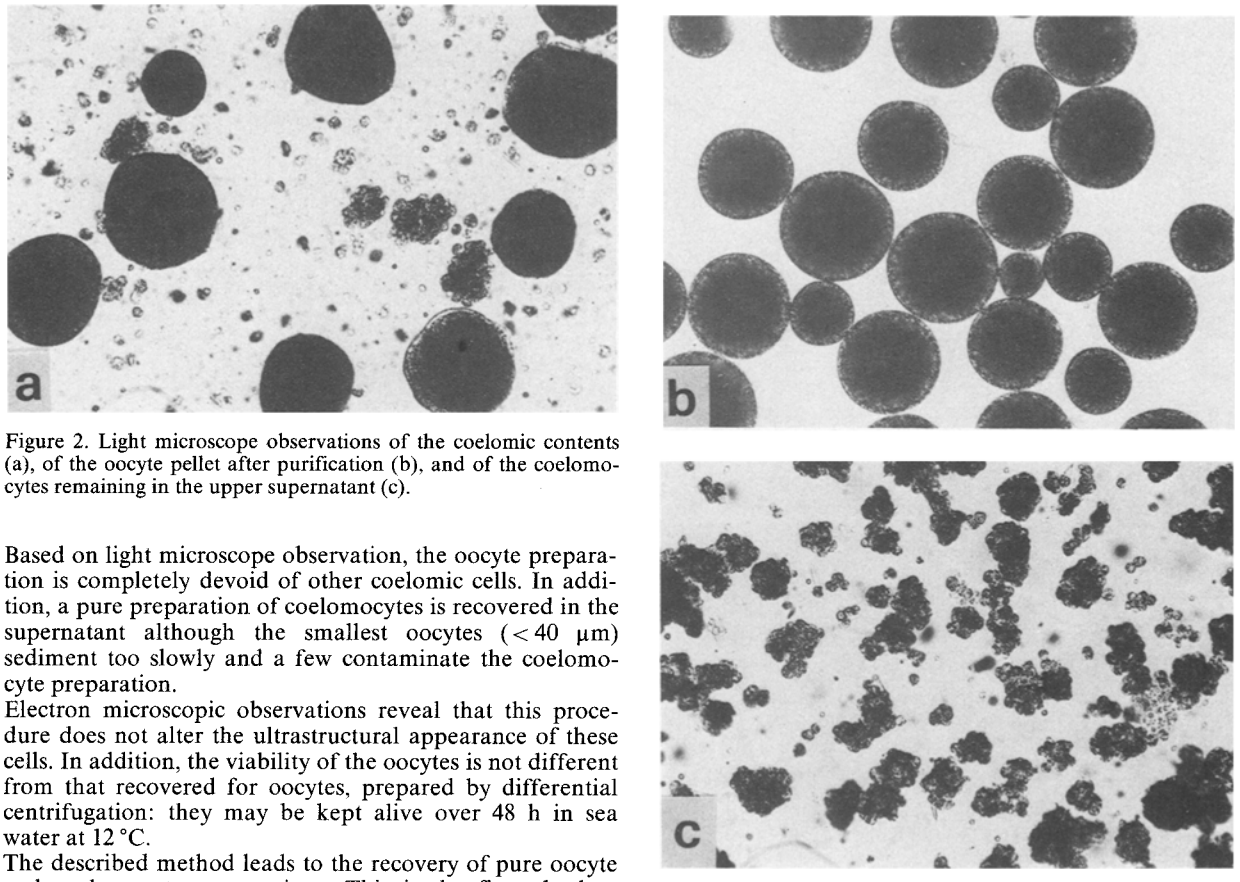

Figure 2. Light microscope observations of the coelomic contents (a), of the oocyte pellet after purification (b), and of the coelomocytes remaining in the upper supernatant (c).

Based on light microscope observation, the oocyte preparation is completely devoid of other coelomic cells. In addition, a pure preparation of coelomocytes is recovered in the supernatant although the smallest oocytes (< 40 µm) sediment too slowly and a few contaminate the coelomocyte preparation.

Electron microscopic observations reveal that this procedure does not alter the ultrastructural appearance of these cells. In addition, the viability of the oocytes is not different from that recovered for oocytes, prepared by differential centrifugation: they may be kept alive over 48 h in sea water at 12 °C.

The described method leads to the recovery of pure oocyte and coelomocyte preparations. This is the first absolute requirement to investigate the proper enzymatic equipment of each cell types and to undertake a clear approach to uncover the biological relationships between each cell of the coelomic contents.

1 Reprint requests to M.P., Service de Biologie Animale, Université des Sciences et Techniques de Lille, F-59655 Villeneuve d'Ascq Cedex (France).

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0014-4754/83/040436-02$1.50 + 0.20/0

Colored filters for microphotography. II. A new method

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Summary. A new method for obtaining colored filters for light microphotography is described. Spectroscopic comparisons of stains and filters derived from them are discussed.

In a previous paper, we reported a technical procedure for preparing specific colored filters for black and white microphotography[3]. However, this technique was found to be rather time-consuming, and required the preparation of large amounts of an agar medium. We have tried to simplify this procedure and we have investigated the use of transparencies as colored filters. In this study, 3 different stains were tested: Ponceau de Xylidine (CI 16150), acid fuchsin (CI 42685) and methylene blue (CI 52015); as these are widely used in our laboratory. Two different films were also tested: Ektachrome 64 (ER 135) and Kodachrome 64 (KR 135).

Preparation of the filter. Aqueous solutions of the stains are prepared and transferred to plastic tissue - culture flasks - with parallel sides[2] (plane area 25 cm^2, volume 50 ml). A stained section is placed on the microscope and strongly illuminated. The tissue-culture flask is placed between the illuminating lens and the condenser. The concentration of the stain in the flask is adjusted when the section is viewed until the background appears as deeply colored as the areas to be masked on the microphotograph.

After removal of the section from the microscope, a slide of the colored light is taken. Films are developed as normal by the manufacturer. Their optical spectra are compared with those of the original stains.

Spectroscopic study of the filters. A classical spectroscope

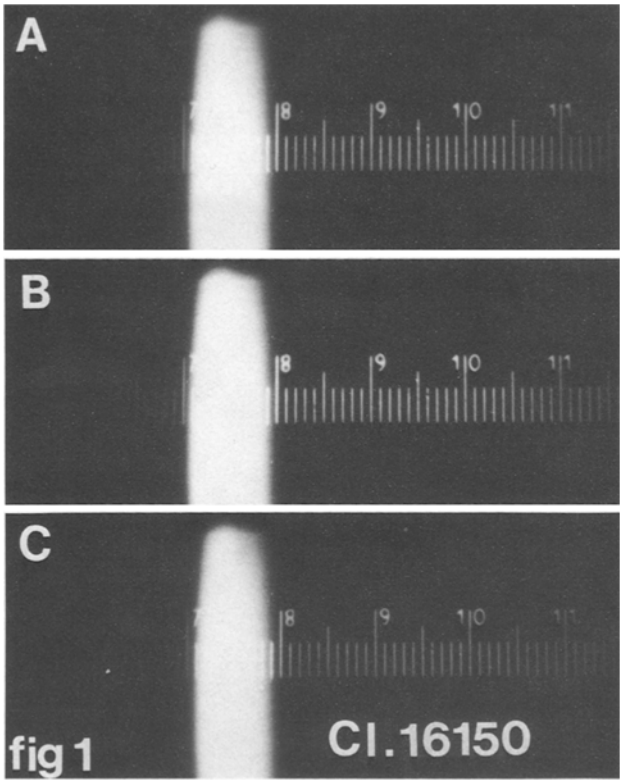

Figure 1. Spectra obtained from Ponceau de Xylindine: *A* Spectrum of the stain solution, *B* spectrum of the Ektachrome slide, *C* spectrum of the Kodachrome slide.

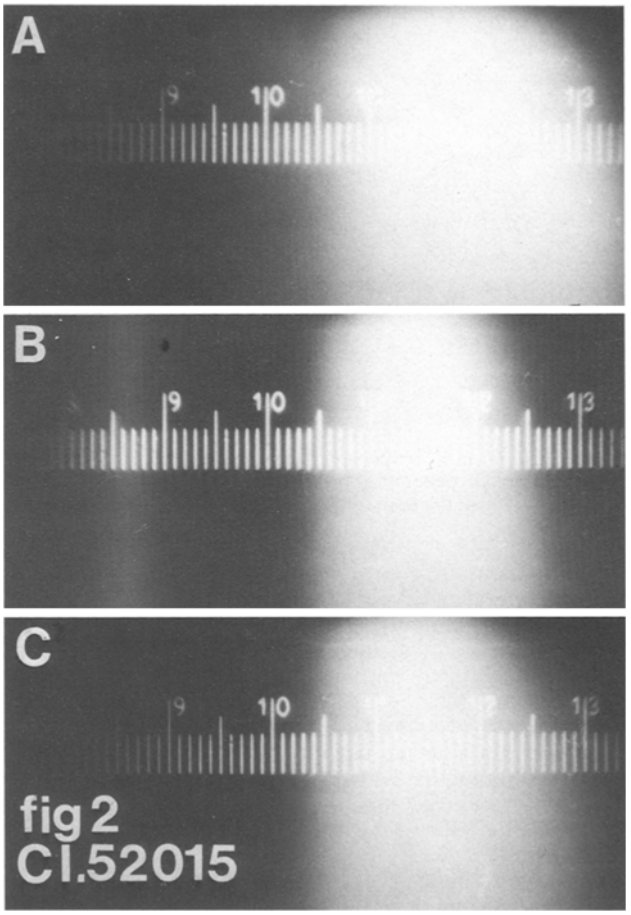

Figure 2. Spectra obtained from methylene blue: *A*, *B*, *C* as for figure 1.

was used, without standardization of its numerical scale by sodium light. The spectra were photographed using a Wild stereomicroscope. We have always studied and compared successively the spectra of the colored solution in the flask and the Ektachrome and the Kodachrome slides prepared as above. Results are shown in figures 1 and 2 for Ponceau de Xylidine and methylene blue.

For the red stains (Ponceau de Xylidine and acid fuchsin), the 3 spectra were exactly similar; no difference was observed between the 2 films. For methylene blue, the Ektachrome transparencies were seen to exhibit a strong additional red line (fig. 2, B). This abnormal line was not present on the spectrum of the Kodachrome slide; this last spectrum could be superimposed exactly upon that of the stain solution.

This simple and reliable technic gives permanent colored filters with spectra exactly similar to those of the stains.

1 Acknowledgments. Authors are greatly indebted to Dr J.C. Healy for his kindness in supplying the spectroscope and for useful advice and discussions and Mr H. Crawford for reviewing the manuscript.

2 Plastic flasks from: Greiner and Son, rue de l'Industrie, 67240 Bischwiller, France; or: Falcon, 1950 William Drive, Oxnard, California 93030, USA.

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0014-4754/83/040437-02$1.50 + 0.20/0

A method for semi-permanent temporary salivary gland chromosome squashes in Diptera[1]

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Summary. A method to preserve the temporary preparations of Dipteran salivary gland chromosomes during longer periods has been described. Departures from the usual procedures are: absence of heat treatment, destaining of cytoplasm with lactoacetic acid and the use of 'rubber cement' as the sealing material. This procedure preserves the banding details, ectopic pairing and clear background for exceptionally longer periods.

Since the time of Bridges[3], temporary squash preparation of salivary (polytene) gland chromosomes have been the most useful way to study the banding pattern of normal and rearranged chromosomes in *Drosophila, Sciara* and other Dipterans. Several improvements of this method[4-9] have been made to increase the resolution of the bands against the background, but no attempt has been reported, except one[10], to prolong the life of temporary squash