off *in vacuo* under nitrogen with an all-glass apparatus. (The water-bath temperature was not above 30° C.) Three 10-ml. portions of absolute ethanol were added and each successively distilled off under the same conditions to effect removal of the water. Finally, the contents in the flask were dried to a constant weight in a vacuum desiccator over phosphorus pentoxide and in the presence of nitrogen. The lecithin, a transparent, colourless and amorphous solid, was dissolved in absolute ethanol, quantitatively transferred to a 50-ml. volumetric flask and made to volume. The weight of the lecithin thus prepared was 1,777 mgm. According to Baer and Kates⁵, the complex has the structure (lecithin)₂ (cadmium chloride)₃. Assuming a molecular weight of 802 for this lecithin, the yield should have been 1,720 mgm. A lecithin containing one molecule each of stearic and linolenic acids would have a molecular weight of 802 and the following calculated analytical figures: P, 3.86; N, 1.75 per cent; iodine number, 95; analysis gave P, 3.83; N, 1.73; choline-N, 1.73 per cent⁶; N : P ratio, 1:1.00; iodine number, 86. (It is not to be construed that this is evidence for the presence of stearic or linolenic acids.) The lecithin is optically active, $[\alpha]_D^{20} = 7.0$ (in ethanol). A 10 per cent solution of the lecithin in ethanol has been kept at room temperature for two months without discoloration.

The method described here, when used in conjunction with the excellent procedure of Pangborn for the purification of lecithin-cadmium chloride, makes possible the easy preparation of naturally occurring, unsaturated lecithins with a high degree of purity.

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¹ Strecker, A., *Liebigs Ann.*, **148**, 77 (1868).

² Bergell, P., *Ber. deutsch. chem. Ges.*, **33**, 2584 (1900).

³ Levene, P. A., and Simms, H. S., *J. Biol. Chem.*, **48**, 185 (1921).

⁴ Pangborn, M. C., *J. Biol. Chem.*, **137**, 545 (1941); **161**, 71 (1945).

⁵ Baer, E., and Kates, M., *J. Amer. Chem. Soc.*, **72**, 942 (1950).

⁶ Handler, P., *Biol. Symposia*, **12**, 361 (1947).

Soil Recolonization Tube for studying Recolonization of Sterilized Soil by Micro-organisms

STUDIES of recolonization by fungi and bacteria of soil sterilized, or partially sterilized, by steam or by various fumigants such as formalin, carbon disulphide and chloropicrin have been made by many investigators. A difficulty common to all such investigations has been that of taking samples of the soil in the process of recolonization for plating out and determination of the recolonizing micro-organisms. If the soil has been treated *in situ* in the field, it is necessary to dig profile pits, from the sides of which samples can be taken¹. If the soil has been treated in laboratory containers, it may be necessary to sacrifice one container of soil for each series of samples taken, and this makes it impossible to make consecutive observations on the soil in a

Fig. 1. Soil recolonization tube: hatched, non-sterile inoculum; stippled, sterile soil

single container. Furthermore, the disturbance consequent upon removal of soil before taking a sample much increases the likelihood of contamination of the soil samples by adventitious micro-organisms. To overcome these difficulties, the 'soil recolonization tube', illustrated in Fig. 1, has been designed and found useful in the study of fungal recolonization of sterilized soil. It is proving equally useful for the investigation of soil recolonization by bacteria. By the use of this apparatus, soil samples can be taken with the minimum of labour and of disturbance to the surrounding soil; moreover, successive samples of soil can be taken from a single recolonization tube for an indefinite period, and the succession of recolonizing organisms in space and time can be properly studied.

The recolonization tube consists of an 18-in. length of 'Pyrex' glass tubing, of 1-in. internal diameter, to which six side-arms, 1 in. long and $\frac{1}{2}$ in. internal diameter, are fused at 2-in. intervals. The tube is packed with moist soil, which has previously been passed through a 2-mm. sieve and adjusted to a moisture content of 40 per cent saturation. Trim and even packing is secured by tamping after each addition of 25 gm. of soil. After the tube has been filled, the open ends of the main tube and of the six side-arms are plugged with cotton wool and the whole tube is weighed; soil moisture content can then be maintained at the original level by periodical weighing and restoring to original weight by addition of sterile water through the side-arms (starting at the end farthest from the inoculum of unsterilized soil). If recolonization of steamed or autoclaved soils is to be studied, the tubes can be steamed or autoclaved in the usual way; for treatment of soil with a fumigant such as carbon disulphide or chloropicrin, the cotton-wool plugs are replaced, for the duration of the treatment, by rubber bungs. After the sterilization treatment has been completed and the necessary additional period allowed for evaporation of a volatile fumigant, an inoculum of unsterilized soil is added at one end of the recolonization tube. At suitable intervals of time, small samples of soil are removed through the side-arms of each tube, and from them 'Warcup soil plates'² are made for determination of the fungus flora².

Consistent results have been obtained with this technique in successive trials of sterilized and re-inoculated Kettering loam soil. After steam treatment at 1 atm. pressure for 30 min. on two successive days, the first fungal colonists following reinoculation with unsterilized soil were phycomycetous species, such as *Pythium acanthicum* and *Zygorhynchus vuilleminii*. These were regularly followed by *Trichoderma viride*, *Mucor ramannianus* and *Fusarium caeruleum*. If the soil was not packed sufficiently firmly, however, *Trichoderma viride* tended to be the first fungal colonizer.

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¹ Warcup, J. H., *Trans. Brit. Myc. Soc.*, **34**, 519 (1951).

² Warcup, J. H., *Nature*, **166**, 117 (1950).