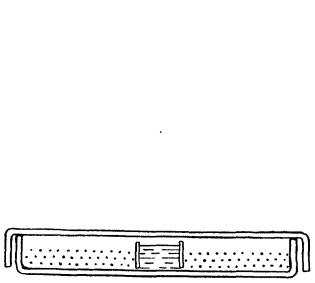
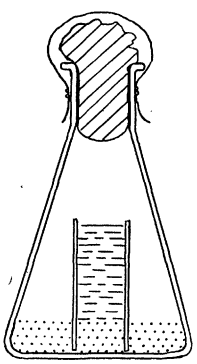
garding various types of fructification can be better secured by observing their development in Petri dish cultures rather than in flask or tube cultures. However, the rapidity with which the substratum dries in cultures of the former type often proves a serious handicap in the study of those species which require long periods of time to complete their life history. This is often true of plates kept at 20° to 22° C., but more often of those incubated at higher temperatures. There has been need of a method for renewing the supply of moisture in the medium without disturbing the growth of the fungus. Such a method would make it possible to continue cultures over a long period of time without either an injury from drying or a disarrangement of conidial fructifications by adding water to the surface of the agar. The following method has been used with good results.

A van Tieghem ring is enclosed in the Petri dish and the two are sterilized together. The ring is easily centered by lightly shaking the dish just prior to pouring the medium. The liquid agar is poured around the ring, spread over the surface of the dish and allowed to harden. The ring thus becomes an empty glass well in the center of the plate (Fig. 1).





FIGS. 1 and 2. Methods for irrigating fungus cultures.

After inoculation and the growth of the fungus, the well is filled with sterile water by means of a sterile pipette before the agar shows any visible sign of drying. From under the lower edge of the ring the water slowly seeps out into the agar, diffusing through the entire plate. This irrigation may be repeated as often as desired, and the life of a single plate culture may thus be greatly prolonged. Since humidity requirements vary with different cultures, a sterile glass cover slip may be placed over the top of the well if it is desirable to prevent too rapid evaporation. If a cover is used it must be so placed as to leave a very small space between its edge and the side of the well, where air may enter as water recedes.

Inoculations are made either at several points immediately outside the glass well, or a continuous circular streak is made with a wire loop carrying a suspension of spores in sterile water.

Van Tieghem rings, sometimes listed as culture rings, vary in size and thickness of glass. Good results have been obtained by using a ring ten millimeters high by eighteen to twenty millimeters in diameter, of glass less than one millimeter thick. The usual ring of thicker glass is more easily lifted from the bottom of the dish by the fluid agar, which then spreads under it, raising the well, and thus opening a wider channel for water seepage. In the case of cultures requiring a very moist substratum this may be desirable; however, for many a culture a slower seepage is to be preferred.

This well can also be used for experiments in nutrition. The acid content of the culture may be altered, or the increasing acidity may be checked. To maintain the purity of any culture a fresh sterile pipette must be used for each irrigation.

Cultures in Erlenmeyer flasks may be similarly irrigated through a piece of glass tubing 18 to 20 millimeters in diameter, inserted into the middle of the agar before it is autoclaved. The length of such tubing must be carefully adjusted to prevent aerial hyphae or fructifications which grow on the sides and the upper edge of the tube from coming into contact with the plug. A two-inch length of tubing is suitable for a flask four inches in height. These flask cultures should be capped with heavy waxed paper or with heavy composition foil before they are sterilized; and all subsequent handling of the plugs should be done without removing this cap from the cotton. This prevents foreign conidia from infecting the plug, which often offers them an ideal chance for germination, and for contaminating growth into the culture below.

DOROTHY PEASE

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