In the Laboratory

A Superior Pith for Free-hand Sections

FERNANDO CARVAJAL
Lawrenceburg, Indiana

The dry pith of mature stalks of the rice-paper tree, *Tetrapanax papyriferum* Koch, has proved superior to commercial pith (elder) for use in the free-hand sectioning of plant material by the writer for a period of years.

This pith is white, devoid of vascular bundles or other hard tissues, and of uniform texture. The plant is in cultivation largely as an ornamental in the southern United States and was first used by the writer at Louisiana State University, where he found it growing on the campus. The pith is extractable from the dead stalks during the winter in straight rods 1.5 cm. in diameter and up to a meter or more in length, the pith being usually continuous and uniform through the nodes.

Under the binocular microscope (wide field, long working distance) it has been found possible to cut satisfactory sections of such fragile materials as leaves, stems, and roots of healthy or diseased plants. For example, this pith has been used to section leaf lesions with fruiting bodies of fungi, galls bearing the so-called X-bodies of a virus (Fiji disease of sugar cane causing galls on leaves), and tobacco stems and leaves affected with the bacteria, *Phytomonas solanacearum*. It has also been used to section roots of plants affected by fungi, bacteria, nematodes, etc., such as roots of sugar cane affected by the fungus, *Pythium arrhenomanes*. It has proved equally successful in working with fresh material from the field, dry, preserved material, or specimens which have been kept in fixing solutions.

Double-edged blades are very helpful for the sectioning. A small piece of material to be sectioned is placed in the pith and held by a clamp which is open at the top (Hoffman Screw Compressor type). The sectioning is done under the binocular microscope. For best results, the sections are made in dry pith and then fixed as soon as they are cut. By means of a needle (wetting the tip of the needle first), the sections are transferred from the pith or blade to a drop of water or stain solution on a glass slide or to the fixing solution. Direct transfers to a drop of lactophenol, with or without cotton blue, and then gently warming the slide gave good results.

This technique was used by the writer in the studies of host-parasite relationships, anatomical and physiological studies of the red rot disease of sugar cane (*1*), and others.

Reference

1. CARVAJAL, FERNANDO, and EDGERTON, C. W. *Phytopathology*, 1944, 34, 206–213, 827–837.

A Culture Method for Certain Marine Algae[1,2]

J. C. STRICKLAND
Virginia Fisheries Laboratory, Yorktown

There is an abundant literature describing the media and procedures for developing unialgal cultures of various marine phytoplankton organisms. Most of these methods demand considerable time as well as specialized equipment or technic. To facilitate studies of the dietary requirements of the larval blue crab, a simple and speedy method was needed for establishing and maintaining unialgal cultures of several plankton organisms at our station on the York River. In meeting a somewhat similar problem, Loosanoff and Engle (*Science*, 1942, **95**, 487–488) found that complete fertilizers[3] of the formulae 5–3–5 or 6–3–6 in solutions of 1 gram of fertilizer to each 1,000 cc. of water provided effective media for growing plankton. Tests of this method favored it for our purpose, but there were two limitations: (1) the undissolved residue obscured early indications of growth and was objectionable to the feeding larvae, and (2) there was an excessive formation of bacterial gloea which inhibited or rendered feeding difficult. The following report describes a logical modification in the preparation of this medium for laboratory use and a simple dilution method used for establishing unialgal cultures.

The usual precautions are taken in cleaning glassware. The water used for rinsing and in the preparation of culture solution is previously filtered through a thick layer of nonabsorbent cotton to remove any larger organisms, silt, or detritus present.

To a flask containing 1,000 cc. of filtered water is added 0.5 to 1.0 gram of pulverized 5–3–5 or 6–3–6 fertilizer and, with frequent shaking, the mixture is brought to a slow boil over a low flame. After several minutes it is set aside and the medium allowed

[1] Joint contribution of the Virginia Fisheries Laboratory (Number 24) and the Department of Biology of the College of William and Mary.

[2] The author is indebted to Dr. V. L. Loosanoff for the supply of fertilizer used and for much valuable information and advice.

[3] Manufactured by the American Agricultural Chemical Company, 50 Church Street, New York City.

to cool at room temperature. When it is cool enough to handle comfortably, the decoction is filtered through nonabsorbent cotton into Erlenmeyer flasks to a depth of about one centimeter. The flasks are plugged with cotton and set aside for 24 hours to permit the solution to cool and become sufficiently aerated.

Plankton is taken at the desired habitat, and inoculations are made as soon as possible by merely shaking the plankton mixture and pouring a small portion of it into the nutrient solution. These gross cultures are exposed to north light at room temperature and allowed to develop for 2 weeks. A microscopic examination will now reveal the organisms which may be most easily isolated and grown. The gross culture is shaken vigorously, portions being poured into watch glasses and, while viewed under a dissecting microscope, diluted to a point providing a satisfactory dispersal of the material. A fine pipette is used to withdraw several of the desired individuals, which are placed in fresh nutrient solution. The inoculated cultures are shaken and exposed to strong illumination from Mazda fluorescent 25-watt lights (each sufficient for 20 to 25 cultures) for about 12 to 14 hours daily. After a week, the cultures are inspected each day for signs of development. When such is detected, a few organisms are removed with a capillary pipette and transferred to fresh media. After three or four subcultures are made in this way, development is allowed to become more extensive until a suitable amount of material can be withdrawn for a thorough microscopic examination. Some cultures will be found to be unialgal at this stage, while others must be subcultured further until they are satisfactory. Vigorous shaking of the culture after inoculation, as well as use of small portions for inoculation, will yield the best results.

The use of artificial illumination materially speeds growth and shortens the time between each subculturing by 5 or 6 days. Unialgal cultures established in this nutrient were subcultured every 60 days. This was done by merely shaking the culture and pouring a small portion into a flask of fresh medium. Uninoculated media may be stored in an icebox for several days without appreciable change.

The combination of this medium and method of culture is suggested as being potentially useful in many ways, such as maintaining organisms for feeding or life-history study, providing large populations of otherwise scattered forms, growing soil algae, or furnishing a constant source of material for teaching purposes.

Pennate diatoms, especially *Nitzschia* and *Navicula* spp., were found to be particularly adaptable to this culture medium. The centric diatoms, *Coscinodiscus, Chaetoceras, Melosira* spp., did not develop so well, and the dinoflagellates, *Ceratium* and *Glenodinium* spp., did not grow at all. It may be noted also that various forms of marine or brackish Myxophyceae, such as *Spirulina subsalsa* and *Lyngbya semiplena,* grow luxuriantly.

A Slide Rule for the Addition of Squares

WILLIAM E. MORRELL
University of Illinois

Some years ago the writer had occasion to solve several thousand problems of the type: $d = (x^2 + y^2 + z^2)^{\frac{1}{2}}$. For the purpose a slide rule with appropriate square and square-root scales was constructed. Its convenience, demonstrated in continued use, leads him to believe that it might be found helpful to others in the solution of problems involving square roots and squares and the addition and subtraction of the latter. Such problems are often encountered in, for example, geometry and trigonometry (*cf.*: $2bc \cos A = b^2 + c^2 - a^2$).

The fixed part of the rule consists of two meter sticks, grooved as shown in diagram (a), and screwed to a baseboard. A hardwood slider about 103 cm. long is grooved to fit between the meter sticks. The cursor (diagram (b)) consists of a piece of glass mounted with Duco cement between two pieces of metal, fitted so as to slide in slits in the edges of the meter sticks. A hair line is scratched on the under side of the glass. About 1.5 cm. to the left of the zero end of the meter sticks a "stop" (see diagram (c)) is screwed to the baseboard.

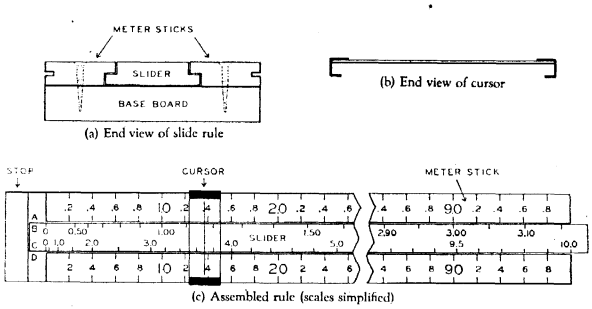

FIG. 1

Decimals added to the numbers on one of the meter sticks give A (diagram (c)) a scale which is linear from 0.0 to 10.0. The D scale, on the other meter stick, is linear from 0 to 100.

The corresponding square-root scales B and C on the slider are from 0.00 to 3.16 and from 0.0 to 10.0, respectively, as indicated in diagram (c). These