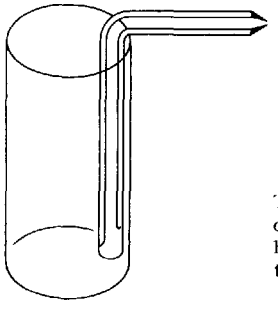
and ileum, and sturdy enough to be used successfully by medical students in 60 classroom experiments.

Construction. (1) Ink delivery tube: The shaft of a 20-gauge needle from which the boss has been removed but with the stylet in place is inserted into a length of closely fitting polythene tubing. The polythene is heated gently and drawn out so as to make a tight fit over the stylet. The stylet is removed, and needle and polythene tubing are bent with the fingers over the nail of the thumb to a right angle 7 mm from the blunt end of the needle: the polythene tube is cut 0.5 mm beyond the ends of the needle.

(2) Ink reservoir: This is made from polythene tubing 3–4 mm internal diameter, by heating this over a microflame and cutting it off at the appropriate length.



The finished inkwriter. The bent ink delivery tube consisting of a 20-gauge hypodermic needle encased in polythene, is glued to the wall of the polythene ink reservoir.

The ink delivery tube is glued to the side of the reservoir and left to dry for 24 h. Finally a length of aluminum wire or thin tubing is fixed to the reservoir, e.g. by inserting the toothed end of the aluminum into the gently warmed wall of the reservoir.

Our inkwriters had a weight of 90–110 mg and a capacity of 0.05 ml, which is enough to record e.g. uterine contractions for a few hours. It is essential to use a freely flowing ink, such as the ink supplied for electric recorders.

Use of this writer in class experiments provided two advantages over smoked paper writers. The work was less messy, and the students could remove their recording immediately after their experiment to take them to a discussion of the results in the lecture room. Especially the latter point was found markedly to stimulate their interest in their work.

Zusammenfassung. Unter Verwendung eines Polythänschlauches wurde ein Tintenschreiber (Gewicht 90–110 mg) zur Registrierung von Kontraktionen isolierter Organe konstruiert. Dieser Schreiber lässt sich mit Erfolg im physiologischen und pharmakologischen Pratikum verwenden.

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Department of Pharmacotherapeutics, University of Amsterdam (The Netherlands), May 8, 1963.

The Preparation of Holey Films for Electron Microscopy

Tiny holes in plastic films are useful for checking the astigmatism of an electron microscope[1], while larger holes are useful for supporting thin sections[2] and negatively stained preparations[3]. There are several methods for preparing films with holes[2,4,5], but we have found them to be unreliable or time consuming compared to the simple methods presented here.

One ordinarily makes a uniform Formvar film by allowing a solution of Formvar in ethylene dichloride to spread on a water surface. If the solution contains water droplets, the film will contain holes. Water can be introduced into the Formvar solution in a number of ways:

(1) One may spread a tiny droplet of 1% Formvar in ethylene dichloride on cold water (4 to 10°C) and immediately condense his breath on the film before the ethylene dichloride has evaporated. This procedure is simpler and quicker than the somewhat analogous method of SJÖSTRAND[2] or HARRIS[5]. However, such a Formvar film often contains bubbles in fields surrounding the zones of holes.

(2) 4–6 tiny droplets of 80% ethyl alcohol are added under shaking to 1 ml of 1% Formvar solution in ethylene dichloride. When the resulting bluish water emulsion is spread on a water surface it forms a very thin fragile film that is full of holes. Such fragile films may be picked up by allowing them to settle onto 200 or 400 mesh grids. Then before the grids have dried, they are touched to a second water surface containing a trace of a detergent so that when the grids are dry, surface tension is less likely to break the holey films.

(3) Finally, a water emulsion can be made by adding 0.001 ml of distilled water to 1 ml of a 1% Formvar solution in ethylene dichloride. The mixture is placed in a plastic tube which is inserted in the water-filled cup of a Raytheon sonic vibrator. After 30–60 sec sonic treatment at 9000 cps, the droplet of water becomes dispersed in the Formvar solution. The resulting milky blue emulsion, which is stable for about 30 min, is then mixed with 1 to 2 vol of untreated 1% Formvar solution in ethylene dichloride. When one drop of this mixture is placed on a clean water surface it spreads to form a Formvar network of veined appearance. The area between the veins contains tiny holes but here the film is again very fragile and should be picked up as previously described.

After such films have been coated with carbon they can be examined in the electron microscope (Figure 1). It is seen that many holes are suitable for correcting astigmatism. The number of holes per unit area can be reduced by decreasing the proportion of water emulsion to Formvar solution. Such mixtures also spread more uniformly on the water surface.

We also use another principle for obtaining holes of different sizes (Figure 2) by exposing the dry Formvar film to droplets of its solvent: 200 or 400 mesh grids are covered with a thin Formvar film. After drying in air, they are exposed to the very fine spray of ethylene dichloride produced by a Vaponephrin nebulizer (Vaponephrin Company, Division of Thayer Labs., Inc., 666

[1] J. HILLIER and E. G. RAMBERG, J. appl. Phys. *18*, 48 (1947).
[2] F. S. SJÖSTRAND in Electron Microscopy: Proc. Stockholm Conf. Sept. 1956, 120 (Almqvist and Wiksell, Uppsala 1957).
[3] H. E. HUXLEY and G. ZUBAY, Proc. Europ. Reg. Conf. Electron Microscopy, Delft 1960, 703 (De Nederlandse Vereniging voor Electronenmicroscopie, Delft).
[4] Siemens Elmiskop I-Information, circular 1, 4 (Siemens New York, Inc. 1960).
[5] W. J. HARRIS, Nature *196*, 499 (1962).

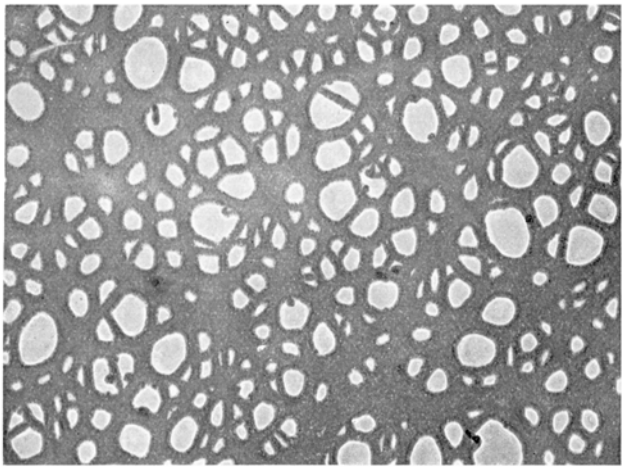

Fig. 1. Holey film prepared with water emulsion (×40000).

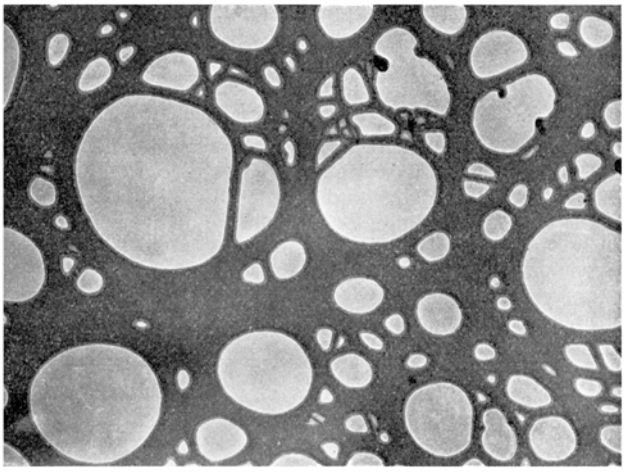

Fig. 2. Holey film. For preparation see text (×40000).

Fifth Avenue, New York 19). We have found it convenient to hold the grid 1 cm from the mouth of the nebulizer and spray three or four times at 10 sec intervals. Too much spray causes the film to break down[6].

Zusammenfassung. Vier verschiedene Methoden zur Gewinnung von Lochfolien für elektronenmikroskopische Belange werden beschrieben. Damit lassen sich unter anderem leicht Anzahl der Löcher pro Flächeneinheit und Lochdurchmesser variieren, so dass eine weitgehende Anpassung der Filmqualität an die sonstigen Versuchsbedingungen ermöglicht wird.

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The Institute for Cancer Research, Philadelphia (Pennsylvania, U.S.A.), February 25, 1963.

[6] This work was supported in part by grant NSF-G12491 from the National Science Foundation.

Preparation of Tritiated Vincaleukoblastine[1] by the Wilzbach Technique

The alkaloid vincaleukoblastine (VLB) has created considerable interest as a chemotherapeutic agent in experimental neoplasias[2]. It was felt that the studies on the mechanism of action of these new agents would be greatly aided if radiolabeled alkaloid were available. Since direct synthesis of specifically labeled VLB is not possible at this time, the use of the Wilzbach gas exposure method[3–5] for tritium labeling was explored.

Experimental and Results. Crystalline VLB:SO_4 (950 mg) and amorphous VLB base (650 mg) were each exposed to 15 c of tritium gas (660 mm Hg) for a period of 14 days at 27° by New England Nuclear Inc. Exchangeable tritium was removed by equilibration with methanol. The sulfate salt was purified by recrystallizing six times from methanol-ethanol mixtures. Insoluble materials were removed by filtration of the hot solutions. The tritiated VLB base was converted to the sulfate salt and it too was purified by crystallizing six times from methanol-ethanol. The progress of the purification was followed by determining the specific activity after each crystallization (Table).

The progress of the purification was also followed by thin layer chromatography on silica gel. A small amount of the hot VLB sulfate was added to a mixture of equal parts of cold VLB sulfate and cold dihydro-VLB sulfate and about 25 μg of the material was applied to the plate. A drop of ether saturated with ammonia was then applied to the spot to liberate the free bases. After drying, the chromatogram was developed with methanol. The VLB and dihydro-VLB spots were then located by placing the plate in a chamber containing iodine vapor. The distribution of radioactivity on the plate was determined by scraping 1 cm lengths of silica gel into counting vials. Care was taken to make a clean separation between the VLB spot and the dihydro-VLB spot. After adding 5 ml of scintillator solution to each vial, the radioactivity was

Specific activities of VLB-t following recrystallization

Recrystallization Number	VLB tritiated as sulfate salt	VLB tritiated as free base
0	193 (950 mg)	1230 μc/mg (650 mg)
1	29.6	143
2	23.3	92.3
3	20.0	78.7
4	18.0	62.8
5	17.5	62.8
6	15.5 (320 mg)	61.4 (40 mg)

[1] Supplied as VELBAN (vinblastine SO_4-Lilly).
[2] G. H. SVOBODA, I. S. JOHNSON, M. GORMAN, and N. NEUSS, J. Amer. pharm. Assoc. *51*, 707 (1962).
[3] K. E. WILZBACH, J. Amer. chem. Soc. *79*, 1013 (1962).
[4] M. L. WHISMAN and B. H. ECCLESTON, Nucleonics *20*, 98 (1962).
[5] S. ROTHCHILD (Editor), *Advances in Tracer Methodology*, vol. 1 (Plenum Press, New York 1963), p. 4.