METHODS FOR DISSECTING SALIVARY GLANDS IN MOSQUITO LARVAE AND PUPAE[1]

DINNIEMAUD V. JENSEN

Although detailed instructions exist for the removal of the salivary glands from adult mosquitoes (e.g., Russell, West, and Manwell, 1946), there seems to be no description of how these glands may be removed from larvae and pupae.

This paper briefly describes and illustrates several methods by which salivary glands may be easily extracted from larvae of all stages and pupae of *Anopheles albimanus* Wiedemann.[2]

EQUIPMENT. The following items or their equivalents are needed for an adequate study: (1) a binocular stereoscopic microscope permitting magnifications of 30–40 diameters, (2) a compound microscope (100×), (3) orangewood sticks, (4) No. 1 insect pins, (5) microscope slide, (6) filter paper (Whatman No. 1), (7) medicine droppers, and (8) 0.85 percent sodium chloride.

Dissecting needles can be made by embedding No. 1 insect pins firmly into orangewood sticks. Both straight and bent needles are necessary. The bending may be done with small forceps or pliers, a 90° bend being most desirable. The tips of the needles should be sharpened on an oil stone or an emery wheel.

PREPARATION FOR DISSECTION. A larva is placed dorsal side up in a drop of water on a glass slide. Pupae are placed in a lateral position. The specimen is partially immobilized by quickly removing the water around it with filter paper cut into small triangular pieces. The slide is then orientated on the stage of a dissecting microscope so that the head of the larva or pupa is to the operator's right. The salivary glands usually can be seen in the intact larva, lying in the thorax (Plate I, Fig 1a).

TECHNIQUES FOR REMOVAL OF LARVAL GLANDS.

First Method:

(a) Hold body firmly in place by putting one bent needle between thorax and first abdominal segment (Fig. 1a).

(b) Sever head with other bent needle.

(c) Place this same needle across metathorax. Push gently downward and slowly and steadily forward (Fig. 1b) so as to express thoracic contents through anterior opening. Straight needles, used in the same manner, are needed in removing glands from young first instar larvae.

(d) Remove fat body and other undesirable material which may obscure glands, using pieces of filter paper.

(e) Add drop of saline with medicine dropper and examine.

Second Method:

(a) Hold body of larva in place as in 1a.

(b) Do not remove the head.

(c) Insert sharp bent needle just under mid-dorsal surface of thorax at posterior margin and make shallow slit up to the anterior end of thorax.

(d) Put elbow of needle between head and thorax (Fig. 2a).

(e) Exert opposite traction on needles as shown by arrows in Fig. 2b so that glands come out still attached to head.

(f) Examine in saline.

Third Method:

(a) Anchor body firmly as in 1a.

(b) Remove head.

(c) Using sharp bent needle, make mid-dorsal longitudinal slit from posterior to anterior end of thorax (Fig. 3a).

(d) Tease out glands in drop of saline with needles as shown in Fig. 3b.

---

[1] From the Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Bethesda 14, Maryland.

[2] The mosquitoes used in this study were reared in the insectary of the Laboratory of Tropical Diseases using methods described by Trembley (1955).

(e) Remove fat body and other undesirable debris.

The first method is especially useful with first instar larvae. The second is used when it is desirable to remove both glands and their ducts. The third is the most efficient and can be used with all stages, except first instars.

TECHNIQUES FOR REMOVAL OF PUPAL GLANDS.

First Method (for pupae less than one day old):

(a) Hold pupa firmly on side with one needle at posterior edge of cephalothorax and remove head as shown in Fig. 4a.

(b) Push another bent needle very gently but firmly downward on middle of thorax and slowly move it forward thus pressing out thoracic contents (Fig. 4b).

(c) Absorb extraneous material with filter paper.

(d) Examine saline mount for degenerating pre-imaginal glands or for developing imaginal glands.

The newly-forming minute imaginal glands are best observed by using a compound microscope (100×). Those in the male appear as two tiny and highly refractile clavate structures; in the female they are minute and tri-lobed.

Second Method (for pupae one day old or over):

(a) Uncurl mouthparts and abdomen and straighten same into adult position (Fig. 5).

(b) Remove legs carefully.

(c) Hold thorax firmly with one needle.

(d) Place another needle at junction of head and mouthparts.

(e) Pull gently with needles in opposite directions (note arrows in Fig. 5) so as to separate head from thorax.

(f) Pull out imaginal glands attached to head.

(g) Examine in saline.

Literature Cited

RUSSELL, P. F., WEST, L. S., and MANWELL, R. D. 1946. Practical Malariology, W. B. Saunders, Philadephia, pp. 245–246.

TREMBLEY, H. L. 1955. Mosquito Culture Techniques and Experimental Procedures, Amer. Mosq. Cont. Assoc. Bull. No. 3, pp. 15–16.

FIG. 1.—Dorsal view of larva with (a) position of dissecting needles at start of dissection and (b) after completion of dissection.

FIG. 2.—Larva showing (a) position of needles at start of dissection and (b) direction of pull exerted by needles as salivary glands and ducts are pulled out attached to head capsule.

FIG. 3.—View of larva showing position of needles (a) while making longitudinal slit in thorax and (b) while expressing salivary glands from thorax.

FIG. 4.—Lateral aspect of pupa with position of needles (a) at start of dissection and (b) just prior to pressing the glands out anteriorly.

FIG. 5.—Lateral view of pupa with mouthparts uncurled, showing position of needles and direction of pull as imaginal glands are pulled out attached to head capsule. S.G. denotes salivary glands.

PLATE I
(Diagrammatic)