with uricase to determine unequivocally that the peak for uric acid was authentic.

The results of these tests confirm that this method could be used to quantify less than 1.0 ng of each of the purines in a variety of biological materials, including individual insect eggs that weighed about 100 µg. If uric acid is the sole compound of interest, 10 pg quantities can be measured using 292 nm rather than 254. However, this study revealed that for the 3 purines studied here, 254 nm is the best compromise wavelength. This method is used in our laboratory to quantify fecal matter production and protein utilization in entomophagous insects, but the extreme sensitivity and the speed and the simplicity of this procedure would recommend it for study of these purines in such minute samples as individual insect organs or even smaller tissue samples.

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2 Cochran, D.G., in: Insect biochemistry and function, p.225. Eds D.J. Candy and B.A. Kilby. 1975.
3 Briegel, H., Experientia *36* (1980) 1428.
4 Ryba, M., and Beranek, J., J. Chromat. *211* (1981) 337.
5 Gehrke, C.W., Kuo, K.C., and Zumwalt, R.W., J. Chromat. *188* (1980) 129.
6 Pachla, L.A., and Kissinger, P.T., Clin. Chem. *25* (1979) 129.
7 Brown, N.D., Kintzios, J.A., and Koetitz, S.E., J. Chromat. *177* (1979) 170.

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Simple procedure to isolate coelomocyte - free oocytes from coelomic fluid of *Perinereis cultrifera* Grübe (Annelida; Polychaeta)

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Summary. Based on a 1-g sedimentation through a Ficoll cushion, a simple and rapid method is described to obtain the complete separation of oocytes and coelomocytes from the coelomic fluid of *Perinereis cultrifera.* Light and electron microscope observations have shown that the 2 cell populations are obtained without any cross-contamination and are perfectly suitable for further biological and biochemical investigations.

In the study of oogenesis in Nereids, the functional relationships between coelomocytes and oocytes contained in the coelomic fluid have become an important issue.

Biochemical approaches to this problem require the separation of well-defined, pure populations of cells. Up to now, the common method to separate coelomocytes from oocytes was based on differential low-speed centrifugation. This approach never leads to a complete separation of the 2 cell types and the oocyte pellet always contains a small percentage of coelomocytes. Although this contamination does not bring large error in determining chemical composition of cellular material[2,3], it may become critical in screening the enzymatic properties of each cell population. This is particulary important to determine enzymatic markers related to the various cell types.

In the present work, we describe a simple and rapid method to isolate large quantities of oocytes completely devoid of coelomocytes from the coelomic fluid of *Perinereis cultrifera.*

Materials and methods. Perinereis cultrifera was collected from the beaches of 'Luc-sur-Mer' (Calvados, France) and the animals were kept in the laboratory in sea-water at 12 °C. The diameter of oocytes from each animals was determined by measuring several oocytes obtained by coelomic puncture[4]. Ficoll solution (Ficoll-400 Pharmacia Fine Chemicals, Sweden) was freshly prepared or kept at 4 °C for no longer than 2 weeks. Electron-microscopic observations were carried out as previously described[5].

Results. The method is based on a 1-g sedimentation of the coelomic contents through a cushion of Ficoll solution (see fig. 1) at 20 °C. The oocytes sediment while coelomocytes do not.

For oocytes larger than 130 µm in diameter, the procedure is as follows: the coelomic fluid from 1 animal is collected by puncture, diluted to 2 ml with filtered sea water and then layered above 5 ml of a 5% (w/v) Ficoll solution in sea water, in a conical tube (2 cm by 8 cm). The oocytes are allowed to settle for 5-10 min; the coelomocytes stay at the top.

In the case of smaller oocytes (less than 130 µm in diameter), the same procedure is applied but 0.1 M sodium phosphate pH 7.4 buffer containing 0.35 M NaCl is used instead of sea water. This phosphate buffer allows dissociation of the coelomocytes which aggregate when the coelomic fluid is taken out of the animal. The concentration of the Ficoll solution is 2.5% (w/v) in phosphate buffer.

After separation, the coelomocyte-containing supernatant (2 ml) is removed with a pasteur-pipette and the remaining medium is discarded, leaving the coelomocyte-free oocyte pellet. Ficoll is removed from the cell suspensions by low-speed centrifugation washings in sea water or phosphate buffer for large and small oocytes, respectively. Figure 2,a shows the content of the coelomic fluid after puncture. As revealed on figure 2,b and c, the 2 cell populations obtained after separation are devoid of contaminants.

Discussion and conclusion. The above described procedure provides a simple and rapid preparation of coelomocytes and oocytes from the coelomic fluid of *Perinereis cultrifera.*

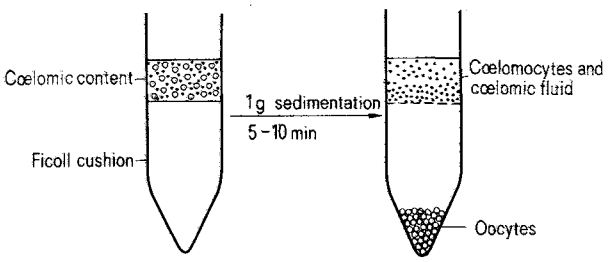

Figure 1. Schematic description of the procedure.

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.

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2 Porchet, M. and Spik, G., Comp. Biochem. Physiol. *59b* (1978) 175.
3 Porchet M., Spik, G., and Dhainaut, A., Biol. Cell *28* (1977) 225.
4 Durchon, M., Annls Sci. nat. *14* (1952) 119.
5 Dhainaut, A., and Porchet, M., Biol. Cell *28* (1977) 233.

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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