

chlorophyll-bearing plant organs. It is not necessary to weigh the copper. A stock of the preservative may be saturated by adding an excess, and the remaining undissolved copper removed, or a lump may be dropped in the preservative along with the specimens and left until proper color fixation occurs. When the latter method is employed the specimens should be shaken after standing a few hours to insure complete distribution of the copper. Successful results also may be obtained even if the copper is not added for six to eight hours after the specimens enter the solution. Some difficulty may be experienced, especially with certain algal cultures in which excess carbonates are present. A bluish-white precipitate (probably copper carbonate) may accumulate if excess copper is added and allowed to remain. Removal of the extra copper after saturation prevents much of this. Ordinarily the usual discoloration occurs soon after specimens enter the preservative, but after three to four days in the F.A.A.-copper sulfate solution usually a green color appears. By watching development of its intensity and removing specimens when the proper color is obtained, excellent specimens may be secured. They are transferred then to a copper-free F.A.A. solution, 70 per cent. alcohol or other preservatives for permanent storage. In a few plants, such as *Berberis* and *Ophioglossum*, some difficulty may be experienced in obtaining sufficient penetration for rapid development of the proper green color. However, if such specimens are boiled in the preservative for fifteen to twenty minutes good results follow. Care must be taken to stop the heating when coloration has developed to the proper point, and to transfer specimens to a copper-free solution. This quick method may be employed wherever heating is not injurious to the plants.

The color reaction may also be hastened by exhausting air from tissues immediately after specimens enter the preservative. This can be done easily by means of the common vacuum pump which is run by water-supply pressure. This is especially usable for fern gametophytes and young sporophytes. In these two cases permanent coloration can be obtained within fifteen to thirty minutes. Thicker tissues should be allowed to stand in the copper solution for a day or so after air exhaustion.

The addition of copper sulfate to Transeau's Algal Preservative also gave similar results as for the above. Dr. E. N. Transeau developed this preservative over twenty years ago. It is an excellent preservative for algae as well as for general preservation. The formula calls for 6 parts water, 3 parts 95 per cent. ethyl alcohol and 1 part commercial formalin. If marine algae are to be preserved sea water is used in making up the solution. After fixation has occurred, 5 to 10 per cent. glycerine may be added to prevent destruction of algal

specimens in case of loss of preservative by evaporation.

Several tissues preserved in F.A.A.-copper sulfate solution have been sectioned and stained. Cellular structure is preserved the same as for straight F.A.A., and no apparent difficulty in staining has been encountered. The copper sulfate even enhances differentiation in some cases. This may be due to the copper salt rendering the tissues more acid. The green color can not be held with sufficient intensity to permit use in the Venetian Turpentine Method without further staining. Efforts in this direction have been made, using fern gametophytes and moss protonemata.

The chief advantages of this method and its modifications are: (1) a green color closely approximating that of ordinary chlorophyll is obtained; (2) preservative ingredients are easily secured and inexpensive; (3) the method is rapid; (4) fixation is sufficiently good for many histological problems; (5) color fixation does not interfere with staining; (6) and the preservative gives successful results with numerous representatives of Algae, Bryophytes, Pteridophytes, Gymnosperms and Angiosperms.

GLENN W. BLAYDES

THE OHIO STATE UNIVERSITY

A MODIFIED QUINHYDRONE ELECTRODE FOR TISSUES¹

THE quinhydrone electrode has enjoyed extensive use in the determination of the pH of physiological systems. Its reliability in solutions containing proteins was investigated extensively by Shau-Kuang Liu² in 1927. Recently, Pierce³ and Pierce and Montgomery⁴ developed a micro-modification of the Cullen⁵ electrode and used it successfully to determine the pH of the glomerular urine of *Necturus* and the aqueous humor of rachitic rats. With this type quinhydrone electrode the broken skin of the animal in contact with the saturated potassium chloride solution completes the junction of the two half cells. To avoid this, which gives rise to erratic potentials with the intact skin and practical difficulties with the broken skin, the following modification of the quinhydrone electrode was designed and found serviceable in tumor tissues where sufficient fluid was present to fill the capillary.

With this quinhydrone electrode a series of Hastings⁶ and Sendroy's phosphate buffer mixtures was measured at 20° C.

¹ The expense of this work was defrayed in part by a grant from the International Cancer Research Foundation.

² Shau-Kuang Liu, *Biochem. Z.*, 185: 243, 1927.

³ J. A. Pierce, *Jour. Biol. Chem.*, 111: 501, 1935.

⁴ J. A. Pierce and H. Montgomery, *Jour. Biol. Chem.*, 110: 763, 1935.

⁵ G. E. Cullen, *Jour. Biol. Chem.*, 83: 535, 1928.

⁶ A. B. Hastings and J. Sendroy, *Jour. Biol. Chem.*, 61: 695, 1924.

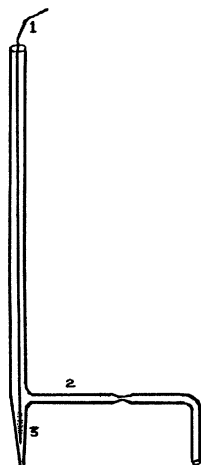


FIG. 1

"1" is a platinum wire 29 gauge, connected with the potentiometer system and extending down through the electrode vessel into the capillary "3." At the lower end it is coated with quinhydrone according to the Pierce technique. "2" is a side arm containing a firm saturated KCl agar bridge, extending into saturated KCl and through this connected with the saturated calomel electrode. The constriction lends immovability to the gel. "3" is a capillary 12 mm long, 0.25 mm at the lower end and increasing to 2 mm at the upper end where it joins with "2." Generally the capillarity of "3" is sufficient to bring the liquid in contact with the bridge in "2," if not, gentle suction may be applied at the top of the electrode vessel by means of a syringe prior to the insertion of the platinum wire.

	Calculated	Determined
	pH	pH
	7.01	6.93
	6.81	6.79
	6.91	6.89
	7.36	7.42
M/20 potassium biphthalate, 25° C.	3.97	3.98

The tumor fluid of Walker rat sarcoma No. 319⁷ showed a pH lying between 7.50 and 7.70.

JOHN C. KRANTZ, JR.
C. JELLEFF CARR
RUTH MUSSER

SCHOOL OF MEDICINE,
UNIVERSITY OF MARYLAND

A SIMPLE CARBORUNDUM PENCIL

ONE of the problems which so frequently confronts the microtechnician, be he botanist, zoologist or bacteriologist, when staining, is to determine on which side of the slide the sections (or bacteria) are. In attempting to insure getting the correct answer to his question he may use a glass or pottery pencil, he may make a scratch on the slide with a small piece of carborundum, he may use the more expensive slides with one end "frosted" or he may merely trust to luck.

⁷ W. Schopper, *Arch. f. exper. Zellforsch.*, 14: 14, 1933.

However, none of these methods is entirely satisfactory when it is necessary to put considerable data upon the slide, such as the cytologist or cyto-geneticist finds necessary in his work.

For myself, I have, while being concerned with a cyto-genetical study, solved this problem of keeping the sections properly orientated by means of a very simple, but practical, tool which was easily constructed.

A small, all-wood penholder was used and the inside of the pen-end was scooped out to a depth of 2-2.5 cm. This cavity was filled with a thick paste of plaster of Paris and a piece of carborundum 15 × 3 mm imbedded in the paste so as to leave about 5 mm protruding beyond the end of the penholder. After the plaster of Paris has formed a rigid matrix one is able to make fine lines, small numbers or letters on his slides.

This device has proven far more satisfactory than any of those methods previously used. Likewise, such a tool is certainly good insurance against loss of valuable sections and data.

ROY MILTON CHATTERS

UNIVERSITY OF MICHIGAN

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