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THE REARING OF ANOPHELES ALBIMANUS WIEDEMANN IN THE LABORATORY

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INTRODUCTION

The efficiency of the Department of Sanitation of The Panama Canal is brought to one's attention in a most striking manner when an attempt is made to collect larvae of *Anopheles albimanus* for experimental work. One must always look for these mosquitoes outside of the sanitated areas if very many are needed. During the rainy season, a fairly satisfactory collecting area was found on the edge of a swamp situated near the ruins of Panama la Vieja; however, an exceptionally heavy rain would flush out this and other breeding places, so that only a few larvae would be encountered. During the dry season one is obliged to make long and often inconvenient trips to localities in Gatun Lake or in the Chagres River, where patches of *Chara*, *Naias*, and *Utricularia* harbor Anopheline larvae. Field collections, then, being extremely unreliable for the maintenance of a continuous supply of *A. albimanus*, it was decided to work out a technique for breeding this species in the laboratory, so that a fresh supply of adults would always be available.

MATERIAL AND METHODS

a. Source of mosquitoes

Two separate laboratory colonies were established; the second was necessary because the first died out, after the fourth generation, due to errors in technique. The first colony was started with a total of 580 adults, bred out from larvae collected from the

[1] The writer is indebted to Dr. M. F. Boyd for many helpful suggestions.

swamp mentioned above, and placed in the breeding cage over a period of two weeks.

More than 1500 larvae were used for establishing the second colony. A little over a thousand of these were collected at Panama la Vieja, and the rest hatched from eggs deposited by wild females, which were caught in traps belonging to the Department of Sanitation of The Panama Canal; then imprisoned in bobbinet cages over water and fed on the blood of a guinea pig.

b. Care of the larvae and pupae

The care of the larvae is almost identical to the technique described by Boyd and Cain (1932), and Boyd, Cain, and Mulrennan (1935). The newly-hatched larvae are placed in hay infusions that are at least six weeks old, contained in white enameled basins, 12 inches in diameter and $3\frac{1}{2}$ inches deep, or slightly larger. The most satisfactory results have been obtained with infusions of alfalfa hay, a bale of which was purchased from the Canal Zone from a supply that had been imported from the United States. Instead of allowing the infusions to ripen in the breeding pans, the writer prefers to place considerable quantities of hay and water in large glass jars, as this saves both space and basins. Such infusions require at least two months, and usually more, before they can be used. When ready for use, a small amount of hay and water is removed from the infusions and placed in a breeding pan, and diluted with the proper amount of tap water. In this manner many basins for the larvae can be made up from a single large jar of infusion.

Heavy cultures of paramecia always develop in the infusions, and the period of usefulness of the water for larval breeding seems to terminate at approximately the time when the paramecium cultures begin to die out. In addition to the protozoa, bacteria, and whatever other types of food there may be in the infusions that can be utilized by the larvae, a small amount of fresh Fleischmann's yeast is fed to the larvae every day. The yeast is placed on a glass slide, which is supported just below the surface of the water by cork floats. This combination of hay infusion and yeast is an excellent source of food for certain species of anopheline larvae, as has been pointed out by Boyd, Cain, and Mulrennan. A small amount of liver extract is being added to a few of the basins, but as yet no definite evidence has been obtained to indicate a more rapid development of the mosquitoes in these basins.

Because of the uniformity of the climate in this region, it is not necessary to regulate the temperature of the breeding water by means of water baths such as those used in Boyd's insectary. It has been found necessary, however, to keep the breeding pans out-of-doors. The windows of the laboratory can not be left open at night because of the heavy rains that fall during the greater portion of the year, and at night the temperature within the laboratory goes up to 89°F., and sometimes even reaches 95°F. and over. Although not in itself fatal to the larvae, this condition appears to favor the growth of micro-organisms to such an extent that it is inimical to the mosquitoes. Regardless of how old or how diluted the infusions are, when kept in a closed room overnight a surface pellicle is invariably found in the morning, which delays growth and eventually kills all of the mosquitoes. The writer is at a loss to attribute this rapid increase of micro-organisms to any cause other than the high incubating nocturnal temperatures. On the other hand, when the breeding pans are placed out-of-doors, the larvae are able to keep ahead of the bacterial and protozoal growth. When first put into use, a slight surface film is always present on the water, but if the infusions are sufficiently matured and diluted, even the newly-hatched larvae can push their way through the film and eat it off in the course of two or three days. Yeast is not fed to the larvae until the pellicle has entirely disappeared. At present the breeding pans are being kept in a room next to the animal house, where they are protected by a corrugated iron roof from rain, but where they are not subjected to high temperatures, as the room is open on three sides. The basins are so situated that the sun can strike them for two or three hours each day. When taken at 8:00 a.m., the temperature of the water sometimes is as low as 21°C.; during the day it usually reaches 27°C. In nature the writer has collected *A. albimanus* in water with temperatures of 28° to 32°C.

The pupae are collected at least once a day and placed in cups of fresh tap water. The adults are allowed to emerge into lantern globes; then they are transferred to the breeding cage.

c. *Care of the adults*

Anopheles albimanus adapts itself readily to laboratory conditions. The adults are perfectly willing to copulate in a relatively small cage, but they must be protected from wind, and they need plenty of moisture. The writer has been keeping his adults in a screen cage, 2 by 2 by 2 feet in size. There was no particular reason for selecting a cage of these dimensions, other than the fact that the laboratory was already in possession of several such cages. One side is taken up by a sleeve made of unbleached muslin. The other three sides are lined on the inside with white cheesecloth, which helps to exclude draft, and affords a suitable surface for the adults to cling to, while the white color facilitates observation. On the outside, these three sides are covered with heavy blue denim, which protects the mosquitoes from wind, and helps prevent a rapid decrease in the amount of moisture within the cage. The floor is covered with heavy white paper in order to keep out draft from the bottom. A wet towel is used to cover the top, and heavy cardboard is placed over the towel, as this seems to diminish the rate of evaporation to the outside. The wet towel is of value in keeping up the relative humidity inside of the cage, but it does not seem to give off quite enough moisture. A section of unglazed drain tile, 12 inches long by 5 inches in diameter, was found to be a very important piece of apparatus within the cage. One end is plugged with paraffin, a rag is packed against the plug, and the remainder of the tile is filled with fine sand. About half a cup of water is poured into this tile each day, and as the moisture oozes out through the tile, the mosquitoes have access to a cool and damp, but not sticky, surface on which to rest. From the number of adults that are constantly seen clinging to the tile, it has been assumed that this is a very popular place. The tile was not placed inside of the cage until some time after the colony had been established, and shortly after it was put into use, the egg production increased so greatly and suddenly that the only conclusion which could be drawn was that the tile kept the moisture content of the cage up to a level where much larger numbers of females were able to live long enough to deposit their eggs.

Because of the small size of the cage, it is impossible to twirl a sling psychrometer; therefore the relative humidity can not be determined from wet and dry bulb readings. Through the kindness of the Chief of Surveys of The Panama Canal, a hygrograph was borrowed for a period of eight days. During this time there was a fairly constant relative humidity of 80 per cent.

The temperature inside of the cage varies from 80° to 86°F. This is several degrees lower than the high nocturnal room temperatures, and is no doubt due to the evaporation taking place from the towel and the tile, while the insulation afforded by the paper, towel, and cloth helps to prevent the atmospheric conditions in the laboratory from having too much of an effect upon those within the cage.

The egg trap consists simply of a crystallizing dish, 7½ inches in diameter and 2½ inches deep, containing tap water, to which a few cubic centimeters of water from a ripe infusion are added. Cork floats afford resting places for the females. In the morning the dish containing the eggs is removed from the cage, and a dish of fresh water substituted. After the larvae hatch out they are pipetted from this water and placed in a breeding pan. It has been found that the egg trap is a cause of considerable mortality among the adults. During the process of copulation many couples drop into the water, where they die. For this reason a round-bottomed flowerpot of unglazed clay is used as a covering. This is inverted and supported above the egg trap on bottles, 4½ inches tall, so that the edge of the pot is two inches above the top of the crystallizing dish. This seems to cut down the number of copulating pairs that fall into the water.

Two or three cellucotton pads soaked with sugar-water are placed underneath the towel on the top of the cage, where the males have ready access to them but can not become stuck to the wet cellucotton, as the pads rest on the screen. The males are also given slices of banana, apples, papaya, grapes; whatever fruit happens to be available in the laboratory. The fruit is changed daily.

The females are fed once a day on the arm of a human blood donor. Although it would be possible to feed them on a guinea pig or rabbit, it is much less trouble for some one to place an arm in the cage for a few minutes; furthermore, since the mosquitoes are being used in experiments necessitating feeding on man, it is thought to be preferable not to risk the development of a strain that might prefer animal to human blood. *A. albimanus* bites severely, but it is not necessary for the arm to remain within the cage for more than five minutes, as from one to two hundred females take blood during that length of time. The donor has become almost completely immune to the bites of this mosquito, and although from time to time there may be a slight reaction, it is hardly noticeable.

DISCUSSION

As has been stated above, the first colony died out because of improper care, and it might be well to mention briefly the reasons for the failure. In the first place, lack of experience resulted in the employment of infusions before they had matured completely, and large numbers of larvae were killed by surface growth of microörganisms. Second, an insufficient amount of infusion made necessary the use of breeding water long past its period of maximum efficiency. It has been found that it is best not to use a basin of water for much more than a month; this observation has previously been made by Boyd, Cain, and Mulrennan (1935). A great source of annoyance were *Psychoda* larvae, which were continually being found in the breeding water. A technician, who tired of picking the larvae of these insects out of the basins day after day, began to cover the breeding pans with cloth or wire screen in order to prevent the females from having access to the water. Not only did this fail to eliminate the *Psychoda* larvae, but pupation of the mosquitoes stopped almost entirely, and very many mosquitoes died before the process was discontinued. In addition to the mistakes in the care of the larvae, the adults died rapidly, so that egg production was very poor. This was before the wet drain tile had been placed in the cage.

By the time these errors were corrected, the first colony had died out almost completely. In the meantime the second colony had been established, and so it was decided to discard the first. At the time of writing, the second colony has been maintained for almost four months, and there never has been any danger of losing it. Infusions are ripened in four large glass jars, so that there is always an abundant supply of breeding water. After two, three, or four batches of larvae have completed development in a basin, the water is discarded. Ten basins of breeding water are in use, and fresh batches of newly-hatched larvae are added to them alternately. As the larvae require a little over a week before pupation, every day at least one of the basins contains many pupae. This number of basins automatically keeps the population of the colony at a constant level. Usually from 200 to 300 pupae are collected daily. About 400 to 600 newly-hatched larvae are added to the basins each day. If more are added, the production of pupae does not increase unless more basins are put into use. A great many larvae in a single basin results in the death of most of them, while those that survive develop slowly, and eventually become very small pupae. Even 400 larvae in a basin are probably too many. The adult population is kept at about a thousand individuals. Egg production seldom drops below 500 a day, and often over a thousand are obtained.

The laboratory colony of *A. albimanus* is serving the purpose for which it was intended. At its present size, it is able to furnish the writer at any time with all of the females he may need for experimental work, and in addition, it is affording an opportunity to study the bionomics of this important Central American vector of malaria.

The cost of the equipment is almost negligible, totalling only a few dollars.

SUMMARY

A technique has been described for the laboratory rearing of *Anopheles albimanus*, involving the use of a small amount of inexpensive equipment.

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