SPECIAL ARTICLES

THE ETHER FREEZING MICROTOME, IN BOTANICAL TECHNIQUE

THE following description of a freezing microtome, and its advantages, is published in the hope that others will derive such benefits and conveniences as I have for some time enjoyed through its use. The microtome is exceedingly simple, and so easily manipulated that an inexperienced person may quickly learn to operate it successfully. It is very rapid in its work, allowing of sections even in less time than is sometimes required for free-hand sections, and does very efficient work. In some kinds of material the sections are superior to those obtained by the paraffine method. It is useful in dealing with a great variety of objects, and is cheap enough to be within the reach of all.

The freezing method of embedding in section work is well known to all biologists, but owing to the use of faulty or cumbersome apparatus, or to the application toward inappropriate ends, it has not become as general in use as it should be. Osterhout[1] found the freezing method absolutely necessary in the anatomical study of the red sea-weeds. No other freezing apparatus with which I am acquainted combines the good features of the machine described below, and I would especially call the attention of all scientists who have used or are using freezing microtomes to these features. The freezing device is exceedingly simple and effective. The knife carriage is also very simple, is accurate in its work, and although very rigid allows of sectioning on any part of the knife, and at almost any angle, vertical or horizontal with little or no delay in adjustment. This latter feature is especially valuable.

It should, first of all, be explained that it is not the intention of the writer to recommend this method as a substitute for the paraffine method. Messrs. Hill and Gardiner, however,[2] have developed this freezing microtome technique in the examination of 'connecting threads' of pine tissues to a high degree of proficiency, and it is to be hoped that their results will be more widely applied. It is not, however, in the study of cytological problems that I have found the technique of most use—though remarkable results have been obtained by Hill and Gardiner—but it is in its use in other lines that it has proved of very great value. The method is capable of application in so many ways, and for such a great variety of purposes that an enumeration of these is deemed advisable. It is chiefly useful for a great deal of work where paraffine sectioning is too slow, and where free-hand sections are difficult to obtain; for example, in sections of certain rust pustules. A great deal of time can often be saved by preliminary sectioning with the freezing microtome before using the paraffine method, in order to determine the condition of the material about to be used. The freezing method can be so developed as to give sections as quickly as, or even in less time than, the free-hand method. The sections, moreover, can be produced in much greater number and are far superior in thinness and uniformity, and in certainty of success. Material can be frozen in eight seconds with an apparatus in good order, so that the embedding can be accomplished in less time than is required for the insertion of the same in pith for free-hand work.

Every mycologist knows how difficult it is to get sections of most fleshy fungi and how almost impossible sections of sporophores of the Tremellineæ and teleutospore clusters of *Gymnosporangium* are, yet these are cut with greatest ease by the freezing method. It is possible to cut a gelatinous sorus of *Gymnosporangium macropus* from tip to bottom together with the hard wood from which the sorus arises, and to preserve it all intact. This seems to me impossible by the free-hand method, and the dehydration in the paraffine method leaves the material in an unsuitable condition for section work. Again, objects of large size can be cut easily, such as small phalloid 'eggs,' entire caps of small agarics, small earth stars, etc., which, if cut at all by the free-hand method would give uneven sections, unless the manipulator is a person of extraordinary skill. I have found

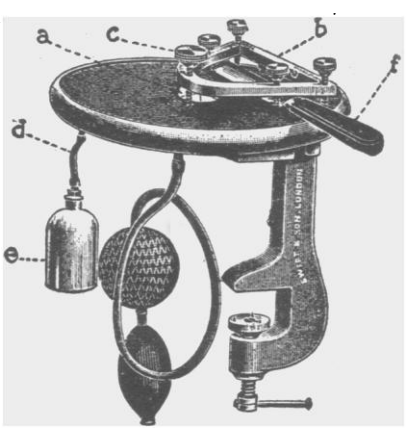
[1] *Bot. Gaz.*, **21**: 195, 1896.

[2] See *Philosophical Transactions of the Royal Society of London*, Series B, Vol. 194, pp. 83–125.

the method of great use in sectioning rust pustules, from both fresh and dried material. It is also of very great value in the study of hard seeds, such as the grains of cereals. The paraffine method, at least as usually practised, is not successful in producing sections of the starchy grains of cereals. Dr. A. Mann, of the Department of Agriculture, however, informs me that he has succeeded by very long infiltration of both xylol and of paraffine in obtaining very satisfactory sections of mature barley grains. By the freezing method excellent thin sections of whole grains (even of large corn) can be cut without difficulty. On the other hand, the method is very useful in cutting hard materials, as small blocks of wood (soaked in water) grass stems, etc. This is made possible by the firmness of the mount, and by the direct and unswerving approach of the knife, which avoids the turning action of the blade, so common in the cutting of free-hand sections from such material. In dealing with such material, moreover, by the paraffine method, dehydration makes the wood so hard that the knife is very easily ruined. In the freezing method, the blocks, on account of their water-soaked condition, are less apt to injure the knife. Again, large soft berries, as cranberries, are easily sectioned whole, and for rapid sectioning of leaves the method is particularly useful. Osterhout's work (*l. c.*) has demonstrated its usefulness in the treatment of red sea-weeds. The method is thus seen to have a wide range of usefulness.

The freezing microtome method should find a place in every botanical laboratory, first as a supplementary aid to the paraffine method, and second, as a quick method of securing sections for ordinary morphological and anatomical work. It sometimes happens that an instructor can obtain only a small amount of material, which may be extremely rare, and by this method he can usually obtain enough excellent sections for a large class in a very short space of time, without the delay necessary for paraffine work, and without the waste of material which is sure to attend the work of sectioning when done by the students. Again, in elementary classes, microtome work of a difficult nature is sometimes necessary, and the students may be unable to obtain satisfactory free-hand sections. In mycological and pathological laboratories the method is extremely useful. It has, moreover, been found of very practical value in medical pathological laboratories, where it is used in rapid histological work.

Perhaps the greatest general good could be derived from its use in high schools, normal schools and smaller colleges, for which the apparatus for the paraffine method is too expensive, where the time is insufficient, and the courses necessarily too elementary to include the study of the paraffine method. It is especially to workers in these institutions that I wish to direct attention to this freezing microtome technique. The method should prove of immense value in such institutions on account of its cheapness, speed and general usefulness. Hundreds of excellent sections can be cut in a very short time and the apparatus is always ready for use. An efficient apparatus such as that shown in the figure, can be obtained for $16 (duty free). It is sold by J. Swift & Son, No. 81 Tottenham Court Road, London.



The apparatus is shown in the accompanying figure. The material to be cut is first placed in a ten per cent. gum-arabic solution. It may be soaked in this for one to twenty-four hours, according to the size of the material. It can, when necessary, be kept in the gum arabic for only a few minutes or even a few seconds, though the longer soaking will

give a better infiltration. It is then oriented in a drop of ten-per-cent. gum arabic placed on the small brass plate (*a*) in the center of the table, and a spray of ether is atomized against the corrugated under surface of this plate, producing the necessary low temperature. The atomizer is usually worked by hand with a double bulb, but I have found a compressed-air tank (such as physicians use) a very great convenience, as such a tank allows of the unhindered use of both hands in the microtome work. It also admits of more rapid freezing. I have been able with this apparatus to freeze material in eight seconds on a warm summer's days in Washington, D. C. A foot pump may also be used in place of the double bulb. The knife (*f*) is carried by a shoe (*b*) and is held in place by adjustable screws. The shoe is supported by three bone-tipped adjustable screws, the forward one of which (*c*) is used to set the knife after each stroke in preparation for the next one. The screws rest on a plate-glass top, which covers the table around the central brass plate. The smoothness of motion is facilitated by oil placed on the plate. The atomizer is of the ordinary type. The intake is shown at (*d*), and the ether bottle at (*e*). The ether should be of good quality (that used in medicine for anesthesia) in order to obtain the best results.

The gum arabic may be kept in stoppered bottles, and can be preserved from mold and bacterial attacks by adding a few crystals of carbolic acid or thymol. The sections after cutting can be handled in the ordinary way with section lifters or with small sieve nets of cloth or other substance. The latter method is very useful if the sections are to be transferred to stains and afterwards washed. Very delicate sections may also be handled by means of a loop of fine platinum or brass wire. The sections are caught up in the water drop and are easily transferred to other dishes or to a slide without the injury which is liable to occur in handling with ordinary section lifters. The sections may be mounted in glycerine or glycerine jelly, and can then be permanently mounted, without having touched alcohol if water stains are used. As Hill and Gardiner point out, the dehydration of sections in alcohol may leave protoplasmic structures in a condition very different from the normal. Of course, the effect of freezing is also one which must be taken into account, though this is seldom, if ever, a serious factor in the morphological and anatomical work for which this method is here recommended. Sections for ordinary anatomical work can be cut from fresh material, or from dry material after soaking in water. The material may also be killed by the ordinary methods, preferably without the use of alcohol, and may then be washed in water in the usual way and preserved indefinitely in a concentrated thymol solution. Such material can be prepared for the knife simply by washing carefully in water.

I wish to acknowledge that my acquaintanceship with the possibilities of the microtome described above was made in the Cambridge (England) botanical laboratory, and I am indebted to Mr. A. W. Hill, of Cambridge University, for many courtesies and favors in my observations and study of this method.

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ASTRONOMICAL NOTES

THE YALE PARALLAXES

Transactions of the Astronomical Observatory of Yale University.—Dr. W. L. Elkin, director of the Yale Observatory, undertook, in 1884, by means of the heliometer, the determination of the parallaxes of the ten stars of the first magnitude in the northern sky. This work was carried out with rare ability and success during the following ten years; but before the completion of this work, it was decided to extend the research by undertaking a survey of all rapidly moving stars not previously attempted, with a view to singling out those which are near enough to show a measurable parallax. This work has been carried on during the last thirteen years, and the results have been recently published as Volume II., Part 1, of the Observatory Transactions, under the title, 'Parallax Investigations on 163 stars mainly of Large Proper-