Staining of Filarial Larvae in Insects before Dissection

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During recent studies on filariasis in Kenya, a simple technique has been developed for demonstrating filarial larvae in preserved mosquitos. After collection, the mosquitos are killed and placed immediately in 80% ethyl alcohol, where they can be kept indefinitely. The specimens are taken through descending dilutions of alcohol to distilled water, stained for three days in Mayer's acid haemalum at room temperature (60°-80°F—15°-27°C), and then differentiated for three days in distilled water. Finally they are transferred to pure glycerol to await dissection. It is convenient to use 3-inch × 1-inch (2.5+7.6 cm) tubes with 100-200 mosquitos per tube.

The mosquitos are dissected with fine needles, using reflected light and 35 times magnification of a stereomicroscope. All stages of developing larvae can be stained and are easily detected; they can be permanently mounted in glycerol for detailed morphological studies. In recently engorged mosquitos the blood-meal becomes fixed, and microfilariae cannot be distinguished in the stomach. Third-stage larvae must be handled with more care than in fresh dissections. Several thousand mosquitos have been dissected by this method, and a collection has been made of several species of filarial larvae.

The technique is equally useful for onchocerciasis investigations. Mr G. R. Barnley, Senior Entomologist, Uganda, sent a collection of *Simulium neavei* in 80% alcohol from Mount Elgon; 56 out of 454 were found to be infected, and two distinct species of third-stage larvae were seen.

The technique is a modification of that used by Lebied [1] for studying filarial larvae in the thoracic muscles of *S. damnosum*. Lewis [2] and Vargas [3] have also demonstrated onchocercal larvae in preserved unstained Simuliidae. The advantages of the method are that insect vectors need not be dissected on the spot, and that more accurate observations can be made on the density of infections, the stages of development, and the species of infective larvae.

[1] Lebied, B. (1950) *Une nouvelle théorie endémiologique* (privately published, Imprimerie Darantière, Dijon)

[2] Lewis, D. J. (1953) *Bull. ent. Res.* **43**, 597

[3] Vargas, L. (1942) *Rev. Inst. Salubr. Enferm. trop. (Méx.)*, **3**, 57