IN THE LABORATORY

A Simplified Diffusion-Dehydration Technique in the Microtomy of Tissues[1]

ALBERT P. LORZ[2]
Purdue University

The procedure described below for the preparation of bundles of root tips for transverse sectioning with the microtome involves a departure from the conventional method of dehydration which can be applied to the preparation of other objects of similar size at a considerable economy of time and materials. The dehydration process from water to 80% alcohol is accomplished in a single step which involves a continuous diffusion of molecules without the production of violent convection currents.

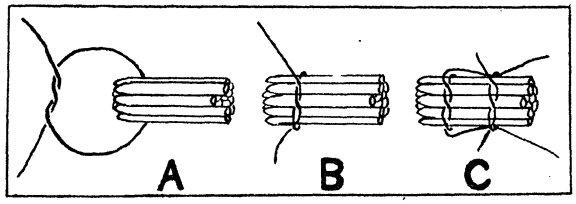
The fixed tissues, after being washed, are placed in 1 ml of 15% alcohol, to which is immediately added 4 ml of 97.5% alcohol in such a way as to prevent the sudden mixing of the two concentrations. A paper disc prevents such mixing and makes it possible to establish an interface between the two concentrations. This interface can be maintained for a period longer than 24 hrs at ordinary room temperatures, but at a temperature of approximately 40° C it will gradually lose its identity as the diffusion gradients are resolved and equilibria are established between the alcoholic and aqueous constituents. The resultant concentration, about 80%, is attained by a gradual and continuous diffusion process which causes no perceptible injury to the tissues. It is generally considered safe to leave tissues in this concentration until it is convenient to process them further.

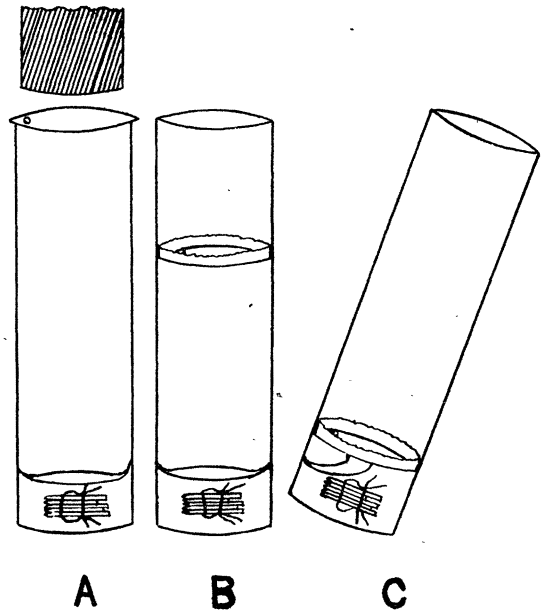
The method is especially valuable for class laboratory routines. There is no need for concern over the possibility of allowing the tissues to remain too long in the lower concentrations of alcohol, since the diffusion process makes the approach to 80% alcoholic concentration automatic and obviates the necessity for students to enter the laboratory outside scheduled class hours in order to change solutions. The full possibilities of the method have not yet been investigated. It may be possible to process larger pieces of tissue than the example described, using the same method but increasing the sizes and amounts of the materials proportionately. The details of the method of dehydration are embodied in the description given for the handling of root tips in bundles with the object of concentrating large amounts of meristematically active tissues on a single slide.

The materials used include: root tips (grown either in water or in a moisture saturated atmosphere); Craf or Navaschin type fixative (*1*); glass slide and No. 50 cotton thread; shell vials, 15 mm in diameter; paper discs, 17 mm in diameter (cut with No. 9 cork borer); 15% alcohol (10% ethyl + 5% normal butyl + 85% water); 97.5% alcohol (equal proportions of 95% ethyl and absolute normal butyl mixed); and 100% alcohol (25% absolute ethyl + 75% absolute normal butyl).

METHOD

(1) Root tips about 10 mm long are fixed for 24 hrs or longer in a Craf or Navaschin type fixative and rinsed briefly in water.





FIGS. 1 (above) and 2 (below).

(2) Five to 10 medium-diameter root tips are placed parallel to each other with their meristematic ends all pointing the same way and flush with each other over one side of an open loop of No. 50 thread, which is spread out on a wet glass slide (Fig. 1 A). The surface film causes the wet root tips already placed to cohere slightly so that they tend to remain undisturbed while others are being added to the bundle.

[1] Journal Paper No. 325, Purdue University.
[2] Now at the Florida Agricultural Experiment Station, Gainesville.

(3) The loop is drawn snugly about the bundle (Fig. 1 B). If the thread is drawn too tightly, the meristems will flare apart. The free ends of this loop are prevented from projecting forward and interfering with subsequent sectioning by being tied back with a second loop placed proximal to the first (Fig. 1 C).

(4) As many as three such bundles are placed in a vial and covered with 1 ml of 15% alcohol.

(5) A hole about 1 mm in diameter is punctured with a dissecting needle in a paper disc about 3 mm from the margin.

(6) The disc is centered, with the rough edges of the hole turned up, over the top of the vial so that the edges of the paper will project uniformly about 1 mm beyond the circumference of the vial (Fig. 2 A).

(7) With a cylindrical plunger (e.g. a No. 6 or No. 5 cork borer) which just fits easily into the vial, the disc, the edges of which are turned up in the process, is forced part way down into the vial (Fig. 2 A and B).

(8) The vial is then tilted (Fig. 2 C) so that the hole in the disc is uppermost as the disc is pushed the rest of the way downward into contact with the liquid. Air displacement through the hole is usually complete if this step is adroitly executed, but small bubbles are not objectionable. Highly absorbent papers are not recommended for the discs, because they are less likely to maintain their shape when wet and their absorbency makes complete air displacement more difficult.

(9) Four ml of 97.5% alcohol is added above the disc. No special care is necessary, since the paper quite effectively prevents the two concentrations from mixing immediately. For demonstration purposes, one edge of the disc may be drawn up very slowly with a curved pair of forceps or a hooked needle and may thus be removed completely, revealing the interface between the two concentrations. In practice it is not necessary to remove the disc, because its freely permeable nature does not interfere appreciably with the diffusion process.

(10) The vial is then stoppered and held at approximately 40° C for a minimum of 24 hrs. A bacteriological incubator set at 37.5° C is satisfactory, but a period of somewhat longer than 24 hrs may be necessary before a complete breakdown of the interface is accomplished and a uniform mixture of the two concentrations is established. It has not yet been determined whether higher temperatures for shorter periods would be equally effective without injury to the tissues.

(11) The tissues may now be left indefinitely in the resultant 80% alcoholic concentration, or the portion above the disc may be replaced with 4 ml of 100% alcohol. After another 12–24-hr period the dehydration is practically complete, but removal of the disc, followed by one or two changes of 100% alcohol at intervals of 2–4 hrs, is advised in the interest of safety.

(12) To the dehydrated tissues in 1 ml of 100% alcohol is added 4 ml of melted paraffin which has cooled to the point where it will partly solidify upon coming into contact with the alcohol at room temperature. There seems to be no injury to root tips occasioned by failure to pass them through pure normal butyl alcohol to eliminate the balance of the ethyl alcohol, and it has been determined experimentally that small amounts of the latter are soluble in paraffin at the usual oven temperatures.

(13) The unstoppered vial is now placed in the paraffin oven for a 24-hr period. After two or more changes of pure paraffin at intervals of 4–24 hrs, the tissues are embedded in the conventional manner in fresh, filtered paraffin containing no traces of alcoholic impurities.

The method just described has been used very successfully for classroom work in the preparation of slides for chromosome studies on a number of different plant species. If the sides of the paraffin block are shaved closely to the root tip bundle, it is usually possible to mount the entire meristematic portion under a 22-mm by 50-mm coverslip, provided the sections are not thinner than 10 μ. Sections thinner than 10 μ are usually undesirable because too many division figures are destroyed by cutting. The initial steps of diffusion-dehydration will doubtless find a wide application in biological microtomy.

Reference

1. SASS, JOHN E. *Elements of botanical microtechnique.* (1st ed.) New York: 1940. P. 19.

Effect of Acetone and Alcohol Fixation and Paraffin Embedding on Activity of Acid and Alkaline Phosphatases in Rat Tissues[1]

ROBERT O. STAFFORD[2] and WILLIAM B. ATKINSON[3]

Department of Zoology, University of Wisconsin

In the course of research involving studies of phosphatase activity in animal tissues, it became of interest to determine quantitatively how much of the enzyme activity present in fresh tissue is preserved in the technical processes of Gomori's histochemical methods (*3*, *4*) for acid and alkaline phosphatases.

It is known that it is more difficult to demonstrate acid phosphatase (AcP-ase) histochemically than alkaline phosphatase (AlP-ase) and that acetone fixation gives better preservation of AcP-ase activity than alcohol. The effect of fixation and embedding on the cytological localization of AlP-ase has been studied in tissue sections by Danielli (*1*) and Emmel (*2*). The only quantitative data on the effect of fixation are those of Gomori (*4*), who showed that alcohol destroys AcP-ase but causes only 20% inactivation of AlP-ase, and those of Danielli (*1*), who estimated that paraffin embedding causes a 75% loss of activity of AlP-ase. To obtain more complete quantitative data on the effects of fixatives and embedding on

[1] Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation, administered by R. K. Meyer and W. H. McShan, and by a grant from the Committee on Growth, National Research Council, administered by E. T. Engle.

[2] Junior Research Fellow, National Institute of Health, U. S. Public Health Service.

[3] Department of Anatomy, College of Physicians and Surgeons, Columbia University.