ULTRA-MICRO PAPYROGRAPHY

PARTITION chromatography (papyrography) with filter paper strips and sheets, first introduced by Consden, *et al.*[1], was carried out by the capillary descent of the solvent. Horne and Pollard[2] and Williams and Kirby[3] brought about a considerable simplification of the technique and apparatus by adopting the capillary ascent of the solvent. The method of Rockland and Dunn[4] represents a micro adaptation of the one-dimensional strip method which also involves capillary ascent. This micro-method was critically studied by Govindarajan and Sreenivasaya[5] and has since been successfully applied to a variety of biochemical problems. Usually spotting is carried out with 2 to 4 μl of the solution which contains about 5 to 15 μg of the substance, as in the case of amino acids and sugars. For a determination of the relative concentration of the various forms of penicillins in a sample of fermented beer, one usually employs 5 units of the antibiotic.[6] A reduction in this quantity was effected by Karnovsky and Johnson[7] who worked with 1-2 units.

In certain types of investigation, *e.g.*, the determination of (1) penicillin-producing capacity of single spores of fungi, (2) penicillin levels in physiological fluids and tissues after injection and (3) allied problems, we were faced with the problem of detecting and estimating smaller quantities of the antibiotic. It was felt that, by using sewing thread of cotton as the cellulosic support in place of the strip of filter-paper, this object of determining the antibiotic in ultra micro quantities might be achieved.

Sewing thread, after successive treatments with (1) benzene, (2) hot water, (3) takadiastase, was washed with water, alcohol and finally with ether. The thread was then impregnated with M/15 phosphate buffer pH 6·2 and air dried. The thread, thus treated (about 400 mm. in length) was used for each experiment. For "ascending" papyrography, the thread (T) (see Fig. 1) was tied to a glass

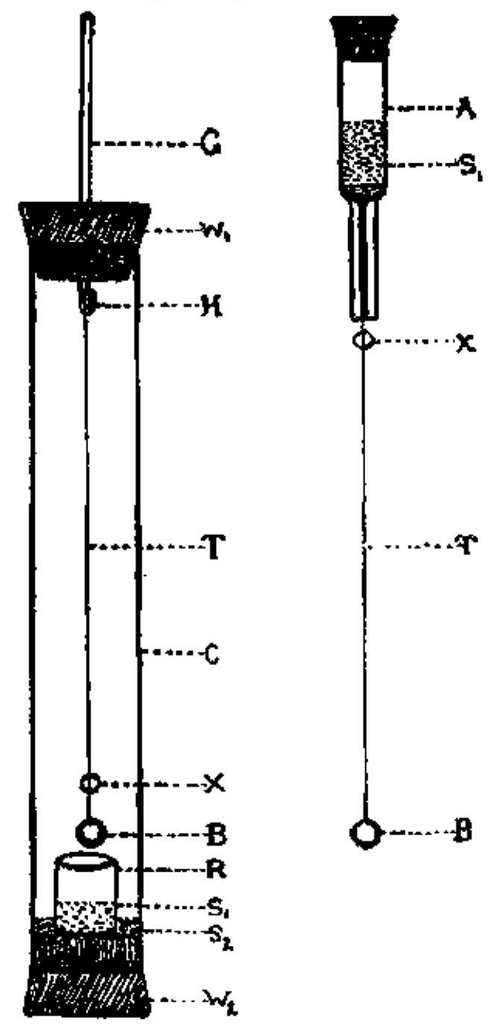

FIG. 1

hook (H) drawn at the end of a glass rod (G) held by a wooden cork (W_1). The other end of the thread is suitably weighted by a glass ring or bead (B) which helps to maintain the thread vertically. The thread is spotted with the test solution at a place (X) about 10 mm.

above the glass ring (B) and air dried. The cork-rod-thread (W_1–G–T) assembly is carefully placed in a chamber (C) consisting of a glass tube (450 mm. × 35 mm.) which is closed at the other end by means of a wooden cork (W_2) which provides a platform for the receptacle (R) containing the developing solvent (S_1) saturated with water. The chamber (C) is lined with filter paper wetted by the solvent-saturated water (S_2) which helps to facilitate rapid equilibration of the system. After a couple of hours during which equilibrium is attained, the thread is carefully lowered into the developing solvent by pushing down the glass rod (G) so that the spotted portion of the thread is well above (at least 5 mm.) the solvent surface. The capillary ascent of the solvent commences immediately and with a solvent like water-saturated ether, the development is usually complete in about an hour. The cork-rod-thread assembly is then carefully lifted out of the chamber, the thread, air dried and bioautographed by the method described earlier,[8] employing a suitable organism. In the case of penicillin we have employed *Strep. aureus* for all the bioautographic tests. The results of one such test is figured in Fig. 2.

The method can be adapted for the "descending" development technique. In place of the glass rod (G), an adapter A (see Fig. 1) made of Pyrex tube (10 × 50 mm.) to which a Pyrex capillary (1·0 mm.) and (50 mm. × 7 mm.) is fused, is employed. The thread is knotted at one end, passed through the capillary and pulled so that the knot plugs capillary. The thread which is thus held in position, is then weighted by a glass ring (B) at the other end. The spotting of the thread with the test solution is carried out at a place a few millimetres (X) below the end of the capillary. A small quantity of treated absorbent cotton is used for reinforcing the plug. The assembly consisting of the adapter and the weighted thread, is held by a wooden cork (W_1) and is placed in the chamber (C) whose other end is closed by a wooden cork (W_2) No receptacle for the developing solvent is necessary. After equilibration, the solvent is placed in the adapter and closed with a cork. The solvent immediately commences to descend and develop the "papyrogram". If flooding is desired, the "development" is permitted to continue; otherwise the thread is air dried and bioautographed.

It will be seen that the method has been successfully employed for the partitioning of the various penicillins in a fermented beer and in quantities which range between 0, 2 – 0·05 units, This ultra micro method is capable of being employed not only for an assay of other antibiotics but also for vitamins and other

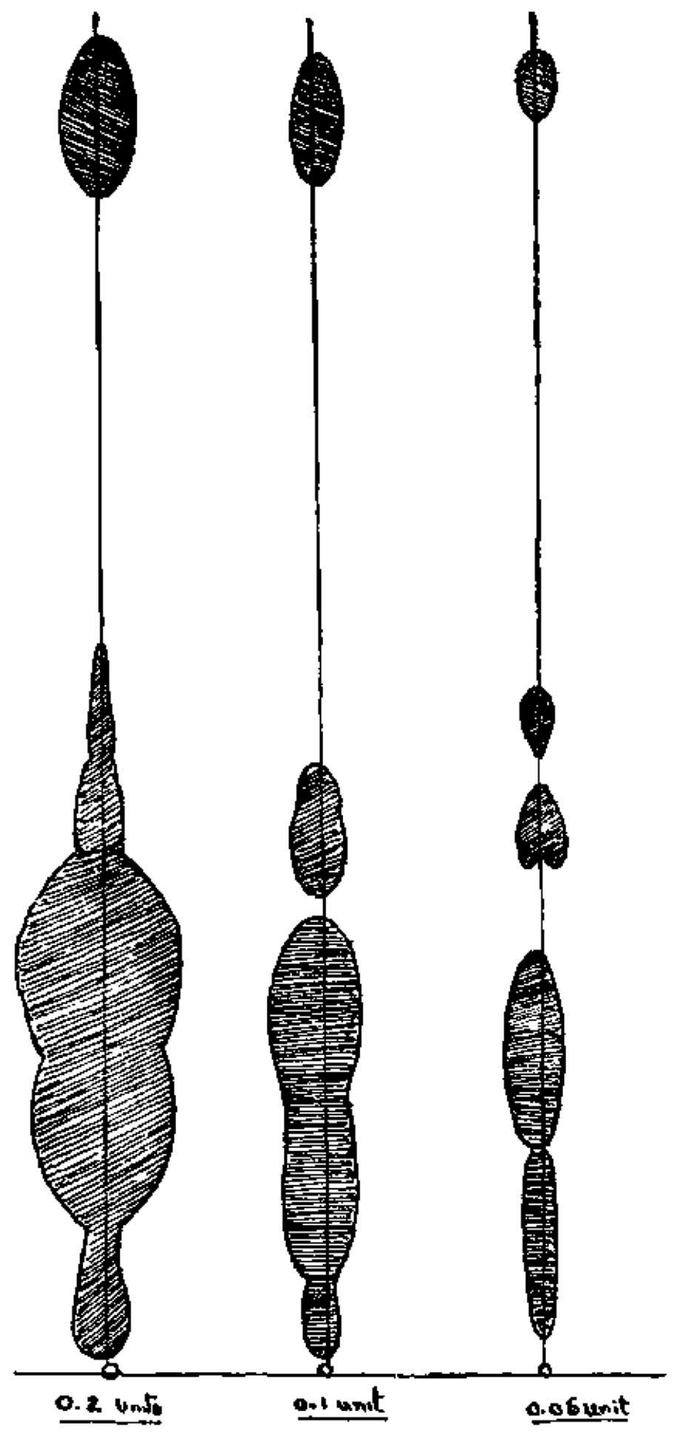

FIG. 2

Penicillium notatum grown on the Basal medium Supplemented with Enzyme-Free Moldy Bran Extract (1·0% solids). Papyrograms were developed on the thread.

Activity tested by Bio-autographic technique using S. aureus as test organism. Shaded areas represent clearance zones.

active principles whose presence can be detected by some type of bioautographic technique involving either an inhibition or growth of a suitable test organism. This method is now being further developed in our laboratories.

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1. Consden, *et al.*, *Biochem. J.*, 1944, **38**, 224. 2. Horne and Pollard, *J. Bact.*, 1948, **55**, 231. 3. Williams and Kirby, *Science*, 1948, **107**, 481. 4. Rockland and Dunn, *Ibid.*, 1949, **109**, 539. 5. Govindarajan and Sreenivasaya, *Curr. Sci.*, 1950, **19**, 39. 6. Goolall and Levi, *Analyst*, 1947, **72**, 277. 7. Karnovsky and Johnson, *Analytical Chemistry*, 1949, **21**, 1125. 8. Venkatesh and Sreenivasaya, *Curr. Sci.*, 1951, **20**, 98.