Tommers. Thinking that eggs could be stained while still in the gonads of the oysters, so that they could later be normally discharged and develop as colored larvae, we placed oysters in solutions of nile blue sulfate and of neutral red. Although the gills and palps of the oysters were deeply stained, the color of the eggs remained unchanged.

The next step consisted in staining unfertilized and fertilized eggs with the same two dyes. They stained well, but, because both stained and control individuals soon died, it was impossible to ascertain the real value of the method. Lack of time and lateness of the season prevented continuation of the experiments. These were, however, resumed by the writers in 1947, the following stains being used: alizarin red S-7, Janus green, neutral red, nile blue sulfate, and trypan blue. They do not, of course, exhaust the list of vital stains that may be tried for this purpose.

The eggs and larvae treated with trypan blue and alizarin red S-7 survived and continued their development, but even after long exposures to these stains the color was too light to be of value in distinguishing stained from unstained individuals. Janus green, generally used for vital blood staining, quickly killed the eggs and larvae. Nile blue sulfate also gave unsatisfactory results because, although the eggs and larvae acquired a deep-blue color, their development was quickly stopped and usually they soon died. In one instance only, the organisms stained as trochophores developed into veligers, *i.e.* the straight-hinge-stage larvae. These, nevertheless, soon died.

In experimenting with neutral red we found that satisfactory results can be achieved, regardless of whether eggs, trochophore, or straight-hinge larvae are used. Staining did not interfere with fertilization. After the addition of sperm, the stained eggs developed normally, the progress of the development was the same as in control eggs, and the mortality was no higher.

In some experiments the oysters were allowed to spawn in a solution of neutral red. The eggs were later fertilized and proceeded to develop normally at the same rate as the control.

The trochophore larvae, developing from the eggs treated with neutral red before or after fertilization, showed a deep-reddish color over their entire surface. After developing into the straight-hinge stage, the larvae still were deeply stained, the color being most intense along the edges of the mantle. Later on, the color became concentrated in several comparatively large areas of their bodies. About 11 days after they were stained as eggs, the larvae were still easily distinguishable from the controls by a large red spot near the stomach.

Trochophore larvae, stained 12 hours after fertilization, developed into normal straight-hinge larvae. They retained the red color the same as the larvae which developed from stained eggs. However, the veligers stained in the trochophore stage retained the color somewhat longer than those which developed from the eggs stained before or soon after fertilization.

In staining young veligers we noticed a certain inconsistency in the results. As a rule, the larvae concentrated the stain in a comparatively large area near the stomach, while the other parts of the body remained almost unstained. On a few occasions, however, the stain was generally distributed through the body, being most intense in the velum and along the edge of the mantle. In either case the stained larvae were easily distinguishable from the controls for a period of at least 10 days.

Older veligers, some as large as 300 μ, were also stained with neutral red. However, although the velum and other parts of the body, such as the mantle, became deeply stained, it is doubtful, because of the dark color and relative thickness of their shells, that they could be easily identified in preserved plankton collections.

In our opinion, the best stage for staining is the trochophore larva. Under laboratory conditions fertilized eggs can be carried to that stage without much difficulty. After staining, the trochophores may be released and should soon reach the early veliger stage.

To stain eggs or larvae we kept them for approximately 20 minutes in a solution of 1 mg. of neutral red in 1 l. of sea water, after which the water was either changed or greatly diluted. Of course, many variations of the method are possible by prolonging the time of exposure or by using stronger concentrations. However, in each case it should be ascertained whether the variation of the method would result in heavy mortality of larvae.

The method offered here presents rather broad opportunities for studies, under natural conditions, of vertical and horizontal movements, distribution, dispersal, and perhaps even the rate of growth and survival of oyster larvae and probably of other lamellibranchs. The latter will be ascertained by our experiments next year. Because our incidental observations showed that many other forms, such as barnacle larvae, copepods, many other crustaceans, ctenophores, worm larvae, etc., remain alive and apparently healthy after staining, the method may also be used for studies of distribution of a wide variety of plankton forms.

Incidentally, in staining the adult oysters we noticed that the boring sponges, such as *Cliona celata*, which inhabit their shells became deeply colored. The sponges retained the stain, especially nile blue sulfate, for several weeks and showed no ill effects of the treatment. Perhaps this observation could find application in some aspects of sponge studies.

A Precision Micro-Pipette Trimmer

KENNETH M. RICHTER[1]

Department of Histology and Embryology, University of Oklahoma School of Medicine

The need for a precise and easily used tool for trimming the micro-pipettes and micro-needles used in micrurgical studies is apparent to all students in this field of investigation. Belkin (*1*) designed a micro-guillotine for this purpose to be used with a Chambers micromanipulator, but the instrument appears not to be adjustable and requires some dissembly of the two-movement manipulator setup. The present paper describes a simply constructed and efficient instrument for trimming micro-pipettes and micro-needles for use with the Chambers (*2*) micromanipulator.

The instrument may be made of plastic or brass. The bulk of the instrument consists of two fitted flat jaws: a U-shaped rear jaw (Figs. 1A, 2A) and a rectangular-shaped front jaw (Figs. 1B, 2A) fitted between the arms of the rear jaw. The two jaws are held in mutual position by two guide bolts (Fig. 1C) which pass through counter-sunk channels drilled through the back edge of the rear jaw and which are screwed into the

[1] I would like to extend my thanks to Ernest F. Hiser, of our Art Department, for the preparation of the illustrations and also to our Pharmacology Department for making its machine tools available to me.

apposing edge of the front jaw (Figs. 2A, 2C). The guide bolts function only to facilitate the general handling of the instrument and to limit the extent to which the jaws may be opened. A centered groove ½ inch wide by $\frac{5}{16}$ inch deep, extending the length of the front jaw and coinciding with a similar but short groove in the apposing edge of the rear jaw, forms a pipette-

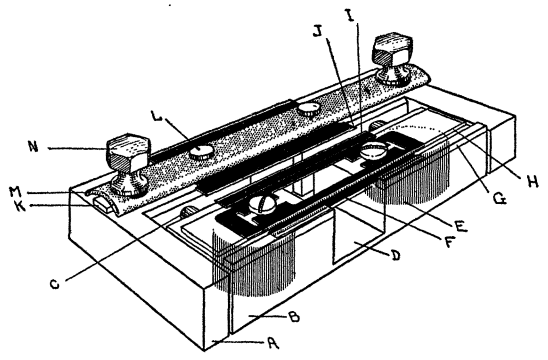

FIG. 1

run (Figs. 1D, 2B). An Alnico steel instrument magnet is cemented into an appropriately sized hole drilled in each half of the front jaw (Figs. 1E, 2A). A standard double-edged razor blade (Fig. 1F) properly oriented by a fixed blade stop (Figs. 1G, 2C) and held down by a clear plastic plate (Figs. 1H, 2C) with two screws provides the functional cutting edge (Fig. 1I) of the front jaw. That of the rear jaw (Fig. 1J) is also provided by a standard, double-edged razor blade. This blade

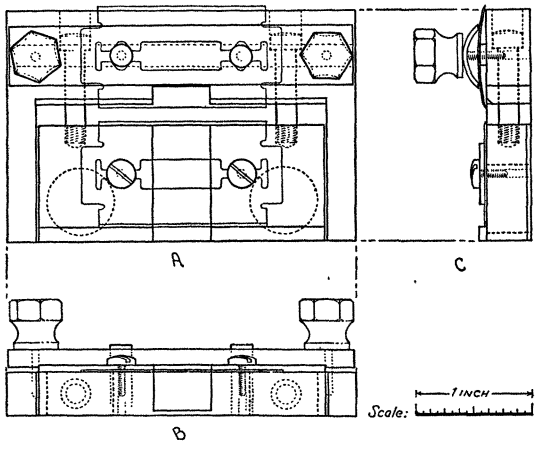

FIG. 2

rests on the top of a narrow ridge (Figs. 1K, 2C) consisting of a plastic strip cemented across the basal part of the rear jaw. The blade is oriented with its functional cutting edge paralleling that of the front jaw by two posts $\frac{3}{16}$ inch in diameter, screwed into the ridge and rear jaw (Figs. 1L, 2A, 2B). The blade is held in position by an arched metal plate (Figs. 1M, 2C) controlled by two thumbscrews (Figs. 1N, 2). By turning the thumbscrews, the arched plate raises or lowers the functional cutting edge of the rear razor blade. This makes it possible to align the cutting edge of the rear jaw with that of the front jaw for maximal cutting efficiency.

It will be noted from study of the construction plan (Fig. 2) that the width and length of the instrument, even with the jaws wide open, and the height of the pipette-run are the same as those of the standard moist chamber used with the Chambers micromanipulator (*3*). Thus, when one with his micromanipulator assembly completely set up finds it necessary to trim the micro-tips, he needs to remove only the moist chamber from the assembly by the usual procedure without disturbing the centered micro-pipettes and to substitute in its place the micro-trimmer with the open end of its pipette-run facing the micro-pipettes. The micro-trimmer is moved forward by the mechanical stage until the two functional cutting edges (which will be closed by this maneuver) are brought into the field of the microscope. The cutting edges are then separated from each other by racking the mechanical stage slightly back. In this maneuver it will be observed that only the real rear cutting edge is moved by the mechanical stage. For this reason, it is referred to as the mobile cutting edge. The real front cutting edge, being quite firmly held by magnetic attraction to the microscope stage, remains stationary and is referred to as the stationary cutting edge. Thus, slight backward and forward racking of the mechanical stage opens and closes, respectively, the functional cutting edges. With the cutting edges almost closed, the mobile cutting edge is lowered by means of the thumb-screw controls until it can just pass above the stationary one. When the cutting edges are satisfactorily aligned, they are opened as indicated above. By means of the micromanipulator controls, the already centered micro-tip which is to be trimmed is elevated to the desired level above and rested against the stationary cutting edge. The mobile jaw is then closed. This closure shears the micro-tip off at precisely the level of the stationary cutting edge. After the pipette has been trimmed, it is lowered by the appropriate micromanipulator control to a position beneath the stationary cutting edge. The micro-trimmer is then removed from the manipulation assembly without disturbing the centered micro-pipettes just as though it were a moist chamber. The moist chamber initially removed is put back into its original place in the complete micromanipulation assembly.

The detailed measurements necessary for the construction of the micro-trimmer may be accurately obtained by applying the 1-inch scale in Fig. 2 to the construction plans (Figs. 2A, 2B, 2C).

In fitting the jaws and the guide bolts, it is essential to reduce all friction to a minimum; otherwise, the magnets cannot satisfactorily immobilize the front jaw at the appropriate times in operational use.

If cylindrical magnets are set into the front jaw, care must be taken to avoid getting like magnetic poles of the two magnets at the same jaw surface. This is necessary, since the complete magnetic circuit when on the microscope is: N-pole of 1st magnet—microscope stage—S-pole of 2nd magnet—N-pole of 2nd magnet—front razor blade—S-pole of 1st magnet. Preferably, any small but powerful instrument magnet of a modified horseshoe shape may be used if its dimensions do not exceed 15 mm. in diameter and ⅜ inch in height.

References

1. BELKIN, M. *Science*, 1928, 68, 137–138.
2. CHAMBERS, R., and KOPAC, M. J. In *Microscopical technique*. C. E. McClung. (Ed.) New York: Paul B. Hoeber, 1937. Pp. 62–109.
3. HOWLAND, R. B., and BELKIN, M. *Laboratory directions for the assembling and use of the Chambers micromanipulator*. New York: New York Univ. Bookstore.