and fifty years. The work of these observers at the Boys' and Girls' Charity

Schools in Santurce is very significant. After explaining that these schools are within half a mile of each other and that mosquito nets are rarely used in either, they describe important differences influencing the spread of filariasis in the two institutions, and in confirmation show marked contrasts in the infection incidences.

For comparison they are as follows:

Boys' Charity School	*Girls' Charity School*
Situated on level land in a densely populated district.	Situated on a well drained slope in an open and uncrowded district.
Mosquito breeding places are numerous.	Breeding places are not numerous.
Mosquito repellents are much used.	Mosquito repellents are not used.
279 children were examined and of these 36 or 13.9 per cent showed evidence of filarial infection.	313 children were examined and of these 22 or 7.01 per cent showed evidence of filarial infection.
28 had microfilariae in the blood.	12 had microfilariae in the blood.
8 had clinical manifestations.	10 had clinical manifestations.
The infection rate showed a marked increase with each year of residence at school.	The distribution of infected individuals was uniform regarding residence.

Tampi [13] found 7.7 per cent of 518 persons from Santurce positive for microfilariae, the highest incidence being 9.5 per cent amongst pupils of the Boys' Charity School who were examined between 8 and 10 p. m.

In the studies here reported, which were carried out during the years 1929 to 1931, no attempt was made to investigate the infection of the entire Island for the following reasons: Firstly, there is much variation in incidence in the different towns owing to great differences in topography and weather conditions; secondly, the rural population far exceeds the urban, and filariasis is essentially an urban problem even in the plains; and thirdly, filariasis is rare amongst the inhabitants of the highest hills and mountains, where there is nevertheless a dense urban as well as rural population.

Examinations to determine the microfilarial incidence in various localities were made between the hours of 8 p. m. and 2 a. m., the majority of them between 9 and 11 p. m. Persons of all ages, from three to eighty-five were examined. Chart II shows the rise in the microfilarial incidence from the age of six. The peak is reached in the second decade;

and although the parasite is found at all ages up to eighty, there is a distinct falling off in incidence in and after the fourth decade. A large number of those examined complained of filarial symptoms, and amongst such persons the microfilarial incidence was usually low, but their relatives showed a higher incidence than in any other group.

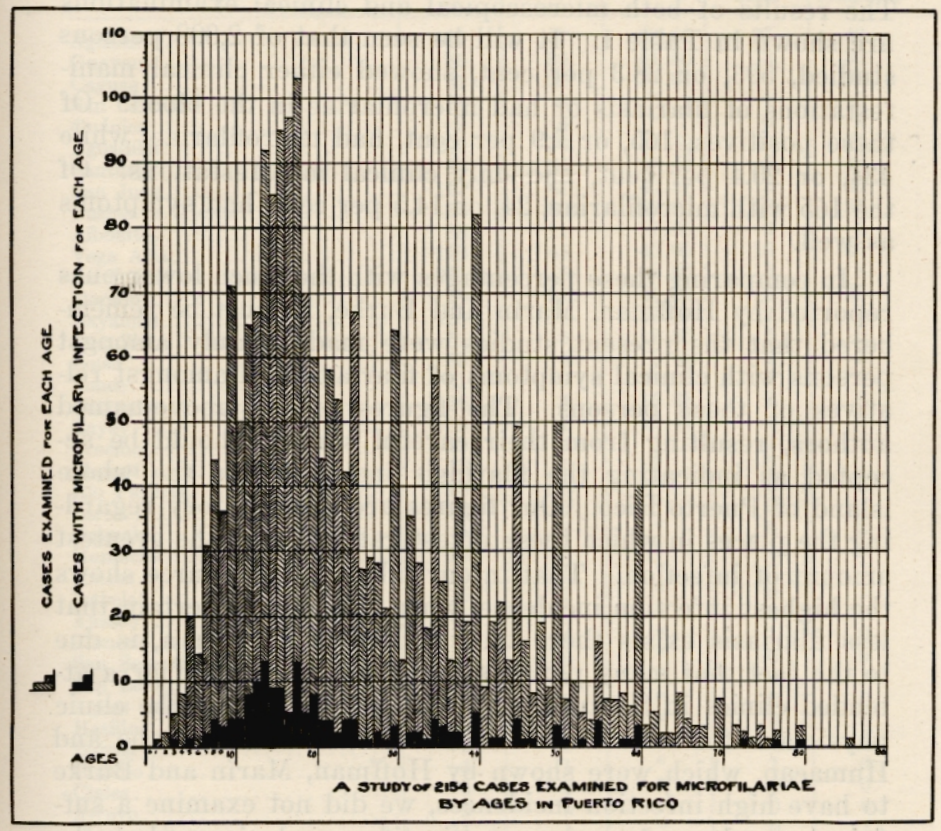

Chart II. Ages of cases examined for microfilariae.
Gráfica II. Edades de los casos en que se pesquisaron microfilarias en la sangre.

Twenty cubic millimeters of blood were taken from the finger in a graduated pipette and expressed on to a microscopic slide as a circular film three-quarters of an inch in diameter. After drying, the film was dehemoglobinized in tap water, dried, fixed in equal parts of ether and alcohol, and dried again. It was then stained with Bullard's hematoxylin for

seven minutes. The stain was flushed off with tap water, and the film was then blued in tap water for ten minutes. Each film was completely examined microscopically under the two-thirds objective with the mechanical stage. All films in which microfilariae were found were mounted, when dry, in Canadian balsam and were kept as records. Complete counts of the number of parasites were made in each case. The results of both microscopical and clinical examinations are shown in Table 1. It will be seen that of 2,098 persons studied, 593, or 28.3 per cent, showed either clinical manifestations of filariasis or had microfilariae in the blood. Of these positives, 165, or 7.9 per cent, had microfilariae, while 434, or 20.4 per cent, had only clinical manifestations. Of the 165 with microfilariae, 24, or 14.5 per cent, had symptoms as well.

In comparing these percentages with the much lower ones reported by Hoffman, Marín and Burke, it must be remembered that the present studies were made mainly amongst persons with clinical symptoms of filariasis and amongst relatives of these persons. The figures of the above-named authors, resulting from their careful survey, should be accepted as indicating the filariasis incidence for the whole island of Puerto Rico. Our figures are confirmatory regarding the places in which these investigators found the greatest amount of infection. That in our tabulation Santurce shows the highest infection incidence, when it is almost certain that less filariasis exists there than in Puerta de Tierra, is due to the fact that many patients living in Puerta de Tierra attended clinics in Santurce and were sent to us by the clinic physicians for examination. In the towns of Naguabo and Humacao, which were shown by Hoffman, Marín and Burke to have high infection incidences, we did not examine a sufficient number of persons to justify conclusions; but the number of purely clinical cases which we encountered confirmed their findings. Our figures show that, as these observers suspected, there is a high microfilarial incidence in Aguada. Of the persons in the mountain town of Aibonito who were found to have clinical symptoms or microfilariae in the blood, many had previously lived in endemic areas of filariasis, where they had probably become infected. This was also true of infected persons in Guayama and Arecibo.

In Sardinera, a rural district in the plains, the three persons with microfilariae among the 293 examined had all lived for a number of years in hyperendemic areas of cities.

TABLE I.—*Persons Found to have Microfilariae in the Blood or Clinical Symptoms of Filariasis in 78 Communities of Puerto Rico.*

Place	Number Examined	Number Positive	Per cent Positive	Number with microfilariae in the blood	Number with clinical symptoms
Aguada	199	67	33.66	27	40
Guayama	86	36	41.86	3	33
Caguas	81	30	37.03	7	23
San Germán	28	8	*	1	7
Guánica	9	4	*	0	4
Aguadilla	209	70	33.49	29	41
Santurce	83	56	67.46	8	48
San Juan	117	47	40.17	13	34
Puerta de Tierra	158	99	62.65	18	81
Aibonito	63	11	17.46	3	8
Vega Alta	110	45	40.90	10	35
Sardinera	291	6	2.06	3	8
Dorado	74	31	41.89	6	25
Bayamón	129	18	13.95	12	6
Luquillo	51	5	9.80	5	0
Loíza	85	5	5.88	2	3
Ponce	31	9	29.03	5	4
Mayagüez	26	4	15.38	2	2
Cayey	9	1	11.11	0	1
Arecibo	19	4	*	2	2
Manatí	12	1	*	0	1
Río Piedras	18	5	*	0	5
Lares	9	0	*	0	0
Naguabo	5	2	*	0	2
Humacao	16	1	*	1	0
Juncos	5	1	*	0	1
Isabela	5	1	*	0	1
Añasco	5	0	*	0	0
Comerío	7	1	*	1	0
San Sebastián	3	0	*	0	0
San Lorenzo	7	1	*	0	1
Aguas Buenas	3	0	*	0	0
Hatillo	1	0	*	0	0
Barros	2	0	*	0	0
Gurabo	4	1	*	1	0
Ciales	4	0	*	0	0
Arroyo	2	0	*	0	0
Barranquitas	11	1	*	0	1
Maunabo	3	0	*	0	0
Patillas	3	0	*	0	0
Quebradillas	5	1	*	0	1
Sábana Grande	4	0	*	0	0
Morovis	4	1	*	0	1
Camuy	1	0	*	0	0
Yabucoa	3	0	*	0	0
Barrio Peñuelas	2	1	*	0	1
Hato Rey	1	1	*	0	1
Sunoco	1	1	*	0	1
Cataño	6	2	*	1	1
San Narciso	1	1	*	0	1

TABLE I.—*Persons Found to have Microfilariae in the Blood or Clinical Symptoms of Filariasis in 78 Communities of Puerto Rico.*—Continued

Place	Number Examined	Number Positive	Per cent Positive	Number with microfilariae in the blood	Number with clinical symptoms
Palo Seco	1	1	*	0	1
Trinidad	1	1	*	0	1
Toa Alta	6	1	*	0	1
Trujillo Alto	5	1	*	0	1
Adjuntas	2	0	*	0	0
Carolina	5	1	*	0	1
Jayuya	5	0	*	0	0
Corozal	9	3	*	0	3
Juana Díaz	2	0	*	0	0
Vieques	3	1	*	0	1
Coamo	2	0	*	0	0
Cabo Rojo	6	2	*	2	0
Trujillo	1	0	*	0	0
Utuado	10	1	*	1	1
Barceloneta	3	0	*	0	0
Yauco	1	0	*	0	0
Cidra	2	0	*	0	0
Moca	2	0	*	0	0
Las Marías	2	0	*	0	0
Toa Baja	1	0	*	0	0
Lajas	2	0	*	0	0
Naranjito	1	0	*	0	0
Río Grande	2	1	*	1	0
Santa Isabel	1	0	*	0	0
Salinas	3	1	*	1	0
Guaynabo	1	0	*	0	0
Guayanilla	1	0	*	0	0
	2,098	593	28.3	165	434

*Small numbers examined do not justify percentages.

In Table 2 the percentage of persons with microfilariae in the blood are listed according to region and sex. It will be seen that the highest incidence was found on the west coast and the second highest on the north coast.** There is apparently little difference in the sex incidence of infection.

TABLE 2.—*Percentage of Microfilarial Infestation According to Region and Sex.*

Region	Total	Males	Females
North Coast	9.1	4.4	4.7
South Coast	6.2	3.5	2.7
West Coast	13.3	7.1	6.2
High Elevation	4.0	4.0	.....
Rural Plains	1.03	0.34	.69
East Coast	3.84	3.84	.....
Central Valleys	5.2	2.2	3.05

**The high incidences were found in towns a few miles inland.

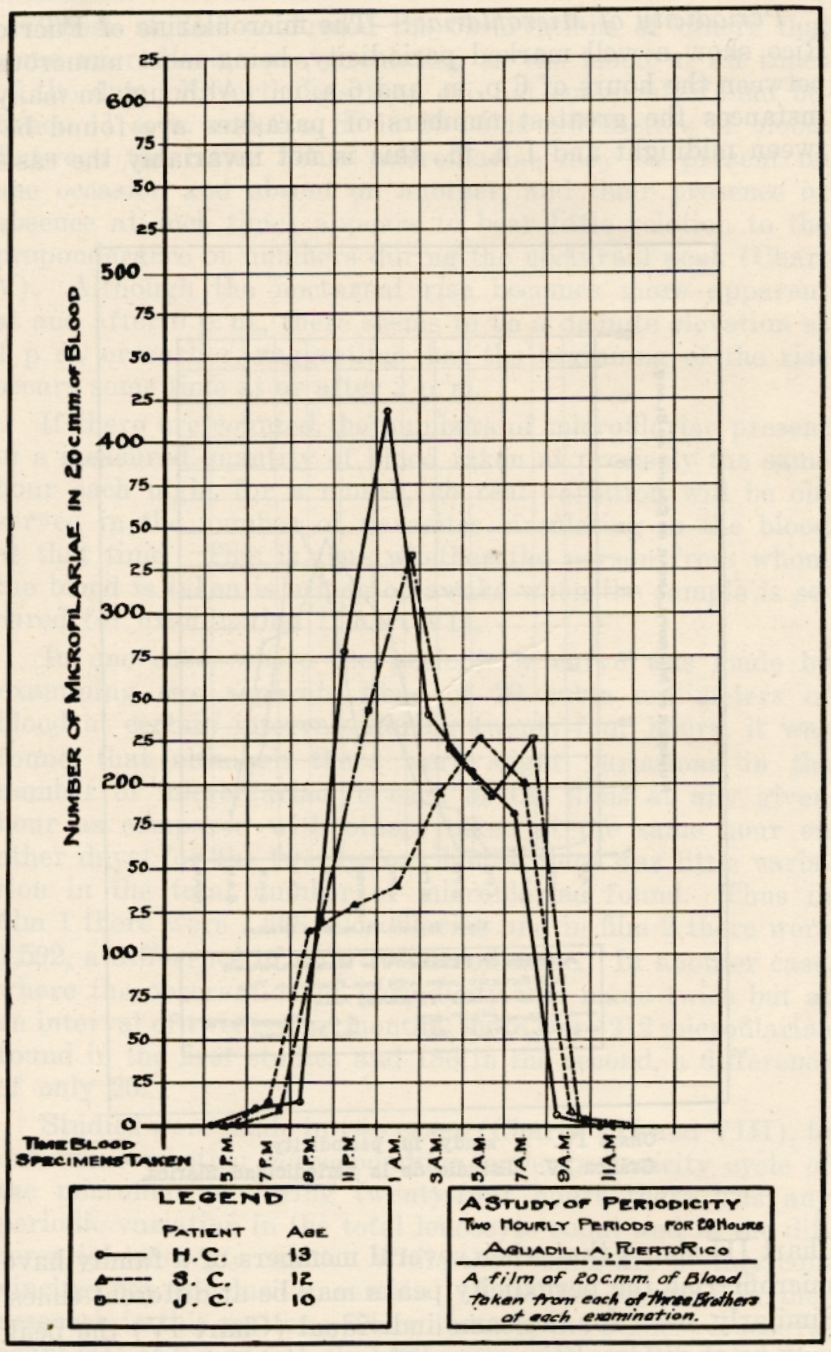

Chart III. A study in periodicity.
Gráfica III. Estudio de la periodicidad filárica.

Periodicity of Microfilariae:—The microfilariae of Puerto Rico show a well marked periodicity, being most numerous between the hours of 6 p. m. and 6 a. m. Although in many instances the greatest numbers of parasites are found between midnight and 1 a. m., this is not invariably the case.

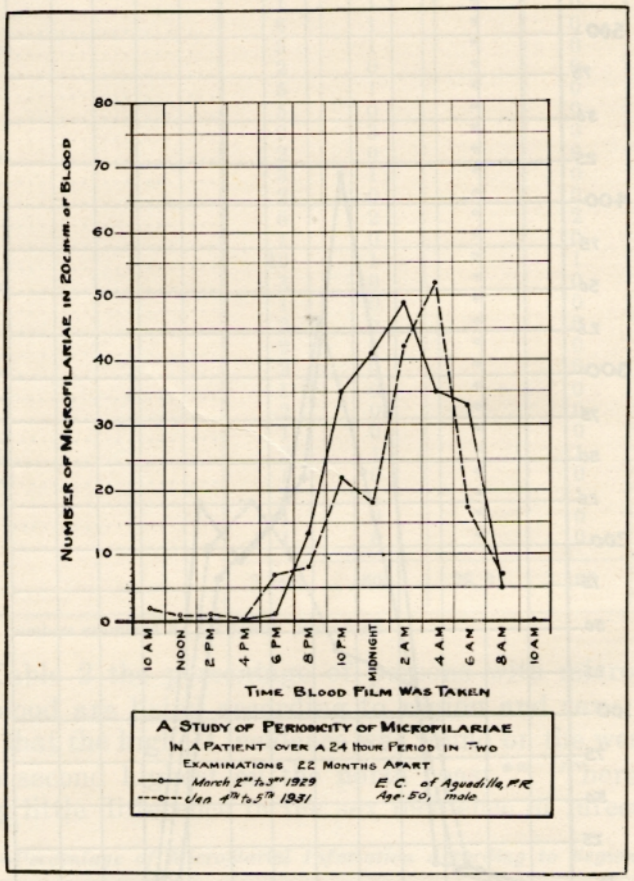

Chart IV. A study in periodicity.
Gráfica IV. Estudio de la periodicidad filárica.

Chart III shows that when several members of a family have microfilariae the periodicity peaks may be at different times. Similarly, even for the same individual (Chart IV) the peak may be at different times on different nights.

These studies confirmed the observations of others that some microfilariae are circulating in the blood at all times of the day. Nevertheless they are not commonly found between 11 a. m. and 2 p. m. in 20 cubic millimeters of blood. Between the latter hours microfilariae may be present on one occasion and absent on another, and their presence or absence at such times appears to bear little relation to the preponderance of numbers during the nocturnal peak (Chart V). Although the nocturnal rise becomes more apparent at and after 6 p. m., there seems to be a definite elevation at 4 p. m. or earlier, suggesting that the beginning of the rise occurs some time at or after 2 p. m.

If there are counted the numbers of microfilariae present in a measured quantity of blood taken at precisely the same hour each night for a month, no real variation will be observed in the number of parasites circulating in the blood at that time. This is true whether the person from whom the blood is taken is asleep or awake when the sample is secured for examination (Chart VI).

In one case, where the periodicity curve was made by examining two separate films of 20 cubic millimeters of blood at certain intervals during twenty-four hours, it was found that although there were slight variations in the number of microfilariae in each of the films at any given hour as compared with others taken at the same hour on other days, for the twenty-four hours there was little variation in the total number of microfilariae found. Thus in film 1 there were 1,668 microfilariae and in film 2 there were 1,592, a difference of only 76 microfilariae. In another case, where the observation of periodicity was made twice but at an interval of twenty-two months, there were 212 microfilariae found in the first studies and 186 in the second, a difference of only 26.

Studies were made in two cases (Charts VII and VIII), to determine whether corresponding to the periodicity cycle of the microfilariae during twenty-four hours there was any periodic variation in the total leucocyte count and in the differential leucocyte count. While the results are neither convincing nor conclusive, certain indications warrant further research in this matter. Thus, in each, the highest leucocyte count was at 6 p. m. A slight temporary drop in lymphocytes

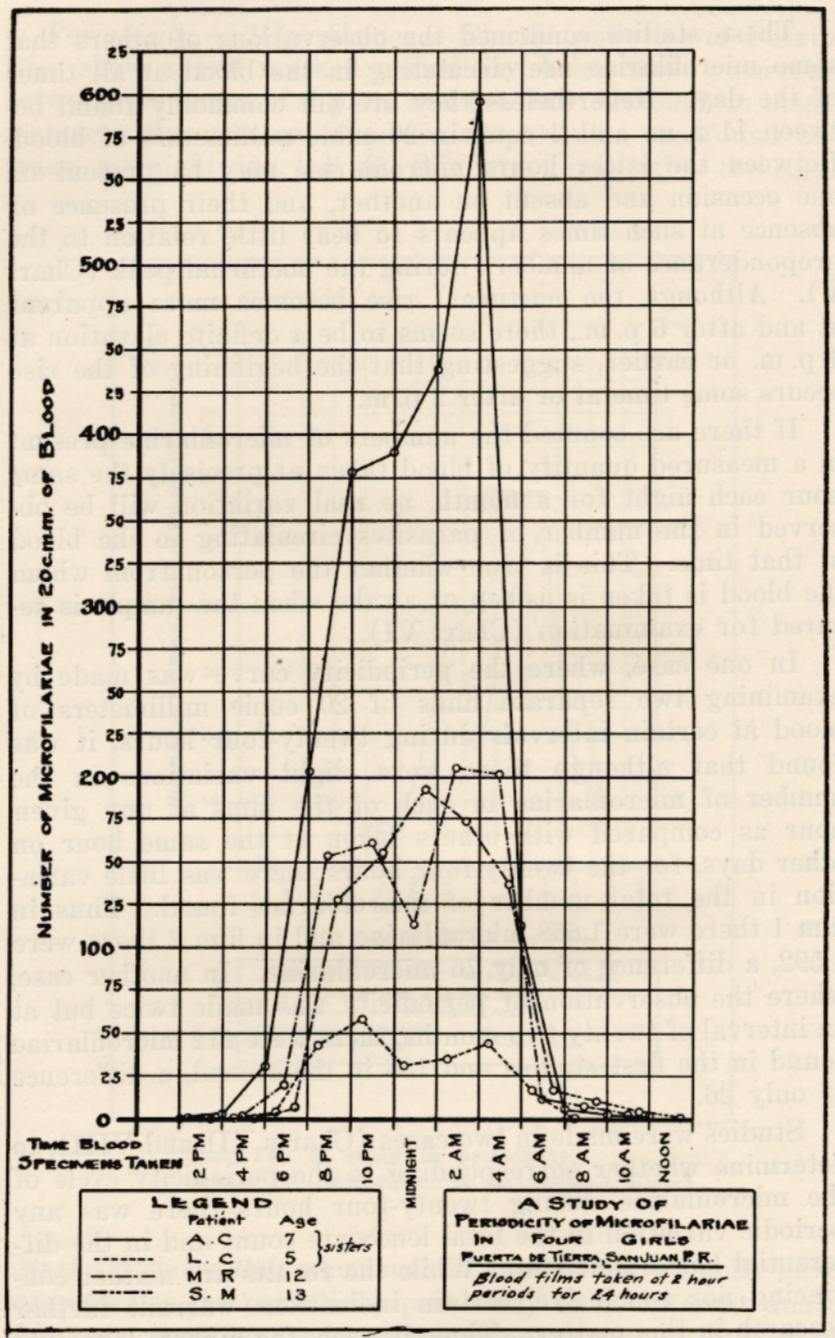

Chart V:—A study in periodicity.
Gráfica V:—Estudio de la periodicidad filárica.

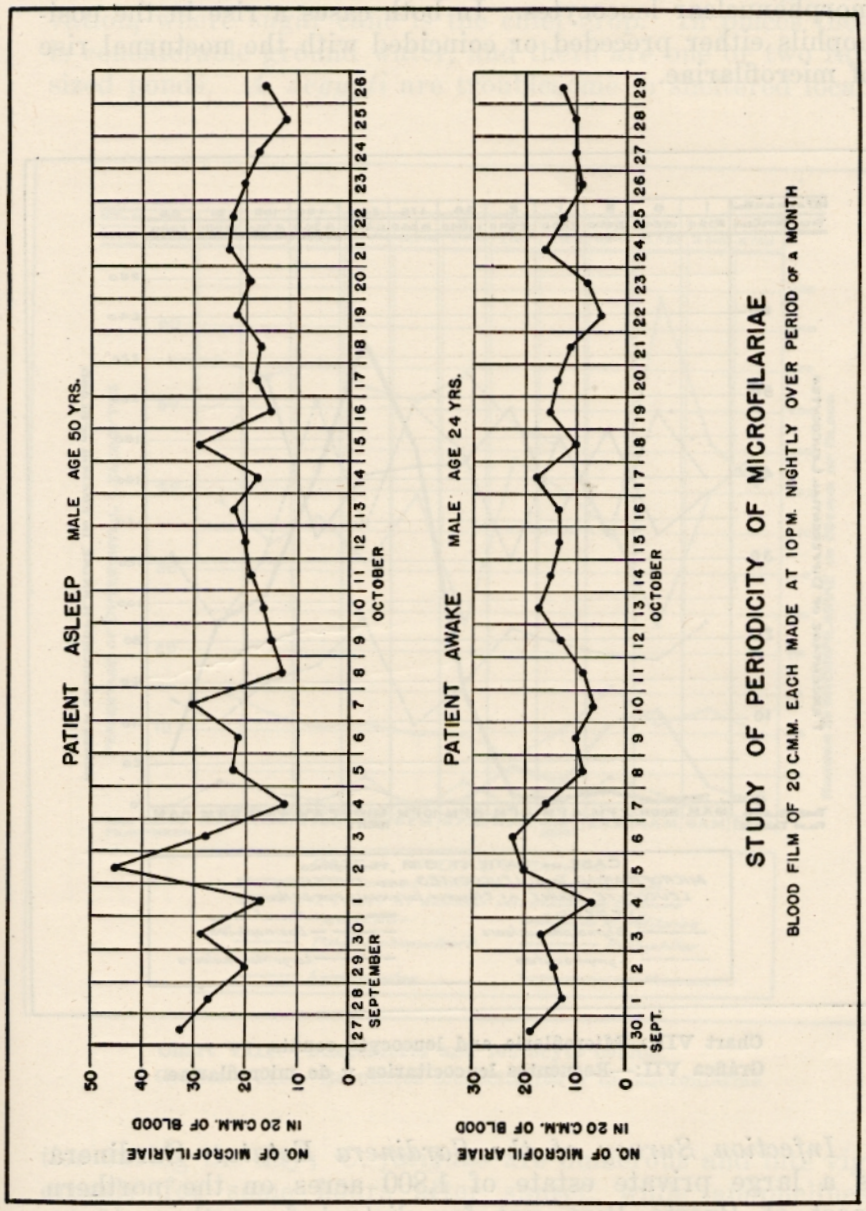

Chart VI
Gráfica VI