thujina was made from dead branches of *Thuja occidentalis* in New York about 1875, and was deposited in the New York State Museum. Positive identification of this ascomycete was made by comparing the type collection (*4*) with the Duchesnay material.

To test the ability of the fungus to produce blue stain, blocks of balsam fir were inoculated with cultures of *Amphisphaeria thujina* derived both from stained wood and single ascospores. After 2 mo, typical blue stain had developed in all of the test blocks. The fungus was reisolated from the artificially inoculated wood and identified as the species first isolated. These experiments prove that *A. thujina* is the causal agent of the blue stain in balsam fir (*5*).

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References and Notes

1. F. Kaufert, *Minn. Univ. Agr. Expt. Sta. Tech. Bull. No. 110* (1935).
2. I. H. Crowell, *Pulp Paper Mag. Can.* **41**, 451 (1940).
3. C. M. Christensen and F. H. Kaufert, *Phytopathology* **32**, 735 (1942).
4. We are grateful to S. J. Smith, curator, New York State Museum, for his kindness in providing the type collection of *A. thujina*. A detailed description of *A. thujina*, including the results of comparative taxonomic studies with Peck's original material, is in preparation.
5. Further laboratory studies are in progress to determine the factors that may influence the establishment of blue stain in the living tree, and to ascertain its effect on subsequent development of decay organisms. This report is contribution No. 723, Forest Entomology and Pathology Branch, Department of Forestry, Ottawa, Canada.

30 January 1961

Freezing-Out, a Safe Technique for Concentration of Dilute Solutions

Abstract. The principle of freezing-out has been adapted for the quantitative concentration of small or large volumes of dilute solutions. Mechanical stirring is an essential part of the process. The method should prove of greatest value where volatile or heat-labile substances are to be concentrated and is applicable to solutions in certain organic solvents as well as to aqueous solutions.

The purpose of this report is to call attention to an exceedingly useful, but hitherto neglected, method for the laboratory concentration of dilute solutions. The method, which possesses advantages over most of those in use, is based on the principle of freezing-out. It is simple, relatively inexpensive, and is almost above reproach with regard to chemical or physical alteration of the substances being concentrated. The method is applicable to a very wide range of volumes, having been used in this laboratory on samples as small as 100 ml and as large as 300 liters. Preliminary work has shown that in addition to its use with aqueous solutions it is applicable also to solutions in organic solvents, such as benzene, which solidify at relatively high temperatures.

Although freezing-out is widely used industrially, it has not become a part of the analyst's armamentarium, and references to its use in the laboratory are rare. As described below, the method is similar to that used in the purification of benzoic acid by Schwab and Wichers (*1*). It differs from the previous applications by Haurowitz (*2*), Gibor (*3*), and Schildknecht and Mannl (*4*), in that mixing is an essential part of the process and part of the solution is left unfrozen. Thus materials which are adversely affected by freezing are not harmed, and substances which dissolve with difficulty are recovered while still in solution. In addition, the volumes which can be concentrated are much greater than in the previous applications.

Volumes of 1 liter or less are treated as follows. The solution is poured into either a glass or plastic bottle, leaving sufficient room for the expansion which accompanies freezing. The bottle is then attached to a mechanical shaker and placed in a freezing chamber. After several hours of gentle shaking, most of the solution will be frozen except for the central spherical or egg-shaped portion which contains the concentrated solutes. This portion is recovered by punching or melting a hole in the ice and pouring it out. The equipment used in this laboratory consists of a freezer cabinet of the type used to store ice cream, operating with a ⅓-hp motor at −30°C, and a Burrell Wrist Action shaker which rests on the wooden cover of the freezer. Arms with clamps attached extend down into the freezer from the shaker. In this way numerous samples of different volumes can be concentrated simultaneously. The results with this method have been excellent, with recoveries of greater than 99 percent after concentrations of as much as 20-fold in volume. However, in order to achieve such recoveries, it is essential to keep a close watch over the bottles so that they may be emptied when cloudy ice indicates that entrapment is occurring.

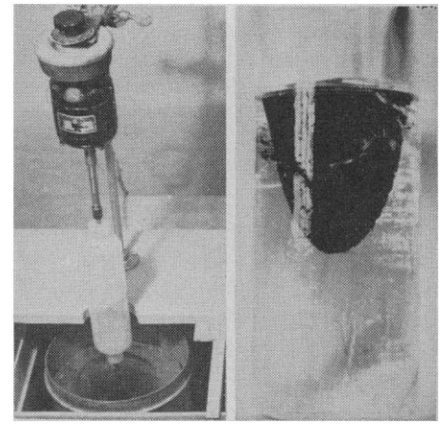

Fig. 1. (Left) A 5-gal container in the freezer chest with the polyethylene bottles on the stirrer. (Right) The block of ice removed from the container showing the concentrate in the core.

Large volumes are treated somewhat differently. The solutions are placed in cylindrical metal containers which are positioned in the freezing chest so that the open tops of the containers are just above the freezing level in the chest (Fig. 1). The solutions are stirred mechanically, and the propeller is raised in several steps as freezing proceeds. Ice forms from the sides inwards and from the bottom upwards, and the result is a smooth, sharply defined shallow conical core in which the solutes are concentrated into a small volume (Fig. 1).

The time required for concentration varies with the volume and the efficiency of operation. For example, with the equipment in use it has been possible to concentrate three 16-liter containers of water to a volume of 1 liter each in about 50 hr. On the other hand, when the solution was placed directly in the compartments of the cabinet instead of in containers, 300 liters were concentrated to 35 liters in the same length of time.

Several refinements are possible. To avoid losses due to splashing of the concentrate onto the top of the already formed ice, two 250-ml polyethylene bottles, bored through the cap and bottom, are slipped onto the stirring shaft (Fig. 1). Thus, as the stirrer is raised the liquid level falls. Recovery of the last traces of concentrate from the core is simplified by gently melting the surface ice with warm air, such as from

a hair dryer. Contact of the solutions with the metal of the containers may be avoided by lining the containers with polyethylene bags. This does not affect the freezing rate, provided that the spaces between the bag and container wall are filled with water. The containers must be nearly filled to begin with, but the necessity of having many different sized containers available can be eliminated by using a core frozen to the proper capacity.

Occasionally entrapment will result in losses, and the easiest way to recover the lost material is to thaw the sample and freeze it again. However, any losses which do occur are nonspecific and do not result in fractionation. Thus any easily measured parameter such as conductivity or optical absorbance will serve to indicate the recovery of the whole. Another phenomenon which sometimes occurs is supercooling followed by the formation of ice throughout the solution. It has been found that this rectifies itself and does not interfere with the final result.

Since the success of the procedure, or at least its application to a larger scale than that of Haurowitz, or Gibor, or Schildknecht and Mannl, depends upon stirring, it is worth-while describing what seems to be a plausible hypothesis for its necessity. In a quiescent solution, as ice is formed, the salts eliminated from it dissolve in the adjacent layer of solution and lower the freezing point of this layer. Thus the temperature of the layer is allowed to fall below the freezing point of the rest of the solution. Then, that part of the solution immediately adjacent to the layer, not having the higher salt content of the layer, becomes supercooled and may suddenly freeze, trapping the layer with its content of salts against the ice, where it too subsequently freezes. Thus the purpose of the stirring is to prevent the formation of such layers. This hypothesis of Himes *et al.* (*5*) is supported by the appearance of ice formed during periods when stirring was interrupted. In these experiments such ice differed from the usual clear structureless ice in being laminated in appearance. The laminations, which were several millimeters in thickness, were blue as a result of entrapment of methyl violet which had been added to the solutions (*6*).

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References and Notes

1. F. W. Schwab and E. Wichers, *J. Research Natl. Bur. Standards* **32**, 253 (1944).
2. F. Haurowitz, *Z. physiol. Chem. Hoppe-Seyler's* **186**, 141 (1930).
3. A. Gibor, *Science* **133**, 193 (1961).
4. H. Schildknecht and A. Mannl, *Angew. Chem.* **69**, 634 (1957).
5. R. C. Himes, S. E. Miller, W. H. Mink, H. L. Goering, *Ind. Eng. Chem.* **51**, 1345 (1959).
6. In the hope that ready availability of the necessary equipment will encourage use of the methods described, arrangements have been made with the Virtis Company, Inc., Gardiner, N.Y., to manufacture and market such equipment. Part of the work was done during the tenure of U.S. Public Health Service research grant No. RG 7229.

20 February 1961

Fractionation of Tracer Effluxes during Action Potential

Abstract. The fluid flowing by the *Nitella* internode or squid giant axon loaded with radioactive cations was fractionated during the action potential. In this manner it was possible to ascertain the time course of efflux of these cation tracers during activity.

In the investigation with radioisotopes of transport phenomena in biological membranes, it is desirable to correlate the movement of radioisotopes with other rapid processes in the membrane, such as changes in electric potential and membrane conductance. It is known that the process of action potential production in the squid giant axon and in other excitable cells is associated with enhancement of loss of various univalent cationic radio-tracers to the extracellular fluid (for example, *1*). It was difficult, however, to decide with certainty in what phase of bioelectric activity this enhancement occurred. Wilde and co-workers (for example, *2*) have had some success in this direction. By perfusion of the coronary vessel of the whole heart, they were able to obtain a detectable efflux of potassium-42. There are, however, obvious ambiguities in their experiment as to the temporal relationship between the efflux and the electric response of the heart because of the impossibility of ascertaining the time required for diffusion of the isotope. In the present study, it has been possible to fractionate the radioisotope efflux during the action potential in single cells (*Nitella* and squid giant axon).

The principle of the method consists in running the fluid medium past the cell under study at a high speed and then fractionating it. A single cell of *Nitella* or a squid giant axon (both approximately 0.6 mm in diameter and 40 mm long) was introduced into a small chamber (1.2 to 2 mm in diameter) made of Lucite. The cell was kept approximately at the center of the chamber. The wall of the chamber was coated with melted paraffin. There were two pairs of platinum electrodes making contact with the fluid in the cavity. The upper opening of the chamber was connected to a large reservoir of fluid and, by virtue of the difference in the hydrostatic pressure, the fluid in the cavity was pushed through at the rate of about 50 to 150 cm/sec in the case of the *Nitella* cell and about 200 cm/sec in the case of the squid axon.

Fractionation of the running fluid was effected by a rotating Lucite disk (about 70 cm in diameter) provided with 40 to 200 small compartments near its edge. The disk was driven by a motor at about 1 rev in 30 to 60 sec in the case of *Nitella* and at the rate of approximately 1 rev/sec in the case of the squid axon. A source of light attached to the rotating disk and a stationary photocell were used to trigger stimuli (as well as the oscillograph used to record the action potential) at a given position of the disk. The Lucite chamber carrying the cell was held by a movable stand so that it could be quickly brought to, or moved away from, the rotating disk.

The middle diagram in Fig. 1 shows an example of the results obtained with a *Nitella* cell loaded with potassium-42. Electric stimuli were repeated at intervals of 2 min for a total period of 80 min. The running fluid (artificial sea water diluted by 500 with distilled water) was fractionated into portions containing effluxes obtained in 0.75-sec intervals. The samples of fluid in individual compartments of the rotating disk thus contained the radioactive potassium released during 40 successive cycles of activity. These samples were dried and measured by a standard radiation counter.

It is seen in the diagram that there is a small efflux of tagged potassium before stimulation and that this level of efflux was markedly enhanced after the delivery of a brief electric shock to the cell. The action potential recorded by this arrangement is "diphasic." With this action potential regarded as composed of two monophasic action potentials (with one of them reversed in phase and delayed by the interelectrode conduction time), the approximate time course of the potential variation under the upper recording