

acetyl derivative melted at 116° (Raistrick *et al.* give 116–118°; Hooper *et al.*, 116–117°). The substance is neutral, is rapidly destroyed by alkali and decolorizes permanganate. These properties correspond with published data on patulin and clavacin, and this substance is, therefore, identical with the other two.

The pure material is quite toxic for mice. L.D. 100=12.5 mg per 20 g mouse. It failed to protect mice against lethal *Salmonella schotmulleri* infections in the highest doses tolerated.

A crystalline substance isolated as above with slight

modifications from culture filtrates of *Penicillium* sp. also proved to be identical with clavacin. The substance melted at 109.5° and showed no depression mixed with authentic clavacin. It analyzed as follows: C, 54.92; H, 4.04. Other properties coincide with those of clavacin.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

INOCULATION OF MEDIA FOR MOLD CULTURE

IN the cultivation of molds in large flasks or bottles it is sometimes difficult to obtain a uniform degree of inoculation and to produce an even growth over the entire surface of the medium. A technique used successfully in this laboratory with several species of *Penicillium* employs a suspension of spores in a medium containing gum tragacanth in which a small amount of lanolin has been emulsified. The particles of lanolin apparently assist in buoying the spores to the surface of the culture medium and holding them there until germinated.

A homogenous emulsion is prepared by warming and stirring 2.5 g gum tragacanth and 0.5 g lanolin in 100 ml of water. Thirty grams of the mixture is placed in a 125 ml Erlenmeyer flask, together with five 12–15 mm glass marbles, the flask is plugged with cotton and sterilized. The flask is then rotated or shaken to emulsify the lanolin while being cooled to 30° C or below. Flasks of gum-lanolin mixture prepared in this way may be stored in the refrigerator indefinitely. To use this gum-lanolin mixture to prepare a spore suspension, the contents of one flask, including the marbles, are poured onto a spore culture grown on agar in a 250 ml flask. The flask is now shaken gently for several minutes with a circular motion in a horizontal plane to cause the marbles to roll over the spore-bearing surface. The spore suspension is further diluted for use by adding 25 ml of sterile water. The resulting suspension measures 45–50 ml and in the case of *Penicillium notatum* suffices to inoculate 15 or more 3-liter Fernbach flasks, the area of the medium in each being about 270 square centimeters. The inoculated flasks are thoroughly agitated by shaking just before incubation and are then allowed to remain undisturbed. This method is readily adapted to a sixfold increase in scale by growing the sporulation culture in a 3-liter Fernbach flask and modifying the rest of the procedure accordingly. For each Fernbach flask use 180 g of tragacanth-

lanolin emulsion, increase the number of marbles to about a dozen and finally dilute with 150 ml of sterile water.

Methyl cellulose in place of gum tragacanth was not satisfactory because it did not properly emulsify the lanolin. Cetyl alcohol in place of lanolin or an eightfold increase in the amount of lanolin inhibited mold growth. If the medium being inoculated contains much suspended matter which settles out, the inoculation is less satisfactory. Presumably the material settling to the bottom counteracts the buoyant effect of the lanolin particles. This difficulty is corrected by filtration of the medium. The incorporation of a wetting agent, such as 0.1 per cent. Ivory soap or 0.4 per cent. Aerosol A.Y. in the gum-lanolin emulsion facilitated the loosening of spores from the mycelium but inhibited spore germination and mold growth.

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