A Concentric Glass Electrode Assembly for pH Measurements

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The electrode assembly described below, which can be made from materials usually available in laboratories, is believed to offer the advantages of good shielding and compactness.

In this design the stem of the glass electrode is placed within a slightly larger tube. The annular space between the two is filled with KCl solution and serves not only as the salt bridge of the reference electrode but also as an electrostatic shield. Such a concentric electrode assembly is particularly convenient for measuring the pH of samples in test tubes. Several electrode assemblies of this type have been built in which the outside diameter is less than 6 mm.

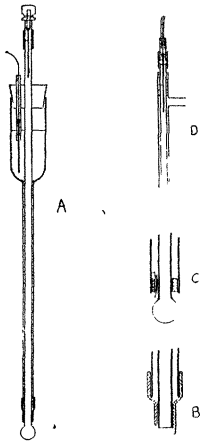

FIG. 1

In the type shown in A, Fig. 1, the annular space is closed at the bottom by a piece of Tygon tubing. This tubing is chosen to fit closely but without stretching over the stem of the glass electrode. It is then softened by steaming and forced over the end of the outer tube. The stem of the glass electrode just above the bulb is roughened on a coarse wheel or by filing (this may be done before the bulb is blown) to provide multiple fine channels through which the KCl can creep and establish a flowing junction with the sample to be measured. The outer surface of the stem of the glass electrode is wet with KCl solution and then slid up through the Tygon bushing. Assemblies which show only a very slow outflow of KCl have functioned satisfactorily. In time the Tygon may contract so as to cut off this flow, but this can be re-established by steaming the bushing in place and putting the lower end of the electrode assembly under gentle suction to draw KCl through.

Several modifications have been developed in this laboratory. A "window" type electrode may be protected by cutting the bushing diagonally and allowing one or more points to project below the delicate glass membrane (Fig. 1, B). In place of the Tygon bushing, a ring of gum rubber may be inserted between the stem of the glass electrode and the outer tube (C). Outflow of KCl is controlled by a tuft of fibrous material such as glass wool, asbestos fiber, etc., placed beneath the rubber ring before the electrode is inserted in the outer tube.

In Fig. 1, A, the KCl reservoir surrounds the stem of the glass electrode. Although this type is simple to make, it cannot be completely shielded, and electrical leakage through the stopper is apt to occur. In the design shown at D the reservoir is put at one side, connected either rigidly by glass or, if at some distance, by flexible tubing. The reference electrode is placed in this reservoir. The annular space for ½–1 inch above the side arm is filled with a mixture of half rosin and half beeswax. As this mixture is a good dielectric and forms a firm, moisture-tight bond with glass, the stem of the glass electrode needs only to extend far enough above the outer tube to fasten the Ag-AgCl electrode to it.

Electrical leakage to the reference electrode is practically eliminated, and, while supplementary shielding is easily accomplished, the short distance which the unshielded conductor is exposed usually makes it unnecessary.

Although no measurements have been made, electrical leakage through the stem of the glass electrode does not appear to be of much practical importance. Successful assemblies as long as 14 inches have been made using ordinary 7- or 8-mm. soft glass tubing for the stem. Heavy-walled barometer tubing has been used but appears to make no difference.

Other types of half-cells could probably be adapted for use with this arrangement, but the author has used silver strips plated with AgCl.

For the reference electrode these are conveniently made by soldering the silver strip before plating to a length of insulated copper wire. This is slipped into a glass tube which is then filled with the molten beeswax-rosin mixture so that the soldered joint is completely covered.

A similar strip may be used to establish contact with the buffered KCl solution within the glass electrode (D), or an insulated binding post (EBY Junior Post) can be used so that electrodes may be readily connected to a pH meter (A). The silver strip is soldered to the brass binding screw before being imbedded in wax. A Tygon collar holds the assembly in place and prevents evaporation of the solution in the glass electrode.

Staining of Oyster Larvae as a Method for Studies of Their Movements and Distribution

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The free-swimming larval period of the American oyster (*Ostrea virginica*) is of considerable duration, sometimes lasting several weeks. During this period the horizontal and vertical distribution of larvae may be controlled by tides, tidal currents, and other factors. Because of different ecological conditions, the behavior of the larvae in different areas may show considerable variations.

In conducting observations on the movements and distribution of larvae, investigators, as a rule, have had to deal with unknown populations, because no method had been developed to mark the larvae of known broods so that they could be easily identified in plankton collections. Such a method is offered here.

Experiments on developing a method for marking oyster larvae were begun in our laboratory in 1944 by Loosanoff and

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

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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

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