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AIDS TO OVARIAN DISSECTION FOR AGE DETERMINATION IN MOSQUITOES

M. E. C. GIGLIOLI
Medical Research Council, Keneba Field Station [1]

INTRODUCTION. Polovodova (1949) and Detinova (1946 and 1962) demonstrated in *Anopheles maculipennis* that age could be determined accurately by post-ovipositional changes in the structure of the membranous tube which joins each ovariole to the inner oviduct or calyx of a mosquito ovary.

Where an ovariole has developed successfully or degenerated, local stretching of the *tunica intima* (Bertram, 1962) around the developing ovariole, followed by partial contraction after ovulation leaves a distinct nodular swelling or dilatation to mark the spot in each follicular tube. Since each dilatation marks one cycle of ovarian development, the greatest number of such dilatations within any one ovariole of an ovary, multiplied by the time taken to complete a gonotrophic cycle equals the minimum age of the mosquito.

In the course of ecological studies on *Anopheles gambiae melas* in the Gambia, West Africa, during 1959 and 1962, over 75,000 mosquitoes were dissected for age determination. This paper describes certain technical difficulties in ovariolar dissection which were met and their solution.

METHOD OF DISSECTION. The ovaries are teased out from the abdomen and freed

[1] From: Medical Research Council Laboratories, Fajara, Gambia, West Africa. Dr. I. A. McGregor, O.B.E., M.R.C.P., Director.

from the ovariolar sheath. A sample of 15–20 follicles per mosquito is examined individually with dissecting microscope under a magnification of X80 or 100, by carefully stretching, without breaking, each follicular tube and its investing muscular sheath (Giglioli, 1959) from the calyx.

The hyaline elastic tube, pedicel and connective stalks (Bertram, 1962), joining the presently developing follicle to the calyx can be seen through the ovariolar sheath. Careful focussing of the microscope will show the presence or absence of bead-like dilatations and, if these are numerous, will allow them to be counted.

DIFFICULTIES AND SOLUTIONS. FOCUSSING. The need for high magnification and resulting shallow depth of field, further aggravated by dissecting the relatively large ovarioles in order to observe the much smaller and transparent pedicel, calls for constantly changing focus, at a time when the operator's hands are totally engaged in maintaining the separation of delicate elastic structures. To cope with this the focussing adjustment of the mircoscope should be independent of the operator's hands.

Figure 1 illustrates a simple method for controlling focus with one's knee, constructed from material readily available in any laboratory or field station.

The lever arm (Fig. 1; fl.) activating the focussing knob of the microscope, is a shortened test tube clamp. A piece of string connects it to a bent iron rod, the knee lever (kl.). The knee lever is located laterally to the microscope on the left hand side of the operator, and is fixed at one end to the lip of the work bench by a horizontal hole which allows it free radial movement. Its free end is bent under the bench to come in contact with the side of the seated operator's knee.

The string connecting the focussing arm to the knee lever passes through a screw eye guide (sc.) which is screwed into the lip or combing of the work bench, in line with the base of the microscope. To operate the focussing device, the test tube clamp is slackened and the microscope focussed manually on a slide on its stage. With the knee lever in a comfortable position (4 o'clock) the test tube clamp is elevated (2 o'clock) and tightened around the focussing knob of the microscope.

A focussing level 5¾" long with insertion of the string 2" from the centre of rotation of the knee lever provides an objective movement of up to 4 mm on a Zeiss dissecting microscope; since objectives are parfocal no resetting is needed for a change in objective magnification.

Although the original apparatus, shown in Figure 1, is fitted on a Zeiss microscope, it has been used successfully on Cooke, Watson and Pryor microscopes with little modification.

STAINING. The ovarioles and sometimes the muscles of the ovariolar sheath and calyx stain with methylene blue and other intravitam stains, but the *tunica intima* forming the tenuous pedicel and small transparent dilatations do not. Since critical definition on this part of the ovariole is needed for the successful application of the Russian aging techniques, many stains were tested in the course of the Gambian studies. Only aqueous Gentian Violet gave satisfactory results, staining all structures in the ovary, giving excellent definition and greatly enhancing "visibility."

Gentian Violet in aqueous or saline solution is used as a dissecting medium; the strength of the solution is determined by eye, depending on the degree of staining required. It cannot be used as a permanent histological stain for permanent mounts in P.V.A. or, after dehydration, in D.P.X., Canada Balsam or Euparol, as it soon becomes destained.

ELASTICITY. Even with the help of knee focussing and clear staining, the tendency of the stretched ovariole to slip from under the dissecting needle and contract against the calyx is exasperating; especially with the older stages of ovarian development, where sudden recuperative movements or heavy handedness may easily rupture the ovariole and release a cloud of yolk granules. These obscure the field and often the granules adhere to the pedicel, making interpretation impossible.

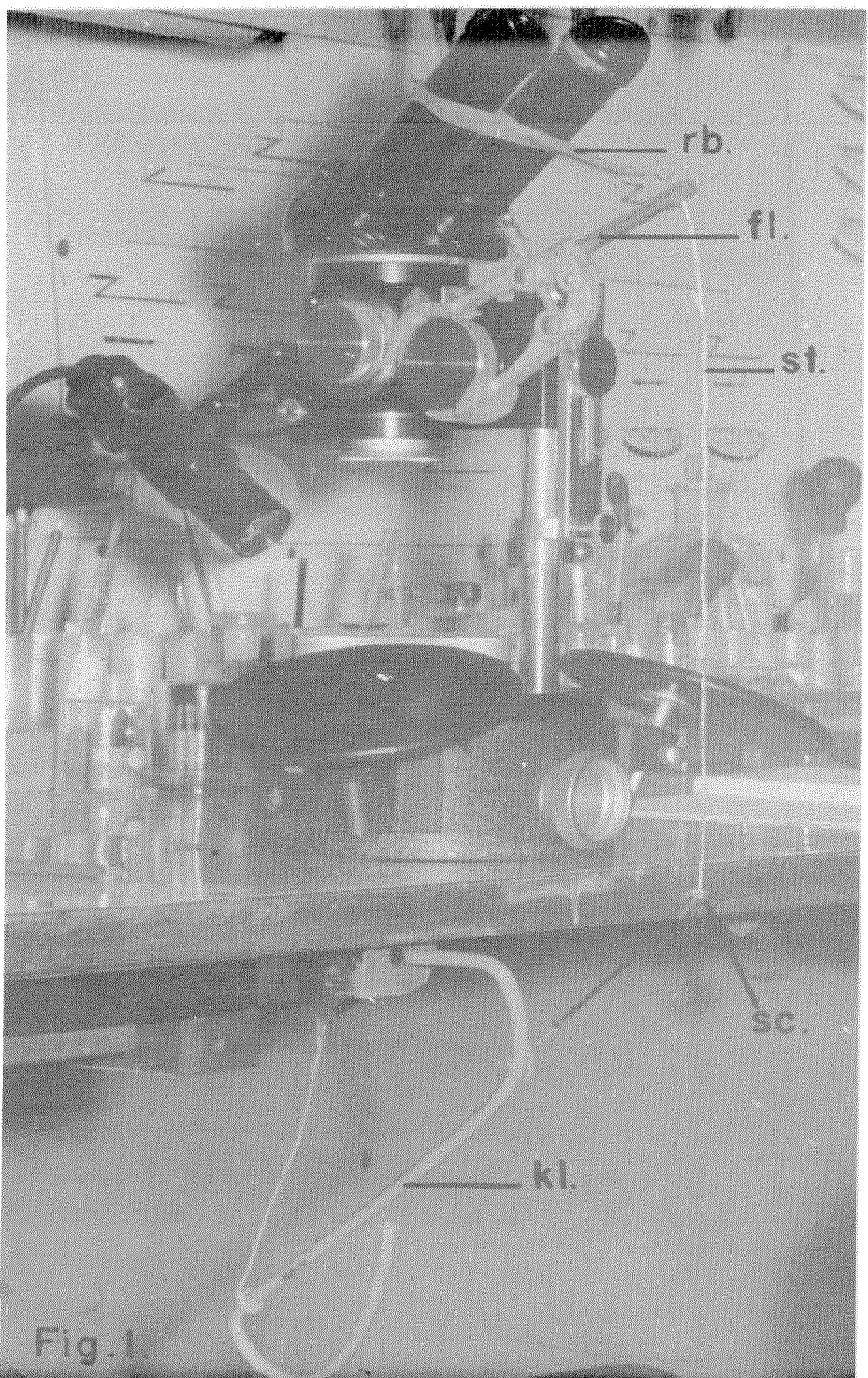

Fig. 1.

Attempts were made, using cardiac and uterine drugs, detergents and salts, to find a medium in which the striated anastomosing ovarian muscles and membranous intima could be relaxed and made easy to handle. The most successful medium found was a saturated solution of sodium chloride.

Using a saturated solution of sodium chloride, it is usually possible to stretch the ovariole and release it without contraction, thus allowing a whole series of ovarioles to be laid out and then examined comparatively. Saturated NaCl does not increase brittleness in the tissues or shrinkage in the membranous tube, though the latter occurs in the developing egg especially if the past Christophers' Stage III of development. An added advantage of this dissecting medium is that the yolk from a ruptured follicle clots rather than spreading all over the preparation.

The disadvantage of saturated NaCl is that it will not hold Gentian Violet in solution, but the combined advantages of the stain and decreased ovariolar elasticity can be obtained by dissecting in a medium of 1–2 parts saturated NaCl and 3 parts Gentian Violet.

CONCLUSION. The major technological difficulties met in ovariolar dissection for age grading anophelines have been simply solved by devising a knee focussing device for the microscope, staining *in vivo* with Gentian Violet and dissecting in high concentration NaCl solutions to achieve increased muscular flagidity.

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FIG. 1.—Zeiss dissecting microscope fitted with simple knee focusing device constructed with a piece of iron rod, a test-tube clamp, string and rubber bands.
fl. focusing lever, a test-tube clamp; *kl.* knee lever of construction rod; *rb.* rubber bands; *sc.* screw eye guide; *st.* string.