In field tests in the Kern Mosquito Abatement District EPN killed 99–100 per cent of these mosquitoes when applied from a jeep at 0.035 pound per acre in emulsion or suspension. Malathon and tetra-*n*-propyl dithionopyrophosphate were about one-tenth as effective as EPN.

Routine applications of an emulsion at 0.075 pound of EPN per acre with 19 jeeps and 1 plane in the summer of 1952 were completely effective against these larvae.

Periodic blood tests of four of the men carrying on these spraying operations have shown no significant reduction in the cholinesterase level of their red cells or plasma.

No adverse effect has been noted in cattle that have grazed in one pasture after six successive applications of this emulsion.

Literature Cited

GJULLIN, C. M., and RICHARD F. PETERS. 1952. Recent studies of mosquito resistance to insecticides in California. *Mosquito News* 12(1):1–7.

LINDQUIST, ARTHUR W., A. H. MADDEN and H. O. SCHROEDER. 1946. Effect of temperature on knock-down and kill of mosquitoes and bedbugs exposed to DDT. *Jour. Kans. Ent. Soc.* 19(1):13–15.

YATES, W. W. 1950. Effect of temperature on the insecticidal action of mosquito larvicides. *Mosquito News* 10(4):202–204.

YATES, W. W., and ARTHUR W. LINDQUIST. 1952. Toxicity of EPN, DDT, and parathion to larvae of various species of mosquitoes. *Mosquito News* 12(4):247.

SOME TECHNIQUES FOR MOUNTING MOSQUITO EGGS, LARVAE, PUPAE AND ADULTS ON SLIDES

GEORGE J. BURTON, PH.D.

Entomologist, Engineer Research and Development Laboratories, Fort Belvoir, Virginia

The methods described in this paper have been used by the writer for years in making clear, permanent slides of all stages of mosquitoes.* Good slides do not always result merely by following certain steps. Manual dexterity and care in handling specimens are of utmost importance, and must be acquired. The loss of hairs or scales results in imperfect specimens which may not be identifiable after being mounted.

The mounting media preferred by the writer include (1) xylene-soluble resins such as Clarite, Piccolyte, Permount, or army issue synthetic resin, and (2) alcohol-soluble gums such as euparal and diaphane. These are preferred because there is no yellowing of preparations with age, and crystallization never occurs. Clarite is colorless, whereas all the other media mentioned have a slight tinge of yellow which is not readily apparent unless the mount is unusually thick. The synthetic resins in the first group are sometimes sold as crystals or nuggets which must be dissolved in xylene to make a mounting medium of desired viscosity. Permount is also available with toluene as the solvent, but xylene is preferred because it does not have the harsh effect that toluene sometimes has on insect tissues. Euparal and diaphane exert a clearing action on the specimen; xylene-soluble media also have some clearing action, as xylene is often used as a clearing agent by itself.

Certain types of equipment are very useful for manipulating specimens, but are not mandatory. The following items have been very useful: minuten nadeln;

* The material presented here in no way constitutes an endorsement by the Department of Defense.

No. 1 insect mounting pins; swab sticks minus cotton swabs; syracuse watch glasses; stender dishes with covers; sharp-pointed tweezers or forceps; a petri dish; fine wire; a small piece of flexible tin, ¾ x ⅛ inch; medicine droppers, some of which have been cut off at the tip to provide a larger opening; a Bunsen burner or alcohol lamp; fine and medium camel's hair brushes; and a pair of fine-pointed jeweler's pliers. An excellent dissecting and manipulating needle can be made by pushing the point of a safety pin into the flat end of a swab stick, then inserting a minuten or No. 1 insect pin into the puncture with the aid of jeweler's pliers. Five or six such needles are very handy in the laboratory. Several manipulating needles made with No. 3 insect pins are often of value when stronger pins are indicated, as in straightening legs. A short piece of fine wire should be inserted into the end of a swab stick, and the end of the wire looped to ⅛ or 1/16 inch; such a loop is very useful in transferring specimens from one container to another. The small piece of tin ¾ inch by ⅛ inch, mentioned above, should be inserted into the split end of a swab stick, and held in place with adhesive tape or scotch tape. The tin should be bent up at the tip, so as to form a J or L. This small spatula is likewise useful in transferring specimens. If desired, sewing needles may also be inserted into swab sticks so as to form manipulating or dissecting needles of various sizes. Such home-made needles can be made at very little cost.

Chemicals required include: 70 per cent ethyl alcohol, 95 per cent ethyl alcohol, xylene, beechwood creosote, and 10 per cent sodium or potassium hydroxide. Absolute ethyl alcohol may be used, if available, but it is not essential. Beechwood creosote may be used over and over again; consequently, a small bottle will last for many months. Clearing solutions of sodium or potassium hydroxide should be changed when considerable amber discoloration is evident. Twenty or twenty-five per cent hydroxide solutions may also be used if quicker action is desired, although it is recommended that the greater dilutions be used over a longer period of time for best results.

The alcohols are used for extraction of water from eggs, larvae, and pupae. Adults are never placed into alcohol. Beechwood creosote is used for clearing larvae and pupae after dehydration with alcohol. Sodium or potassium hydroxide is used only when beechwood creosote does not clear the specimen satisfactorily. Xylene is used as a premounting dip, and also for removing excess mounting medium as necessary. Ninety-five per cent alcohol is used also for removing excess euparal or diaphane from around the cover glass.

If practicable, eggs, larvae, and pupae should be carried from the field in the water of their natural habitat. Wide-mouth jars are preferred to vials, so as to lessen the possibility of hairs being knocked loose. Judgment should be used concerning the number of specimens per container. Specimens may also be killed in 70 per cent alcohol in the field, but it is preferred that live specimens be brought to the laboratory and killed in hot water. When dropped into hot water just short of boiling, eggs, larvae, and pupae are killed instantly. When they are dropped into alcohol, they twist and turn violently, making it possible for hairs to become detached during their struggles.

A stender dish, 35 x 18 mm. or 50 x 25 mm. is excellent for permanent storage of beechwood creosote used for clearing, since it has a tight-fitting cover which can be easily lifted up for removal. It is recommended that dehydration be carried out also in a stender dish, since a syracuse watch glass does not have sufficient capacity. Larvae and pupae are lifted and transferred with a small wire loop or tin spatula; if a camel's-hair brush is used, hairs may be dislodged. A medicine dropper may also be used for transferring specimens, but there is less chance of hairs or scales being dislodged when a loop or spatula is used. Adults should be lifted by the legs with a pair of tweezers or forceps. After specimens have been

placed on the slide, orientation is accomplished with minuten nadeln. It is essential that adjustment of hairs or parts be done while the specimen is completely covered by the mounting medium, which keeps the hairs in a flexible condition.

Standard histological techniques should be scrupulously followed for cleaning slides and cover glasses, and keeping them free from subsequent contamination with lint, grease, dust and debris.

MOUNTING EGGS. Eggs should be mounted in euparal or diaphane, preferably. They are picked up and transferred with a fine camel's-hair brush or medicine dropper, never with needles. It is not usually necessary for eggs to be cleared; however, egg rafts are often so heavily pigmented that clearing is necessary. Mosquitoes of the genera *Culex, Mansonia* (*Taeniorhynchus*), *Uranotaenia,* and *Culiseta* (*Theobaldia*) form egg rafts, but of the latter the subgenus *Culicella* does not. In other genera eggs are laid singly. Often, when a viable egg raft is immersed in 70 per cent alcohol, the unhatched first-instar larvae are forced out of their egg shells for about three-fourths of their length. This results in a mount showing both the egg raft and the mass of first-instar larvae. Clearing of rafts or individual eggs, if necessary, is accomplished in beechwood creosote. Eggs of *Anopheles* and *Orthopodomyia* should be handled very carefully, so as to prevent folding of the lateral membranous flanges of the latter and of the floats of the former. A piece of hollow glass tubing, drawn out very finely, may also be used for transferring individual eggs. Eggs of *Aedes* and *Psorophora* are easily handled, as they have no external membrane. An egg raft may be mounted so as to show either the upper or the lateral aspect. If the egg mass is too large, it may be separated with needles into smaller groups.

MOUNTING LARVAE. Usually only fourth-instar larvae and other skins are mounted, although it may be desired to mount the other instars so as to show changes in size, proportion, and chaetotaxy. Preferably, larvae are killed in hot water; if they are killed in 70 per cent alcohol, they may fail to clear properly, as in many species of *Aedes* and *Culiseta.* Larvae should be transferred individually, first to 70 per cent alcohol for at least two hours, then to 95 per cent alcohol for at least two hours. Results are best when the dehydrating time is increased up to 24 hours. It has been found that absolute alcohol is not necessary, but may be used if desired. No difference in results has been observed when maximum dehydration has been accomplished with 95 per cent alcohol, as compared with absolute alcohol.

After the initial dehydration, in order to avoid handling of larvae, the writer prefers to remove the 70 per cent alcohol from the stender dish, using a pipette. No hard and fast rule can be laid down about dehydration time, but in general, the larger the larva the longer the time necessary for dehydration. Ninety-five per cent alcohol is poured gently into the stender dish to replace the 70 per cent alcohol removed. After at least two hours, the specimen is lifted up with a wire loop or spatula and transferred to the stender dish containing pure beechwood creosote. The specimen may tend to float for a while, but if it has not sunk below the surface after ten minutes, it should be pushed below the surface of the creosote with a needle or loop. It is not absolutely necessary for the specimen to remain on the bottom, as sufficient clearing will take place even if it remains suspended in the creosote. Some larvae become cleared in ten minutes, whereas others are cleared in one to two hours or more. No definite clearing time can be advocated; frequent examination is necessary. Very often it has been observed that the clearing time varies within the same batch of larvae of a particular species.

When clearing has been completed, the specimen is lifted out of the stender dish, and placed into a syracuse watch glass containing xylene. After one minute, it is removed and placed on a clean slide. The xylene evidently counteracts the effect of minute amounts of water remaining in

the tissues, and is also preparatory for mounting in a xylene-soluble medium. It has been frequently observed that very large larvae, such as those of *Culiseta,* tend to retain minute amounts of water within the tissues, regardless of how many hours dehydration has taken place, even in absolute alcohol. This "bound water" often will cause a whitish precipitate to form around and over the specimen if the one-minute immersion in xylene is omitted. A precipitate seldom appears if this step is followed. Although specimens may be left in beechwood creosote indefinitely, it is recommended that mounts be made as soon as time permits. Occasionally, the beechwood creosote causes the specimen to become brittle, but this does not occur very often.

The mounting medium should not be too viscous, as it will not flow properly when the cover glass is applied. If it is too thin, the medium will seep out, often displacing eggs or larvae toward the periphery of the cover glass. The best viscosity may be determined by trial and error.

A larva must be examined under the stereoscopic microscope prior to being mounted, in order to ascertain whether all hairs are attached. If many specimens of a particular species are available, imperfect specimens may be discarded; however, if a comparatively rare specimen has been found, all such larvae should be mounted, providing the essential characteristics are still apparent. When a rare species is involved, a slightly damaged specimen is better than no specimen at all.

After ascertaining its condition, the larva should be placed upon a clean slide, dorsal aspect up. In culicine larvae, the dorsal aspect is obvious when the respiratory siphon is seen sticking up toward the observer. Any excess xylene remaining around the larva should be removed with any absorbent material. Several drops of mounting medium are then placed directly upon the specimen, so as to cover all the hairs. A sharp razor blade with edge held parallel to the slide, is then placed over the sixth or seventh abdominal segment, and with gentle pressure the larva is cut through. This procedure will enable the respiratory siphon to flop over on its side, thus bringing into view the pecten teeth, siphonal tufts, anal segment, anal brush, and the comb scales of the eighth abdominal segment. If desired, only a partial cut may be made, thus permitting the posterior segments to remain attached to the anterior portion. In any event, a clear, lateral view of the eighth and ninth abdominal segments is necessary for proper identification.

Anopheles larvae have no respiratory siphon; consequently other means are used to determine whether the specimen has been placed with the dorsal aspect up. Often the palmate hairs are visible without the aid of a microscope; a ten power hand lens will bring these clearly into view. Orientation of *Anopheles* larvae may be accomplished simultaneously with the examination for completeness of the specimen. Since *Anopheles* larvae rotate the head 180 degrees during feeding, they are often killed with the head upside down. An attempt should be made to twist the head back to normal if it is found to be reversed, but this is often difficult to do, and it may be necessary to sever the head from the thorax in order to orient the head properly. Palmate hairs of the right and left sides should be present, but if the specimen is rare it should be mounted regardless of condition until others are obtained.

After the posterior segments have been turned laterally, additional drops of mounting medium should be applied. With the aid of a small glass rod, the medium is then drawn over the slide so as to form a square pattern exactly the size of the square cover glass. This is done for two reasons: to reduce the possibility of the specimen moving while the cover glass is being lowered, and to prevent the formation of bubbles. It is important to have sufficient mounting medium directly upon the specimen, especially if it is a large larva. If the technician is right-handed, he should touch the left edge of the cover glass to

the left edge of the medium, then lower the right edge toward the slide surface with the aid of a needle supporting the right side. If the person is left-handed, the right edge of the cover glass is placed down first, and the corresponding procedure should be followed. There should be little or no movement of the larva, but occasionally, the severed posterior portion becomes disoriented. It can be reoriented as follows: Hold a No. 1 insect mounting pin parallel to the cover glass, carefully insert it under the cover glass from an appropriate direction, gently draw the moved portion into position, then remove the pin. Any specimen may be oriented in this manner. It is important that the pin be perfectly parallel to the slide, otherwise the cover glass will be pushed off. No ringing of the cover glass is necessary. The slide should be left in a horizontal position in a drawer or slide box for several days to a week, depending on the thickness of the mount. Usually, the medium begins to harden within twenty-four hours; when Clarite is used, the setting time is usually much shorter. Larval skins are also mounted as described above.

Only by experience will the person making slides learn just how much mounting medium is necessary for any particular specimen. The length, breadth, and height of the specimen will assist in approximating the amount which is necessary. If too much has been added, the excess will seep out quickly at the edges of the cover glass. If this happens, the slide should be kept perfectly horizontal, and, as seepage occurs, the excess should be wiped away with a small piece of paper towelling or a small square of cloth. When the seepage ceases wipe away traces of the medium at the right and left edges, using a cloth dipped in xylene. Never clean away any excess from around the top and bottom edges, as there is usually very little free space between the cover glass and edge of the slide, thus making it easy to move the cover glass and spoil the mount if care is not taken. When using diaphane or euparal, excess medium should be removed with a cloth dipped into 95 per cent alcohol, never 70 per cent alcohol. If a round cover glass is used instead of a square one, the same procedures are to be followed, except that the mounting medium should be spread to the size and shape of the cover glass prior to lowering the latter.

Often the amount of mounting medium is underestimated, but the remedy is simple. The slide is kept in a horizontal position, and slight pressure is placed upon the cover glass with the tip of a needle until all the existing medium moves to the lower half of the mount. Using a thin glass rod, additional medium is applied beneath the top edge of the cover glass, one drop at a time, until the empty space becomes filled with the medium. The slide may be tilted very slightly, if necessary, to assist the newly-added medium in flowing down to mingle with the medium already on the slide. A slight excess should be added to compensate for evaporation. The additional medium may also be added with a medicine dropper having a narrow opening, but extreme care should be used to avoid bubbles, and to avoid dropping the medium on top of the cover glass.

The removal of an air bubble depends on its location. A bubble located near the edge of the cover glass may be removed by inserting a needle beneath the cover glass and working the bubble toward the edge. When it reaches the edge, touch it with the point of a twisted piece of paper towelling, or small cloth; the bubble will usually be absorbed. If the air bubble is located too far in from the edge, and is in the upper half of the mount, tilt the slide slightly by holding the lower edge of the slide to the table and raising the upper edge. The bubble will then travel toward the upper edge of the cover glass, from whence it may be removed with the point of a twisted paper towel. If the bubble is in the lower half of the mount, the opposite procedure is followed; i.e., the lower edge of the slide is raised, and the bubble will flow gradually toward the lower edge. In other words, a bubble tends to work

its way toward the edge which is raised.

Another convenient method of removing an air bubble is to use a very fine pipette made by drawing out a piece of glass tubing. Insert it under the edge of the cover glass and aspirate the bubble out. The bulb should be squeezed *before* inserting the pipette underneath the cover glass, otherwise bubbles will be added instead of removed. Should there be too many bubbles, or too large a bubble, it is recommended that the cover glass be removed, the bubbles wiped away, and a new cover glass added.

Very often, when a slide is examined the day after being prepared, evaporation at the edges or corners may be observed. Additional mounting medium should be added with a medicine dropper or narrow glass rod. It is a good idea to add a little of the medium to excess. Once the medium has hardened, it is not feasible to attempt to remove any excess from around the cover glass, as it may be covered over when slide labels are affixed. If removal of hardened medium is absolutely desired, it may often be accomplished by scraping carefully with a single-edged razor blade, taking care not to touch the cover glass.

There should be no halo around the specimen. When mounted, the larva should present a clear outline, just as if it were alive and being examined in water. Should a whitish halo appear after hardening of the medium, the cover glass should be removed, the larva should be replaced into xylene for one minute, and then removed. Should the halo persist after the larva has been mounted for the second time, it is evident that incomplete dehydration has taken place, consequently, the larva should be either replaced into 95 per cent alcohol, or else discarded if many such specimens of the same species are on hand.

If desired, the larva may be mounted in euparal or diaphane from 95 per cent alcohol, with or without clearing in beechwood creosote, as the alcoholic mounting media act as clearing agents themselves to some extent. Such a mount will, however, take much longer to dry than when a xylene-soluble medium is used.

The specimen should be mounted directly in the center of the slide. The writer prefers to use two labels. The label to the left of the cover glass should have printed upon it the complete scientific name of the specimen, the date collected, the locality where collected, the type of water accumulation from which collection was made, and the name of the collector. The label at the right should have printed upon it the chief characteristics used in identifying the specimen to the particular species, so that any person can readily check the identifying characteristics against the specimen itself. This procedure is valuable in teaching taxonomy, and avoids constant reference to keys; it is also useful when a field worker sends a mounted specimen to his headquarters located far away. It is entirely possible that the field entomologist may have keys, whereas his headquarters may lack them. If the label at the right is included, the receiving entomologist will have all information available on the slide mount. If the field entomologist had sent the specimen with only the determined genus and species, and the receiving entomologist had no key for determination of the specimen, the receiving entomologist might not know which features of the larva were diagnostic for the particular species.

MOUNTING PUPAE. The pupal characteristics of value in identification are the size and shape of the respiratory trumpets, the shape of the cephalothorax, and the chaetotaxy of the abdominal segments and paddles. Ordinarily one does not attempt to determine the species of a particular pupa, for the adult will emerge soon and lend itself more readily to identification.

A pupa is ordinarily too bulky to be mounted on a flat slide without being modified first, but such a mount is possible with slight distortion. After being dehydrated in 95 per cent alcohol, a pupa is placed between two slides in a petri dish containing 95 per cent alcohol. Using slight but continuous pressure upon the

upper slide directly over the pupa, the latter may be flattened slightly without being crushed. The abdomen may be shown either in its natural lateral aspect, or may be twisted around so as to show the dorsal chaetotaxy. The specimen is then placed into beechwood creosote for clearing, and later mounted as described for larvae. The mount may tend to be high, but pressure on the cover glass will usually produce an acceptable mount. More mounting medium is required in mounting pupae than in mounting larvae, due to the height of the pupa. It is better to add excess medium and then wipe the excess away, than to underestimate and attempt to add medium later. As the pupa becomes enveloped by the mounting medium, it will tend to flatten. Evaporation of some of the medium often takes place by the next day, due to the height of the mount, consequently additional medium should be applied as evaporation occurs. Complete drying of such a high mount requires several weeks to a month with all media except Clarite, the latter usually drying in one to several days.

If desired, a pupal skin may be mounted instead of the entire pupa, after emergence of the adult. Clearing is not necessary in this case, as the skins are quite transparent. The dorsal chaetotaxy may be made apparent by detaching the cephalothoracic portion, or by twisting the abdomen while the cephalothoracic portion is attached. The skin may be mounted in euparal or diaphane directly from 95 per cent alcohol, or in a xylene-soluble medium directly from xylene. Labels should be affixed as described under mounting of larvae.

MOUNTING ADULTS. Mounting of adults on slides should be a supplement to, not a substitute for, pin mounts. The writer developed this method as a means of preserving permanently certain anatomical features of mosquitoes, especially for use in teaching. An entire male or female mosquito may be mounted on a flat slide, making the following features easy to study: wing scale patterns, ring patterns on legs, color patterns of tergites and sternites (to some extent), mouth parts, antennae, eyes, head tufts, and lateral thoracic bristle groups. White stripes, white bands, and other whitish or yellowish markings usually retain their color, but in the genus *Aedes* the white markings become cleared. It has been found that white markings of *Culiseta* (*Theobaldia*) and *Anopheles* show up very well.

Only reared, unfed adults are mounted, as they possess all the bristles characteristic of the species, are quite transparent, and have a minimum of water in the body. Following emergence from the pupal case, the adult is killed with ether or chloroform. As soon as it is dead, it is placed on its side on a clean slide, and synthetic resin mounting medium is dropped upon it, thus gluing it down. Additional drops are applied upon the head, legs, and abdomen, but most of the medium is concentrated upon the heaviest parts of the body. The wing nearest the technician will give the greatest trouble, as it tends to fall so as to obscure the thorax and abdomen. The opposite wing will give less trouble, as it can be glued down readily with the mounting medium. Fracturing the axillary sclerites at the wing base often tends to permit manipulation of the wings as desired. Pins should not be pressed against the wing membrane, as it will tear. Insert the pin next to the costa for easiest manipulation of the wing. The mount may not be very high, as the adult has not been given the opportunity to feed. Only a square cover glass should be used, and should be lowered in the manner already described. Should the wings, legs, antennae, or mouth parts tend to move out of position after the cover glass is applied, they may be returned to their former position by using a No. 1 insect pin carefully inserted beneath the cover glass.

It is usually unnecessary to dissect out the individual mouth parts, as they tend to separate away from the labium of their own accord when the mounting medium is dropped upon the proboscis. Should separation not be complete, it should be accomplished with minuten nadeln under

a stereoscopic microscope. The labrum-epipharynx (U-shaped in cross section), mandibles (sharp-pointed), maxillae (distally finely serrated), hypopharynx (stylet-like) and labium (sheath-like, terminating in a pair of labella) should be discerned after the mount has been made. When making mounts of male mosquitoes, the mouth parts may or may not separate out. Details of the antennae and palps are readily discernible in all mosquitoes if the mounts are prepared properly. Mounting of an adult on a slide may take about 15 minutes or more, but the results are worth the effort. Dorsal and ventral views are best seen in mosquitoes mounted on pins. It is to be re-emphasized that slide mounts are supplements to, not substitutes for pinned mosquitoes.

Any part of the mosquito body may be mounted separately. Heads, legs, and wings may be dissected from the body and mounted alone. Wings must never be put into liquids. To mount a pair of wings, remove them carefully from the thorax, orient them on a clean slide, spread a few drops of a xylene-soluble medium over the wings, and apply a cover glass. Should the wings move out of position, insert a No. 1 insect pin beneath the cover glass, and carefully move them back into position. If mosquito heads or legs are to be mounted individually, they should be separated from the body *prior* to clearing in beechwood creosote, as it is more difficult to perform the dissection after clearing.

To mount male genitalia, snip off the tip of the abdomen prior to dehydration, clear in beechwood creosote, and mount after drawing out the parts with fine needles. It is often advantageous to clear with hot or cold 10 per cent sodium or potassium hydroxide instead of beechwood creosote, for better depigmentation. The clearing time may be shortened considerably when the hydroxide is warm or hot. The freshly-clipped genitalia should be placed into the hydroxide solution, and after clearing has progressed to the desired point, the genitalia should be placed in tap water or distilled water for one or two hours. Dehydrate with 70 per cent alcohol for two hours, follow by 95 per cent alcohol for two hours, and then mount in euparal or diaphane; otherwise the genitalia may be transferred to xylene for one minute following 95 per cent alcohol, and mounted in one of the synthetic resins. Drawing out of the parts of the genitalia may be easier to accomplish after washing in water, instead of after dehydration. The hardening action of the alcohol may tend to keep the parts in place.

SUMMARY. Some successful techniques for mounting mosquito eggs, larvae, pupae, and adults on slides have been presented. Briefly, the steps are as follows:

Mounting Eggs. (1) Kill in hot water (preferably) or 70 per cent alcohol. (2) Dehydrate in 70 per cent alcohol for two hours. (3) Dehydrate in 95 per cent alcohol for two hours. (4) Mount in euparal or diaphane.

Mounting Larvae. (1) Kill in hot water (preferably) or 70 per cent alcohol. (2) Dehydrate in 70 per cent alcohol for 2–24 hours. (3) Dehydrate in 95 per cent alcohol for 2–24 hours. (4) Clear in beechwood creosote for at least 10 minutes. Remove when clearing is complete. Store indefinitely in beechwood creosote, if desired. (5) Place in xylene for 1 minute. (6) Mount in a xylene-soluble medium, such as synthetic resin, Piccolyte, Clarite, or Permount; *or,* mount in euparal or diaphane, omitting step (5).

Mounting Pupae. (1), (2), (3)—same as for larvae. (4) Place pupa between 2 slides in petri dish filled with 95 per cent alcohol. Apply slight pressure so as to flatten pupa slightly. (5) Follow steps (4), (5), and (6) under *Mounting Larvae.*

Mounting Adults. (1) Kill emerged, unfed adult with ether or chloroform. (2) Place on slide as desired, cover with synthetic resin mounting medium or equivalent. (3) Orient wings, legs, head, and mouth parts, and apply cover glass.

Continued practice is necessary for achieving success in mounting. The technician will often develop variations in technique or new techniques after con-

siderable experience. Manual dexterity is necessary for orientation of parts of the specimen which are comparatively fragile. Various mounting media other than those mentioned herein have been used by entomologists, and may be tested by the newcomer to slide-making. The methods presented here have been time-tested, and, if followed faithfully, should result in permanent slides of mosquito eggs, larvae, pupae, and adults, of which the entomologist can be proud.

A TECHNIQUE FOR MICROMANIPULATION OF MOSQUITOES[1]

ROBERT CHARLES WALLIS[2]

The importance of insects in basic research has become more generally recognized in recent years. Many possible lines of investigation have been pointed out in physiological research by Roeder (1952). In one of these, using the mosquito as an experimental animal, Roth (1952) describes physiological experiments requiring microsurgery of the adult mosquito. Shambaugh (1952) points out the importance of microinjection techniques for studies in toxicology and physiology, and describes a microinjection technique for *Aedes aegypti*. However, in studies involving mosquito microsurgery and microinjection, extreme difficulty is encountered in the handling of the mosquito due to its very fragile structure and small size. Physical damage of mechanically holding the mosquito, surgical shock, heat desiccation, and prolonged anesthesia in such operations all tend to produce a high operative mortality rate. Studies in this laboratory requiring micromanipulation of living mosquitoes for microsurgery have resulted in an improved, simplified technique that has effectively lowered post-operative mortality, and reduced preparation time for experiments that require operations on large numbers of mosquitoes.

This success is due principally to the introduction of a suction pipette as a holding device to replace pinching or other friction holding instruments for securing the anesthetized mosquito while operating under a dissecting microscope. The suction pipette, pictured in Fig. 1, was made by drawing out a quarter-inch glass tube to a small oval tip sized 1 by 2 millimeters. This tip was then covered with grid by cementing on a piece of 51 gauge nylon cloth material. The pipette was attached with rubber tubing to a water pump suction device, and sufficient suction maintained to securely hold an adult mosquito to the grid tip of the pipette.

In use, the adult mosquitoes were exposed briefly to CO_2 gas in small cartons, and when anesthetized, the rubber tubing to the pipette was pinched to shut off the suction, the tip of the pipette placed close to the scutum of the mosquito, the tubing released to start suction in the pipette so that it firmly secured the mosquito against the grid tip, as shown in Figure 1. In this manner the mosquito is held in place by gentle pressure against the relatively strong thick integument of the scutum, with little damage to the mosquito. Also, as shown in Figure 1, there is a minimum surface area occluded by the holding device, and the legs and other appendages are left freely accessible. Holding a mosquito thus, it could be picked up and manipulated freely under the field of the dissecting microscope without physical damage. Also, held securely in this man-

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[2] Department of Parasitology, Sch. of Hyg. & Pub. Hlth., The Johns Hopkins University, 615 N. Wolfe Street, Baltimore, Md.