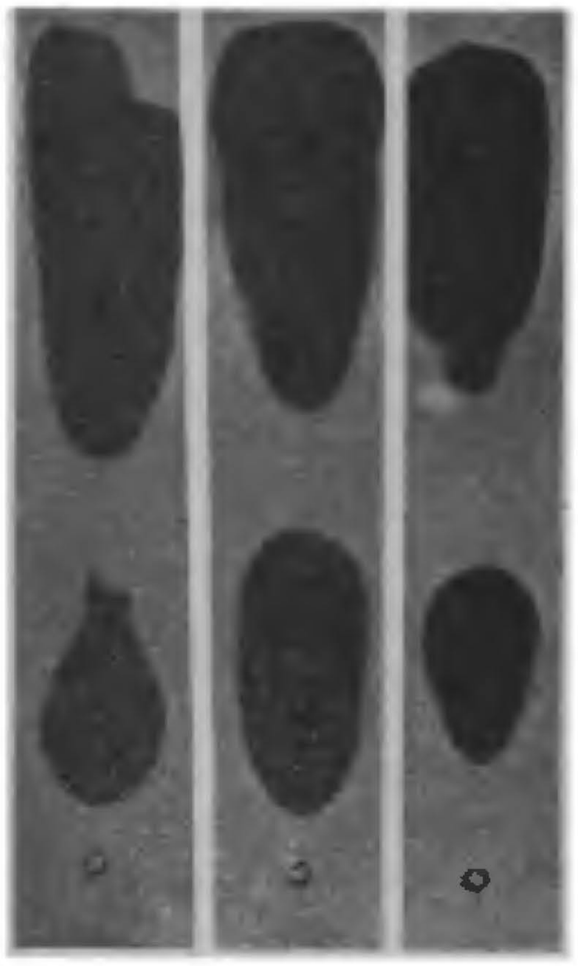
A MODIFIED AUTOBIOGRAPHIC TECHNIQUE FOR THE LOCATION OF "PHYSIOLOGICALLY ACTIVE" SPOTS ON PAPYROGRAMS

IN the course of our studies on the papyrographic separation and quantitation of antibiotics, vitamins and other growth factors, we were confronted with the problem of locating the 'physiologically active' spots on the papyrogram by the technique of bio-autography. Since rectangular dishes (45 × 25 × 2 cm.) usually employed for such tests[1,2] have not been readily available to us, we tried to use stainless steel 'Dishes' (40 cm. × 10 × 2 cm.) made to similar specifications. Our first trials with penicillin papyrograms developed according to the micro-method of Rockland and Dunn[3] as modified by Govindarajan and Sreenivasaya,[4] showed that it was difficult to locate the "active" spots. The technique was, therefore, modified by first layering the "dish" with a rectangular sheet of cellophane, with its two shorter ends over-reaching the lip of the dish. The nutrient agar medium inoculated with *S. aureus*, was then poured on to the dish and allowed to set. The papyrograms (1 cm. × 19·5 cm.) after developing were placed on the agar surface, gently pressed with a flattened glass rod to facilitate intimate contact of the paper and allowed to remain there for 30 minutes. During this period, the active principles from the 'spots' were expected to diffuse into the agar. The strips were then removed and the test "dishes" incubated at 37° C. for 18 hours.

With the help of the extra length of cellophane allowed to over-reach the lips of the dish, the agar layer can be easily lifted and held vertically for inspection. The "active" spots can be easily located as clear and transparent spots on the agar layer. Permanent records of such papyrogram preparations can be obtained by making contact prints on "Ilford reflex contact Document No. 50" (see Fig. 1).



1 2 3

Penicillium Notatum grown on the basal medium, supplemented with:—

1. Lac Washings. 2. Aqueous green gram extract. 3 Enzyme-free moldy bran extract.

Circles in the figure represent the areas spotted with the beer.

This method has been successfully applied for the characterisation and quantitation of the penicillins in the fermented beers and also, for the separation and identification of growth factors in the physiological fluids and tissue extracts.

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1. Karnovsky, M. L. and Johnson, M. J., *Anal. Chem.*, 1949, **21**, 1125. 2. Kluener, R. G, *Jour. Bact.*, 1949, **57**, 101. 3. Rockland and Dunn, *Science*, 1949, **109**, 539. 4. Govindarajan, V. S. and Sreenivasaya, M., *Curr. Sci.*, 1950, **19**, 39.