data have been analysed in such a way that the mean position of apparent-continuity, together with its standard error and variance, can be clearly seen. In order to consider the comparative extents of the illusion in the two conditions, it must be borne in mind that the data do not refer to independent samples, because it is most likely that the two performance parameters under consideration are correlated. Any sequential changes in the extent of the illusion are also likely to be correlated. Thus, it is necessary to apply here a statistical procedure relevant to consideration of differences between correlated variables. A convenient procedure is one which tests the hypothesis that differences in the performance of subjects in the two conditions have arisen by chance. The mean difference  $(\overline{D})$  between the extent of the illusion in the two conditions reaches a value of 0.02, the standard error  $(S.E.\overline{D})$  associated with which is 0.07; this yields a value of t of 0.29, the probability of occurrence of which on the null-hypothesis being greater than 0.25.

Thus, these data indicate first that for each of these subjects the illusion is apparent in the visual condition; secondly, that for each of these subjects the illusion is apparent in the tactile-kinaesthetic condition; and finally, that for the group of subjects taken as a whole, the extent of the illusion does not differ significantly in the two conditions. It would seem to be justifiable to conclude that a tactile analogue of the Poggendorff illusion in vision has been demonstrated.

This work was supported by grants from the Science Research Council and the Ministry of Defence.

GERALD H. FISHER

Department of Psychology,

University of Newcastle upon Tyne. <sup>1</sup> Fisher, G. H., Amer. J. Psychol., 75, 321 (1962).
<sup>2</sup> Fisher, G. H., Amer. J. Psychol., 77, 2 (1964).
<sup>3</sup> Burmester, E., Z. Psychol., 12, 355 (1896).

## GENERAL

## Preparation of Films with Holes for Electron Microscopy

PERFORATED, or "holey", supporting films are constantly required in electron microscopy. They are necessary for the correction of astigmatism, and are invaluable for obtaining the best resolution, because they simplify focusing at high magnification, and allow areas of the specimen to be examined unimpeded by supporting film.

In the preparation of good films, however, it is difficult to control the size and distribution of the holes, and the smoothness of the edge of the perforation is frequently inadequate. The best quality of perforated film for astigmatism correction that I have seen was made by a method described in the operating instructions supplied with the Siemens Elmiskop electron microscope, using brominated glacial acetic acid and 1 per cent collodion in butyl acetate. Unfortunately, it is not easy to make this method work; furthermore, the intense irradiation in the electron beam which seems necessary to form the holes in the plastic film can give rise to an undesirable level of X-ray emission.

Films with holes are usually obtained by condensing water droplets on to a glass slide on which a chloroform solution of 'Formvar' is evaporating<sup>1,2</sup>. Collodion solutions can be used in a similar fashion<sup>1</sup>. A better method has been described by Harris<sup>3</sup>, in which glycerol droplets are used instead of water.

The present method also uses glycerol emulsions, but in addition it etches the cast perforated film so as to remove the fine pellicles which are believed frequently to overlie the glycerol droplets, and which cause both false perforations and the ragged edges of genuine perforations.

A glycerol emulsion is formed by adding 0.05 ml. AnalaR' glycerol and 0.05 ml. 'Teepol' to either 6 ml. of 0.25 per cent 'Formvar' in chloroform, or to 6 ml. of chloroform. The 'Teepol' used is a blend of anionic and



Fig. 1. Portion of a carbon film, the perforations of which were formed by diluting 0.5 ml. of the original emulsion, as described in the text  $(\times 20,000)$ .

non-ionic detergents and is first diluted with distilled water to quarter strength. Vigorous shaking will yield a milky emulsion; ultrasonic disintegration has been used, but with no apparent advantage. To prepare a film with small holes suitable for the correction of astigmatism up to  $\times$  80,000, 0.35 ml. of this emulsion is added to 10 ml. of 0.25 per cent 'Formvar' in chloroform. The resultant suspension, which shows no evidence of being an emulsion, is poured on to a new glass slide. The chloroform evaporates and a plastic film remains on the glass. For consistency it is important to shake the suspension vigorously before pouring-a precaution which should be taken also before removing any of the original emulsion for dilution.

The slide is now immersed for a time, say 30 min, in an etching solution of about 25 per cent amyl acetate in benzene. It is then removed, and quickly dried by waving in the air. After scoring the border of the slide with a needle, the membrane can be floated off on to a water surface and used for filming grids before carbon-casting in the usual manner<sup>4,5</sup>.

If a network, rather than a perforated film, is required, the original emulsion made in 0.25 per cent 'Formvar' can be used undiluted; various degrees of coarseness of perforation can be obtained by various dilutions of the original emulsion. For the usual film with holes, however, so little of the original emulsion is added to the diluting 'Formvar' solution that it is quite satisfactory to make the emulsion in chloroform alone.

When coarser perforations are being prepared, more glycerol will be left on the slide after the film has been cast, so it is probably worthwhile to give the slide a brief rinse in benzene containing about 15 per cent acetone before etching. This rinse should not be prolonged or the film may not float off the slide when it is later submerged in distilled water.

Although ragged holes can still be obtained with this method, smooth ones predominate. With the simple shaking precaution mentioned here, it is not difficult to achieve an acceptable degree of consistency in the preparation of perforated films.

Note added in proof. Subsequently it has been found more convenient to etch for 10 min in a solution consisting, by volume, of 20 parts chloroform, 5 parts acetone and 75 parts benzene.

## ARWYN CHARLES

Medical Research Council Unit for Research on the Experimental Pathology of the Skin, Medical School, University of Birmingham.

Bradley, D. E., in *Techniques for Electron Microscopy*, edit. by Kay, D., chap. 3 (Blackwell, Oxford, 1961).

<sup>a</sup> Pease, D. C., *Histological Techniques for Electron Microscopy* (Academic Press, New York, 1960).
<sup>a</sup> Harris, W. J., *Nature*, 196, 499 (1962).

- <sup>4</sup> Bradley, D. E., Brit. J. App. Phys., 5, 65 (1954).

<sup>5</sup> Watson, M. L., J. Biophysic. Biochem. Cytol., 1, 183 (1955).