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TECHNICAL MANUSCRIPT 407

LABORATORY STUDIES OF MOSQUITO FLIGHT: I. A FLIGHT MILL SYSTEM

Wayne A. Rowley

Charles L. Graham

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JULY 1967

DEPARTMENT OF THE ARMY

Fort Detrick

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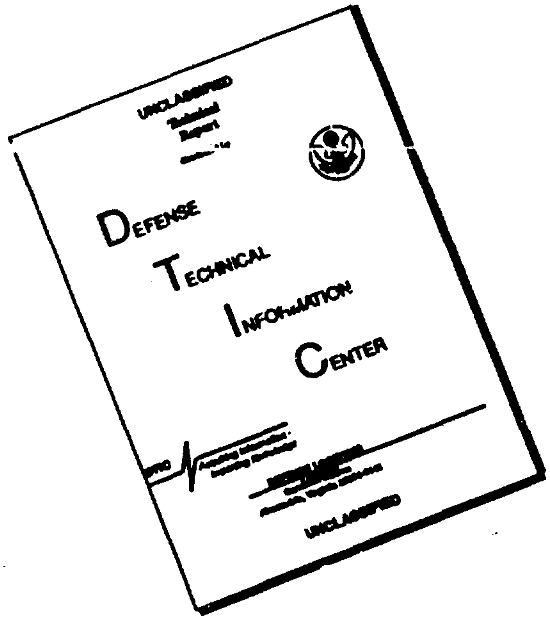

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Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORY

Project 1C522301A059 July 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

A flight mill system is described for the quantitative assessment of mosquito flight performance under controlled laboratory conditions. The system includes a device which insures uniform attachment and orientation of mosquitoes to flight mill arms. Attachment and orientation of the mosquito prior to flight are second only in importance to the over-all efficiency of the flight mill itself. Data-recording equipment accurately measures the distance flown, the flight duration, and the speed of mosquito flight.

I. INTRODUCTION

For more than 60 years, biologists have been aware of the importance of mosquito flight. The significance of mosquito flight range in the transmission of disease agents was realized soon after anophelines were incriminated as arthropod vectors of malaria. In view of its importance to the epidemiology of mosquito-borne diseases and to mosquito control and abatement programs, it is surprising that little progress has been made in developing effective means for evaluating mosquito flight. Presently, there is no single approach to insect flight studies that affords a suitable method for estimating quantitatively the flight ability of mosquitoes.

Virtually all data concerning mosquito flight have been derived from field observations. These studies generally involve mass rearing, marking, and releasing of thousands, or even millions, of mosquitoes and the use of elaborate techniques and equipment for recapture.[1-3] Generally, only a small fraction of the marked specimens are recovered, and almost all of these are taken within a relatively short distance from the release point. Provost[3] recovered only a small percentage of 1.5 million marked mosquitoes; Eddy et al.[1] released 25,500 marked mosquitoes, representing three species, but failed to recover a single marked specimen. Wolfinsohn and Galun[4] contributed significantly to the existing knowledge of Aedes aegypti (L.) flight range, but pointed out that their results represented only 0.24 and 0.28% of the individuals released. These figures were consistent with the average percentage of catch reported by other workers for the recapture of marked mosquitoes. In most cases, however, a few specimens were recovered at sufficient distances from the release point to indicate that some individuals can and do fly longer distances. Provost,[3] studying the dispersal of Aedes taeniorhynchus, recovered two marked specimens in light traps 24 and 25 miles from the release point.

Kettle[5] discussed the difficulties of determining flight range by recapture methods. He pointed out that the variable usually measured is "population density" rather than "flight range." Therefore, Kettle reserved the term "flight range" to describe the mean distance flown by individuals of the same species.

Various means have been used to study insect flight in the laboratory. A common method for determining flight duration, energy utilization, and some biochemical characteristics of insect flight has used the tethering of insects to some type of stationary mount. Krogh and Weis-Fogh[6] developed an ingenious "roundabout" and flew several migratory locusts at a time. Weis-Fogh's subsequent studies represent the only comprehensive evaluation of the flight activity of an insect species.

Faust[7] and others used high-speed cinephotography and other photographic techniques to investigate such aspects of insect flight as flight attitude, aerodynamic drag, and wing-beat form. Other authors[8-10] utilized stroboscopic techniques to measure wing-beat frequency as a function of flight ability in different species of insects. Reed et al.[11] used wing-beat frequency determinations to separate species, races, and geographic varieties of *Drosophila*. Kennedy[12] used a wind tunnel to measure the speed at which *Aedes aegypti* flew in still air, in winds of various velocities, and against moving and still backgrounds, but he did not determine endurance or distances flown by individual mosquitoes.

Hocking,[13] in his monumental study of the intrinsic range and speed of flight of insects, devèloped a flight mill capable of recording the distances flown by insects. He demonstrated the utility of his mill by flying field-caught specimens of northern species of *Aedes* and several other dipterous species. Clements[14] used a flight mill similar to Hocking's for determining sources of energy for mosquito flight. Chapman[15] and Atkins[16] also used flight mills to study the flight range of bark beetles. The apparent success of these studies suggests that, if properly designed and controlled, flight mills can be a useful laboratory tool for estimating some parameters of insect flight. However, one must be careful not to confuse flight capabilities and performance on a flight mill with the species' flight range in its natural habitat. In nature, a number of intrinsic and extrinsic factors influence mosquito flight. The ability to fly a certain distance does not imply that the species or individuals of the species will "need" to fly that distance. In other words, "flight range" cannot be mistaken for "home range." The extent to which non-appetential flight occurs among mosquitoes in nature is not known, and, possibly, it does not occur in most species.

II. PRINCIPLE OF TETHERED INSECT FLIGHT

Mosquitoes, like many other insects, react according to Fraenkel's[17] postulated tarsal reflex phenomenon. Thus, removal of contact between the tarsi of the insect and a substrate initiates an immediate wing movement. In some mosquitoes, the tarsal reflex is sufficient to maintain flight until exhaustion.[18] A reverse tarsal reflex response occurs if mosquitoes are permitted to re-establish tarsal contact with a solid object. The curved nature of the aluminum foil wedge (described later) used in attaching mosquitoes to flight mill arms precludes contact of the wedge with their metathoracic tarsi. Once a mosquito's initial flight phase ceases, a gentle stimulation (puffs of air or re-establishment and removal of tarsal contact) will reinitiate flight until complete exhaustion is achieved.

Hocking[13] listed the desirable requisites for an insect flight mill system in order of importance: (i) low aerodynamic and frictional drag; (ii) convenient and readily adjustable attachment procedure; (iii) durability; and (iv) low moment of inertia. The flight mill system described here incorporates all of these features plus uniform orientation, standardized attachment procedures, and accurate data recording. Hocking theorized that an insect harnessed to a flight mill arm, although potentially supported by the arm, supports its own weight while flying. Therefore, Hocking has assumed that an arm which is balanced before an insect is attached should remain in balance with an insect attached and flying.

III. FLIGHT MILLS AND RELATED EQUIPMENT

A. FLIGHT MILL ARM AND SUPPORT

Arms used for flying mosquitoes are fabricated from extremely lightweight non-magnetic stainless steel. Specifications for flight mill arms and supports are given in Figure 1. Aluminum foil wedges, 10 mm in length with a 1-mm base, are preshaped into semicircles and attached to the mill arm. Wedges are attached with wax at right angles to the longitudinal axis of each arm. Each mosquito is attached to a mill arm by the foil wedge.

A plastic cylinder (Fig. 1) is mounted at the gravitational center of each arm. This cylinder serves as a housing (hub) for a precision engineered sapphire jewel bearing.* Specifications for the jewel bearings are: outside diameter 2.0 mm; 0.58 inch thick; 85±5 degrees; radius 0.006 to 0.009 inch; and depth 0.027 to 0.032 inch. Jewels are set into a plastic housing (hub, Fig. 1) that in turn is placed over a fine, pointed, 1-inch long, stainless steel pivot. Pivots are precision matched for each bearing by the manufacturer. Only the jewel comes in contact with the pivot point. Pivots are supported on brass tubes that also support a photoelectric cell system. The photoelectric apparatus (Fig. 1) consists of one 28-v lamp (GE-327) and a Clairex hermetically sealed 75-milliwatt photoconductive cell (No. 603A) with a peak spectral response of 7,350 A and a rise time of 0.001 second. The photoconductive cell response time is adequate to record any possible speed flown by mosquitoes.

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* Sapphire VEE Jewels, Part No. RB 202732, manufactured and engineered by the Richard H. Bird & Co., Inc., Waltham 54, Mass.

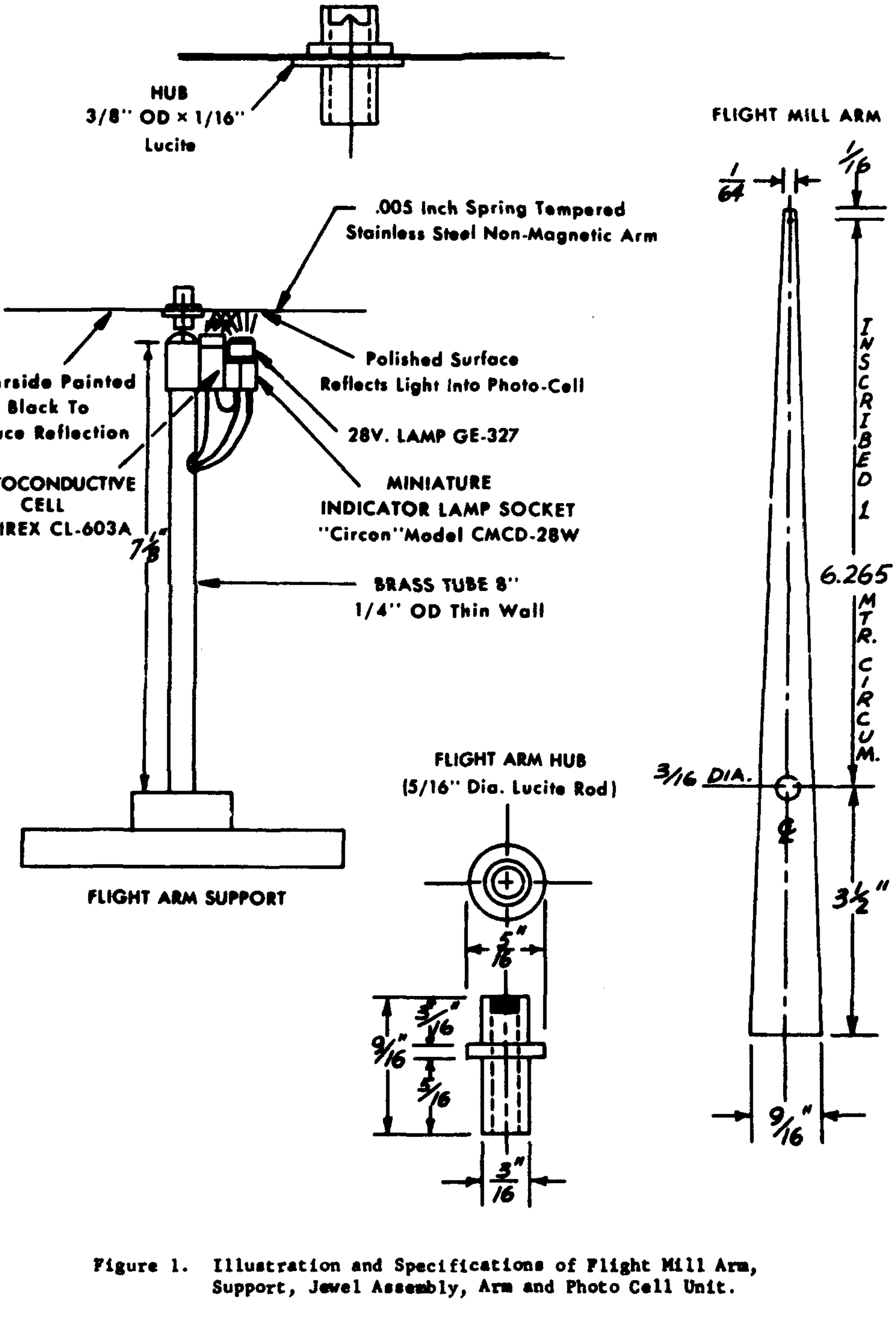

Figure 1. Illustration and Specifications of Flight Mill Arm, Support, Jewel Assembly, Arm and Photo Cell Unit.

The underside of each mill arm is painted flat black on one side of the jewel assembly and polished on the other. As the polished portion passes through the light beam of the photocell, light is reflected back into the photoconductive cell, activating the data recording equipment. The painted portion of the mill arm does not reflect the light beam and, consequently, the system is activated only once per revolution of the mill arm. One complete revolution of the mill arm equals 1 meter of flight.

Each flight mill is enclosed in a plastic hood (Fig. 2A) to eliminate wind resistance caused by air currents in the incubator. Four flight mills are placed in a walk-in controlled environmental chamber where predetermined climatic conditions can be maintained.

B. DATA RECORDING EQUIPMENT

The quantitative assessment of mosquito flight performance under laboratory conditions is dependent on the accurate measurement of at least three variables: distance flown, duration of flight, and speed of flight.

Speed as such represents the distance traveled in a given time. In studies concerned with exhaustive flight, speed per se probably has little or no significance. However, the linear rate of change in speed at particular times during flight may prove valuable in comparing the flight performance of individuals. To determine linear rates of change in flight speed, it is necessary to mdasure accurately the distance traveled at different times throughout the flight.

C. POWER SUPPLY UNIT

Figure 3 is a schematic diagram of the power control circuit and recording equipment with one flight mill. The power unit serves all mills. However, each mill can be operated independently. Included in the power supply unit is a totalizer (counter) for each mill (Fig. 2B) that maintains a running count of the total meters flown by each mosquito.

D. MODIFIED ESTERLINE ANGUS RECORDERS

Figure 2C shows a modified Model AW Esterline Angus chart recorder. Recorders are equipped with a continuous belt of recording paper 45 inches long. Three inches of chart represent 1 minute of flying time. Graphic records of flight can be used in establishing speed of flight, length of flight, and duration of rests. They also provide a permanent visual record of each flight for reference and comparison. By simultaneously recording the flights of four mosquitoes on charts it is possible to follow the progress of each mosquito by observing flight patterns on the charts. Thus, it is not necessary to maintain a constant surveillance of the mosquitoes in the controlled environmental chamber.

Figure 2. Flight Mill System. A. Plastic hood with enclosed flight mill. B. Power unit for data recording equipment (unit also has a totalizer for meters flown on each mill. C. Modified A.W. Esterline Angus chart recorder. D. Digital printout recorder.

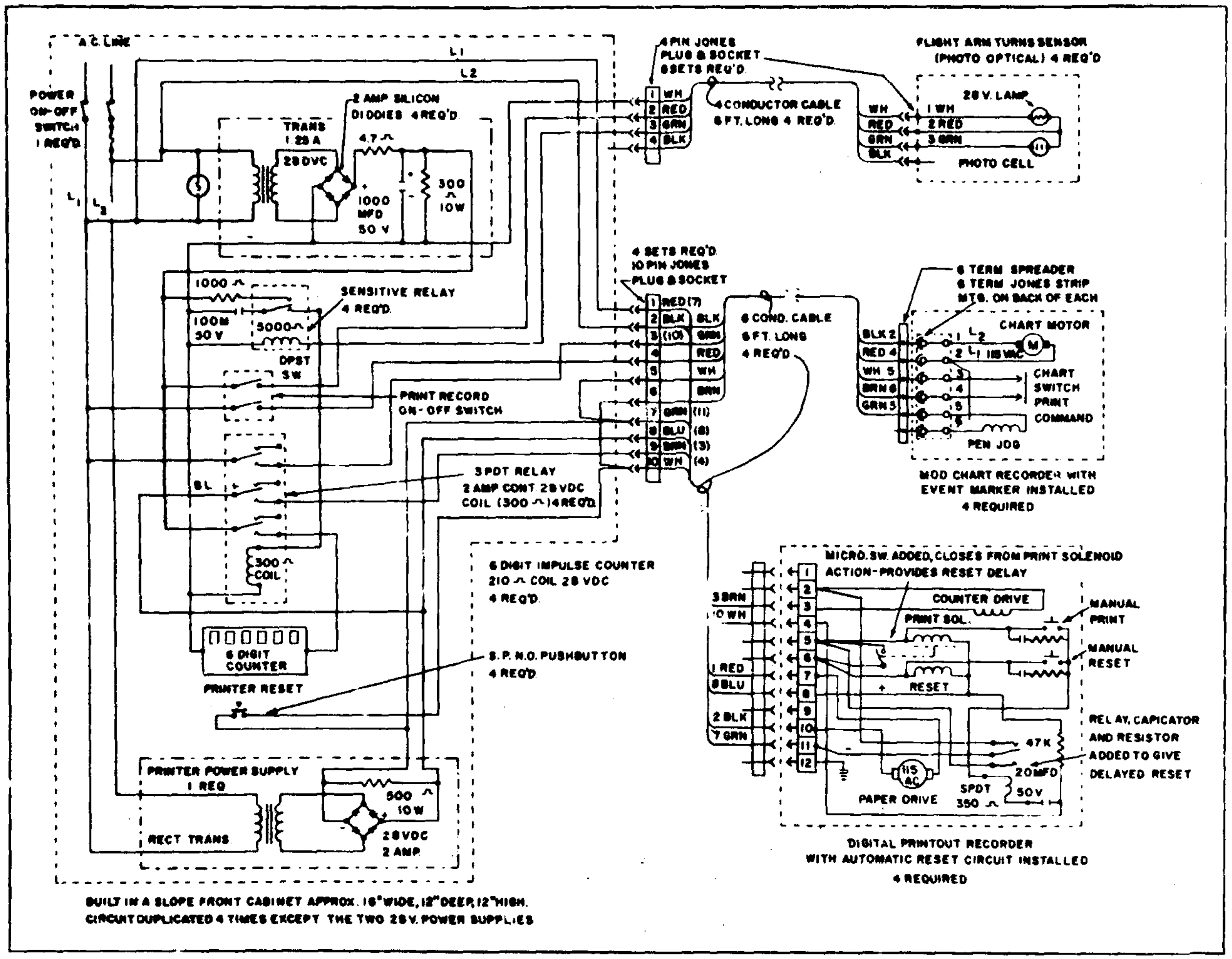

Figure 3. Wiring Schematic of Flight Mill and Associated Equipment.

E. DIGITAL PRINTOUT RECORDER

Figure 2D shows a modified digital printout recorder.* Printers can be preset to record the number of meters flown during any given interval. In our studies, distance and speed of flight are determined by setting recorders to printout and reset every 15 minutes.

IV. ATTACHMENT AND ORIENTATION

Attachment and orientation of mosquitoes to flight mill arms are second in importance to the aerodynamic efficiency of the flight mill system. If attachment interferes with the thoracic cuticular flexions essential in insect flight, the data collected may have little value. Also, if orientation is such that the angle of flight differs from the normal 1-meter circles in which the mosquito is constrained, a considerable amount of flight energy is wasted in an attempt to achieve a normal flight attitude. Performance under such conditions would not reflect a true estimate of the flight potential of the mosquito on test.

Attachment of mosquitoes to a finely balanced arm and adjustment of weights opposite the mosquito showed that A. aegypti females attempt to adjust their flight attitude to fly in a horizontal plane. In other words, mosquitoes attempt to fly in a normal horizontal position, not tilted to one side. Moreover, high-speed cinephotographs (film speed of 15,000 frames per second) were examined of female A. aegypti in free and tethered flight, although a critical analysis of wing-beat form was not undertaken. Wing-beat frequencies were almost identical under both conditions. Therefore, attachment and orientation of mosquitoes probably exert a more pronounced effect on the flight performance of a tethered insect than does tethering as such.

To insure proper and consistent orientation the following procedures and techniques were developed. Mosquitoes are carefully removed from holding cartons. Preferably, a mosquito is trapped in the glass stem of an aspirator and transferred to a plastic vial because forced aspiration may injure the insect and alter its flight performance. The plastic vial containing a mosquito is placed in a freezer at -4 C for 2 minutes. The cooling is sufficient to inactivate a mosquito and enable attachment without allowing it to escape into the laboratory. Recovery from cold inactivation is rapid and has no apparent deleterious effect on flight performance.

* Texas Instruments Incorporated, Dallas, Texas.

Each mosquito is accurately weighed on a Mettler microbalance prior to being flown. After weighing, the mosquito is oriented gently, dorsum up. A saddle (Fig. 4) formed from a 19 G hypodermic needle and attached to a vacuum of 3 to 4 pounds per square inch is placed over the dorsum of the abdomen posterior to the thorax. The saddle is shaped to fit snugly over the abdomen of the mosquito and holds the wings in a folded position. The vacuum is just strong enough to keep the mosquito inactive during attachment.

Figure 4. Vacuum Saddle for Holding Mosquitoes During Attachment.

After a mosquito is secured by the vacuum, the saddle holding the mosquito is placed in a notched pedestal mounted on the stage of a dissection microscope (Fig. 5). The grooved pedestal insures identical presentation of each mosquito for attachment to flight mill arms. Slught adjustments in the lateral orientation of individuals can be made with a jeweler's forceps.

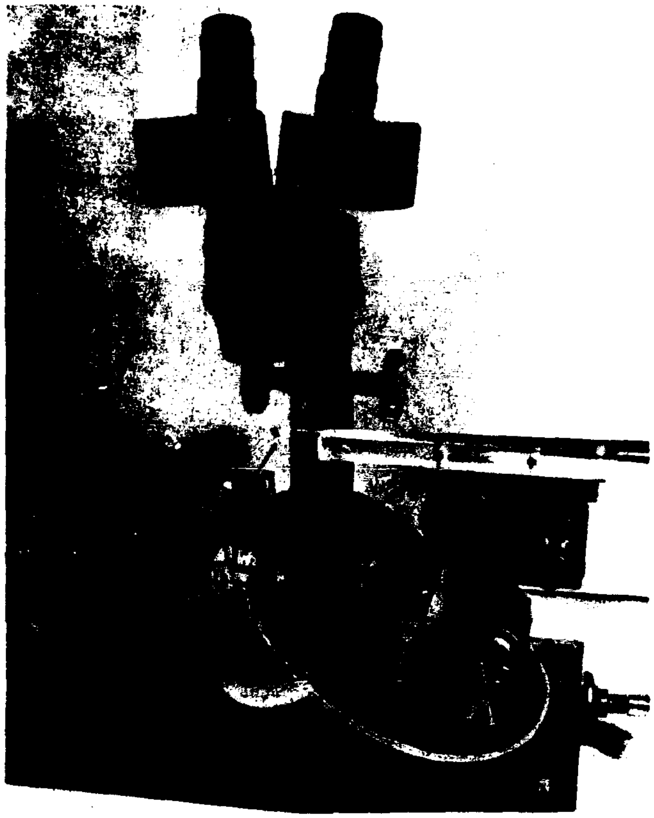

Figure 5. Dissection Microscope with Modified Stage and Micromanipulator for Standardizing Mosquito Attachment to Flight Mill Arms.

A flight mill arm is positioned in a channel on a modified Brinkman micromanipulator (Fig. 5). The arm is moved into position so that the foil tip is just above and behind the thorax of the mosquito. Figure 6 shows a mosquito in position prior to attachment to the mill arm. A minute drop of low melting-point wax is placed on the mesonotum of the mosquito with an electric needle (Fig. 7). After the wax is in position, the foil tip is moved forward and lowered into the drop of wax. The cold foil initiates immediate solidification of the wax and the mosquito is secured for flight. The vacuum is turned off and the mosquito is gently removed from the saddle. During attachment, the mosquito completely recovers from the cold and is ready to fly.

Figure 6. Mosquito Secured in Vacuum Saddle with Curved Foil Wedge on Mill Arm in Position for Attachment. The drop of wax on the mesonotum of the mosquito was greatly exaggerated to facilitate photography.

A minute drop of wax effectively attaches an aluminum foil wedge to the thorax of the mosquito without altering the function of the thorax. A wax with a relatively low melting point must be utilized because a hot wax would injure subcuticular tissues. Also, the wax must remain liquid until the arm tip can be brought into a correct position on the mesonotum. A temperature-indicating wax* with a melting point of 113 F satisfies the above criteria. Figures 7 and 8 diagram the electric microprobe and the power output necessary to melt and transfer a minute drop of wax.

* Tempil Corporation, New York, N.Y.

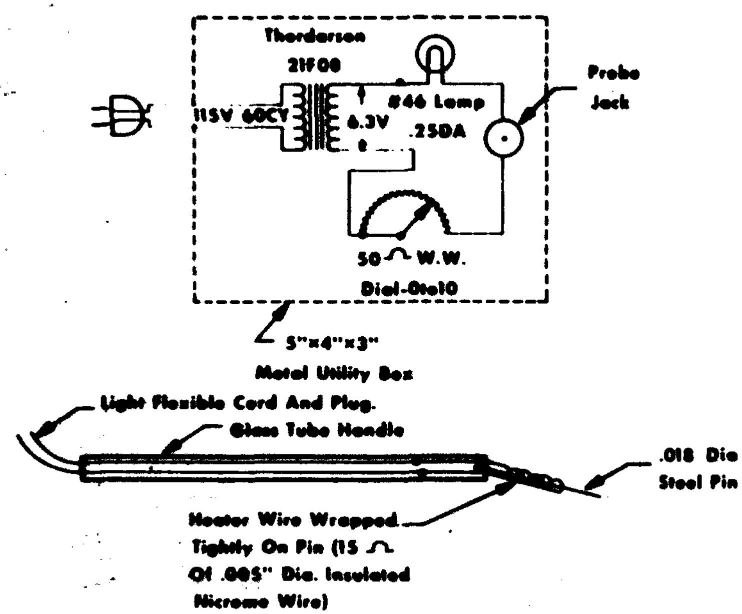

Figure 7. Schematic Diagram of Power Supply and Microprobe Used to Melt and Transfer Wax to Mosquito's Thorax.

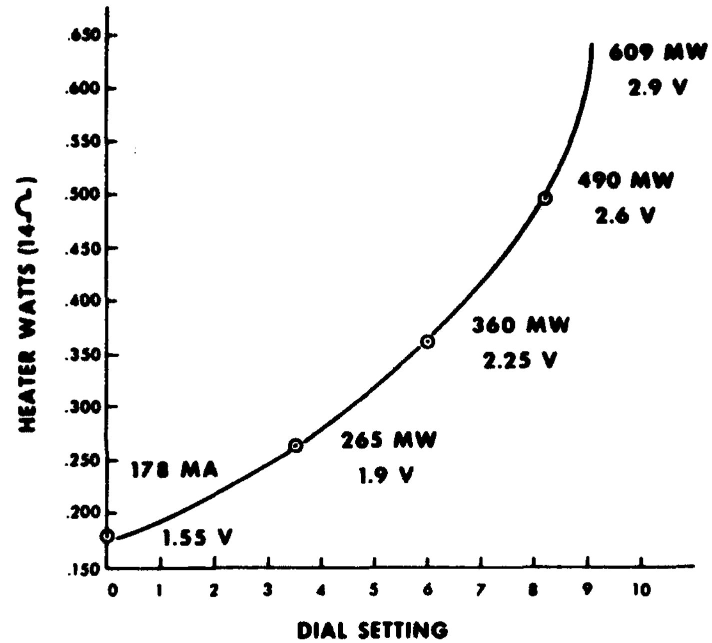

Figure 8. The Power Characteristics for Electrically Heated Microprobe are Also Given.

V. DISCUSSION

Pringle[19] states that the wing-beat form of a tethered insect is different from that observed during free hovering or free forward flight. Thus, he is critical of flight studies involving tethered insects, especially when such studies concern aerodynamic efficiency or energy utilization. However, little evidence exists that demonstrates wing-beat form during free or hovering flight. Weis-Fogh and Jensen[20] maintain that it is impossible to calculate energy changes in insect flight and that neither individual contributions nor their sums can be estimated. Therefore, they suggest a more rigorous definition of the term "efficiency" or elimination of its use altogether. At present, it is difficult, if not impossible, to measure the aerodynamic forces acting on a flying mosquito or its efficiency during flight. The aerodynamic and energetic aspects of insect flight are intriguing basic problems, but they are, in fact, academic in nature. In lieu of a better method for studying the flight potential or performance of mosquitoes, we feel and shall demonstrate that an efficient flight mill system can be utilized as an effective laboratory tool to estimate and compare many heretofore unmeasurable parameters of mosquito flight.

The influence of age, temperature, and humidity on the flight performance of insects can be determined in the laboratory. Information gained from these studies answers important questions, especially with regard to mosquitoes that are vectors of viral or other pathogenic disease agents. Many other aspects of mosquito flight can be investigated in the laboratory with a flight mill system. Laboratory flight studies can serve as good and probably reliable indices of the actual flight potential of mosquitoes in nature, until augmented or replaced by sound field studies considerably more efficient than those in present use.

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13. ABSTRACT

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*Mosquitoes
*Flight mill

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