SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SENSITIVE HUMIDISTAT

THE unreliability and low sensitivity experienced with a commercial hair humidistat led to the development of a control operated by the differential in temperature between ether-filled wet and dry bulbs. The difference in vapor pressures of ethyl ether contained in the bulbs (see diagram) due to the temperature difference displaces a mercury column across the platinum contacts sealed in the connecting tubing.

Approximate adjustment for the desired humidity range is obtained by varying the amount of mercury in the manometer tube by adding or subtracting from a reserve supply stored in the bulbs. Final adjustment is made by swinging the instrument about a pivot. The 13 cm distance apart of the manometer legs facilitates the changing of the mercury level with respect to the upper platinum contact. With the instrument illustrated, a removable paper scale serves for approximate testing, but final adjustment must be made by trial and error for precise values, since the wet-dry bulb differential as well as the ether vapor pressure difference depends to a degree upon the environmental temperature.

The large reservoir serves to supply the wick cup with distilled water over an extended period of time. The constant-level device utilized is indicated in the diagram.

The practical sensitivity of the control depends to a large extent upon the rate of change of humidity in the environment and air flow over the bulbs. Sling psychrometer readings indicate, however, that in a closed constant-temperature room, control has been obtained well within 1 per cent. relative humidity of a desired value. In the original design, the instrument was adjusted so that one end of the mercury column moved along only a slight upward incline. Such an arrangement gives approximately twice as much movement of the mercury column for a given pressure differential as the normal arrangement. Further increase in sensitivity might be obtained by utilizing the principle of the sloping manometer.

The vertical manometer tube must be sufficiently long so that the lowest humidity will not cause displacement of the mercury into the wet bulb and necessitate readjustment. In the instrument illustrated the 21 cm is sufficient for stability down to approximately 32 per cent. relative humidity at 25° C. If the instrument is to operate at low humidities, it would be essential to extend the tubes for a distance of about 35 cm above the level of the top platinum contact. Calculations of the height required are easily made for a given set of conditions by reference to data upon vapor pressure of ether[1] and to psychometric tables.[2]

No difficulties have been encountered due to the inflammable nature of ether. The instrument was filled with dry ether after addition of sufficient mercury. The outlet was sealed off after slowly boiling

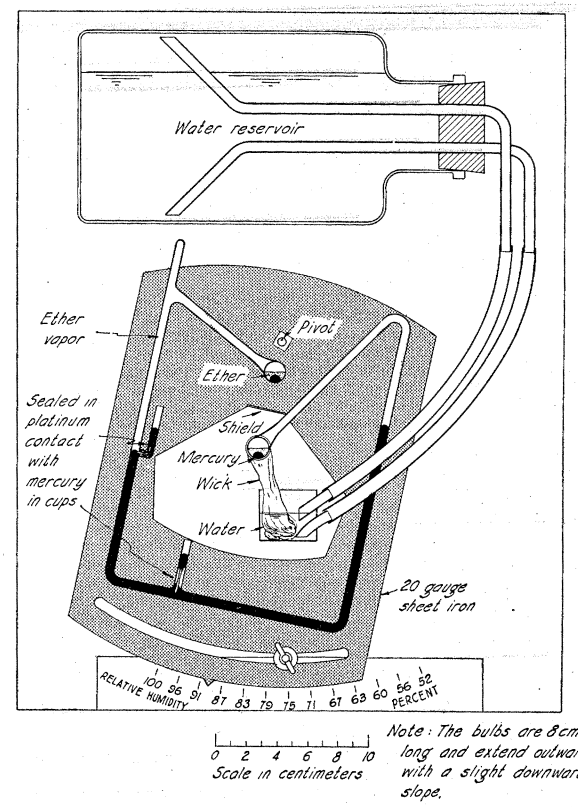

FIG. 1

away the excess ether under reduced pressure, leaving only enough ether to slightly less than half fill both bulbs, which are about 8 cm long and extend outward sloping slightly downward. (Not clearly illustrated in the sketch.) The sealing off is carried out with the ether still boiling so that any residual air would be insufficient to cause an explosion or to exert a partial pressure which would interfere with the operation of the instrument. While the relatively nonexplosive chloroform might be utilized, the vapor pressure change at room temperature is only about one third that of ether. The reduced sensitivity would probably not be of importance for many applications but would be advantageous in order to reduce the tube length required at low humidities.

[1] International Critical Tables.
[2] *U. S. Dept. Agr. Weather Bureau Bul.* 235. 1937.

A sensitive relay is required, as otherwise the heating at the mercury-platinum contact is sufficient to cause trouble in the operation of the instrument; however, either a 2 ma 110 V. AC or a 10 ma 6 V. DC relay has been found satisfactory. The relay should be normally closed for the instrument as illustrated if used in a humidifying system.

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SECTIONING AND STAINING REFRACTORY MATERIALS IN PARAFFIN

MANY tissues, as, for example, the lens of the vertebrate eye, are difficult or impossible to section in paraffin with ordinary methods. Such materials may be cut in celloidin, but the celloidin method has several disadvantages, chief of which are (1) the impracticality of cutting thin sections and (2) the difficulty of keeping serial sections in order.

None of the steps in the paraffin method to be described here are new—in fact, all are to be found in the tenth edition of Lee's "Microtomist's Vade-Mecum"—but they have been combined in an unusual way and the results obtained have been more than satisfactory.

The steps in the method, as it was finally developed, are as follows:

(1) Fix material in Bouin's solution;

(2) Transfer, without washing, to 100 per cent. dioxan and change once during an eight-hour period;

(3) Transfer to paraffin containing 0.5 per cent. beeswax and change twice during an eight-hour period;

(4) Embed in the usual manner and then expose tissue by cutting away one side of the block;

(5) Soak block in water for at least twenty-four hours before sectioning;

(6) Section;

(7) Place section (or sections) on water on clean slide and warm gently;

(8) Allow to cool and replace water with solution No. 1 of Mallory's triple connective tissue stain. Stain for five minutes;

(9) Drain off first stain and replace with Mallory's solution No. 2. Stain for five minutes;

(10) Drain off second stain and replace with water;

(11) Drain off water immediately and replace with 95 per cent. alcohol;

(12) Drain and repeat with absolute alcohol;

(13) Center section on slide and run over it a few drops of 0.5 per cent. celloidin (dissolved in equal parts of ether and alcohol);

(14) Dry for several hours or overnight;

(15) Clear in xylol;

(16) Mount in balsam or clarite.

The materials used in testing the method consisted of the following: frog heads (adult *Acris gryllus* and recently metamorphosed *Rana pipiens*); skin from frog (*Rana pipiens*); skin from seven-day old rat; grasshopper eggs (*Melanoplus differentialis*); amphibian eggs in early cleavage stages (*Triturus* sp.); compound eyes from grasshopper (*Melanoplus differentialis*); compound eyes from beetle (*Dytiscus* sp.); human lens with cataract; and pathological human liver tissue.

All were sectioned at 4 and 6 micra with unbelievable ease except the *Dytiscus* eyes which could not be cut successfully at less than 8 micra. On the other hand, frog's skin and frog's head, including the lenses of the eyes, were sectioned at 2 micra.

Cellular details were found to be even better than those obtained with ordinary paraffin methods. Such structures as intercellular bridges, rods and cones of the retina, the cytoplasm of the cells in the lacunae of cartilage, ciliated epithelium of the oral cavity, muscle striations and the cells surrounding the lens in the sections of the frog's head were in excellent cytological condition. Mallory's stain, when used as described above, shows greater delicacy and precision than is obtained when sections are stained after the removal of the paraffin. Another advantage of the method lies in the fact that tissues such as vertebrate lens and insect cuticle remain flat and do not curl away from the slide as they almost invariably do when sections containing them are spread and dried in the usual way.

The method outlined above is rapid, simple, gives perfect, thin, serial sections of materials ordinarily very difficult to cut and insures fine cellular detail.

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