bent at 120 degrees and the other end of the rod fixed to a pulley (*F*) to provide movement in the horizontal plane. The wing tip is oriented along the long axis of the capillary.

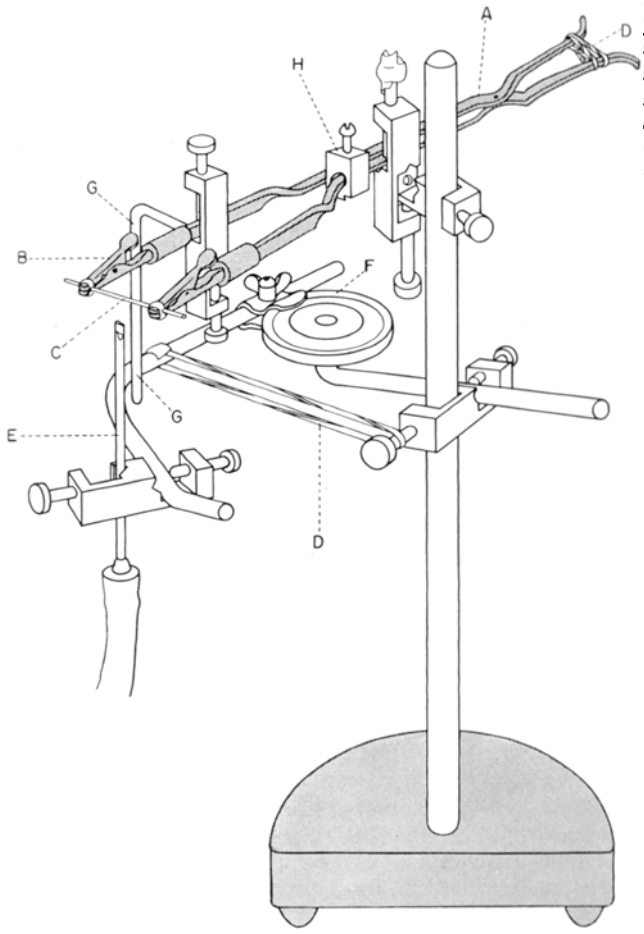

Diagram of micropipette puller.
A Tong like holder, *B* Alligator clamp, *C* Capillary tubing, *D* Rubber band, *E* Microburner, *F* Pulley, *G* Vertical rod, *H* Movable stop

A vertical rod (*G*) is fixed to the movable arm of the tongs through a 90° angle and rides in the angle of the horizontally oriented rod holding the microburner. The vertical rod functions as a stop for positioning the microburner and also pushes the microburner aside in the final stage of pulling. In order to align the flame consistently with respect to the capillary tubing, a rubber band (*D*) may be attached between the horizontal rod and a clamp on the stand. A movable stop (*H*) on the stationary arm of the tongs is a convenient aid in the initial alignment of flame and capillary tubing.

The size of the flame and its distance from the tubing seem to be the only critical variables. In general, a small blue cone flame gives the most satisfactory results. Electrodes of less than 0·5 μ can be fabricated routinely.

The filling of capillary micropipettes under reduced pressure has been in use for some time[3]. It appears worthwhile to describe in detail a simple filling technique which does not seem to be widely known. The capillaries in a plastic rack are held vertically with tips down and shank ends covered by filtered 3 *M* KCl. The top of the rack is a flat disc of the same diameter as the containing vessel, a heavy-walled Pyrex cylindrical jar (4″ × 6″), filled to a level about $^2/_3$ capacity. The cuff of a large sized heavy rubber glove is fitted on the wide end of a Büchner funnel and then stretched down over the Pyrex jar to give a vacuum seal. The solution is heated to almost boiling and reduced pressure then applied from a water pump while the heating is continued. After 5 min air is admitted to the system. It is important that the electrodes are completely immersed in solution at all times during the filling process. Cooling to about 4°C will usually eliminate a small air space which may remain occasionally in an electrode.

S. E. Kirk and G. Falk

Department of Pharmacology, University of Washington, School of Medicine, Seattle (Washington), July 11, 1957.

Résumé

Nous décrivons un appareil facile à construire pour étirer des microélectrodes capillaires et une manière simple de remplir ceux-ci sous pression réduite.

[3] R. D. Keynes and H. Martins-Ferreira, J. Physiol. *119*, 315 (1953).

PRO LABORATORIO

A Perspex* Moist Chamber for Micromanipulation

A modification of the moist chamber described by Fowell[1], for the isolation of yeast ascospores and vegetative cells has been constructed from perspex. Incorporated in the design is an easily removable lower floor of glass. This facilitates initial inoculation, final isolation and any intermediate macromanipulation that may be required, without the removal of the chamber roof, on the underside of which the micromanipulation has been carried out.

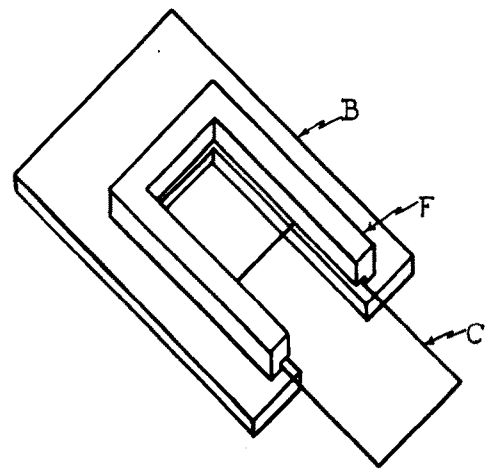

Fig. 1.

The U shaped frame (*F*) is made from $^1/_4$ in. square section perspex and is mounted on the base (*B*) made from $^1/_8$ in. sheet material. The portion of the base lying

* = Plexiglas.

[1] R. R. Fowell, J. appl. Bact. *18*, 149 (1955).

between the arms of the frame is cut away. The lower inner edge of the frame is rebated to enable a 2 × $^{15}/_{16}$ in. coverslip (*C*) to be inserted and act as the floor of the chamber. The roof consists of two $^{7}/_{8}$ in. coverslips or a single 2 × $^{15}/_{16}$ in. coverslip these being lightly cemented in position with small spots of a lanolin-resin cement. Inoculation, final isolation, and other macromanipulation is carried out by sliding out the basal coverslip to the required distance.

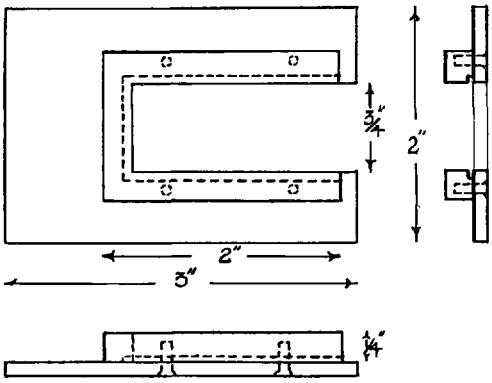

Fig. 2.

P. A. DIXON

Birkbeck College, University of London, March 19, 1957.

Résumé

Une chambre humide pour la micromanipulation a été construite en Perspex. Elle est munie d'un plancher inférieur mobile en verre, qui facilite l'inoculation initiale, la macromanipulation intermédiaire et l'isolement final.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Metabolic Products of *Penicillium patulum* and their Probable Interrelationship[1]

By E. W. BASSETT and S. W. TANENBAUM[2]

During the course of a study of the biosynthesis of patulin in *Penicillium urticae*, Bainier (syn. *P. patulum*) strain 2159A[3], several closely related compounds were found to accumulate in the growth filtrate. Many of the metabolic products had previously been identified, including gentisic acid, gentisaldehyde, and gentisyl alcohol by BIRKENSHAW *et al.*[4], and 6-methylsalicylic acid by EHRENSVÄRD[5]. In our experiments using this nonpigmented mutant strain, the following additional compounds have been identified: 6-formylsalicylic acid, 3-hydroxyphthalic acid, pyrogallol, *p*-hydroxybenzoic acid, anthranilic acid, and an aliphatic substance which we have tentatively named 'pre-patulin'.

Pathways for the biosynthesis of patulin have been proposed first by BIRKENSHAW[6] and later by EHRENSVÄRD[7]. Their salient features involve the oxidative rupture of gentisaldehyde followed by rearrangement and closure to patulin. EHRENSVÄRD's scheme places 6-methylsalicylic acid as the productof a side reaction and does not recognize a direct relationship between this C_8 metabolite and the C_7 aromatics postulated as patulin precursors. With the finding of 6-formylsalicylic acid and 3-hydroxyphthalic acid, heretofore unrecorded in biological systems, we are able to fit all of the known metabolic products of this mold into a logical sequence.

Results.—The identification of 6-formylsalicylic acid (6-FSA) was made by comparison with authentic[8] material synthesized by the method of ELIEL, RIVARD, and BURGSTAHLER[9]. A list of the physical and chemical characteristics of the isolated and authentic materials is given in the Table (p. 40). The ultraviolet absorption spectra are presented in the Figure. By comparison with synthetic material prepared from 3-aminophthalic acid, 3-hydroxyphthalic acid was identified. Both the synthesized and isolated compounds had the same properties, i.e., m.p. 154°, λ_{max} = 323 mμ, as well as identical paper chromatographic and color reactions. Further fractionation of the culture filtrates afforded pyrogallol in good yield (m.p. 134°, m.p. of triacetate 162°); while *p*-hydroxybenzoic acid and anthranilic acid were detected by paper chromatographic and spectrophotometric analysis. These last two acids have long been known to be intimately related to aromatic biosynthesis from shikimate both in *Aerobacter*[10] and in *Neurospora*[11]. To the authors' knowledge, pyrogallol, the classic example of the higher plant aglycones, has not previously been found among the fungi.

From cultures grown on glucose in the presence of $CaCO_3$ and which failed to show evidence of patulin formation by the usual criteria, a waxy material (λ_{max}

[1] Supported by a grant (NSF–G2914) from the National Science Foundation.

[2] Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, N.Y.

[3] We should like to thank Dr. C. W. HESSELTINE of the NRRL, Peoria, Ill., for sending us this culture.

[4] J. H. BIRKENSHAW, A. BRACKEN, S. A. MICHAEL, and H. RAISTRICK, Lancet *245*, 625 (1943). – J. H. BIRKENSHAW, A. BRACKEN, and H. RAISTRICK, Biochem. J. *37*, 726 (1943).

[5] G. EHRENSVÄRD, Exp. Cell Res., Suppl. *3*, 102 (1955).

[6] H. J. BIRKENSHAW, Ann. Rev. Biochem. *22*, 371 (1953).

[7] J. H. BIRKENSHAW, A. BRACKEN, and H. RAISTRICK, Biochem. J. *37*, 726 (1943).

[8] We are indebted to Prof. E. L. ELIEL for a generous supply of this compound.

[9] E. L. ELIEL, D. E. RIVARD, and A. W. BURGSTAHLER, J. org. Chem. *18*, 1679 (1953).

[10] B. D. DAVIS, J. Bact. *64*, 729 (1952).

[11] E. L. TATUM, S. R. GROSS, G. EHRENSVÄRD, and L. GARNJOBST, Proc. nat. Acad. Sci., Wash. *40*, 271 (1954).