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Electron Probe Micro-X-ray Analyses of Electrolyte Composition of Fluid Microsamples by Use of a Sephadex Bead

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Obtaining samples of certain body fluids for determination of the ionic content can involve great difficulties if the available amount of fluid is small and difficult to reach by a micropipette. For instance, samples of uterine secretions of rodents can not be obtained without a serious risk of contamination by interstitial fluid or cell sap.

In order to circumvent these problems, we have developed a new technique where we use Sephadex beads as carriers of the fluid to be analyzed. In short, a hydrated Sephadex bead is inserted at the place for study and after an appropriate time for equlibration between fluid and bead, we have recovered the bead in a volatile, hydrophobe silicon oil and then prepared it for micro-X-ray analyses in Philips 400 electron microscope. This paper aims to give the details of the preparation technique used to obtain a qualitative information, the procedures for quantitative analyses will be reported later. We have taken the secretion of the mouse uterus as a model, but the technique is applicable also for analyses of other body fluids.

A few Sephadex G 150 beads (Pharmacia Fine Chemicals AB, Uppsala, Sweden) are soaked in bidestilled water for about 15 minutes. Six to 8 hydrated beads of sizes around 100 um are selected and aspirated in a glass capillary, connected by a plastic tubing to a micrometer-controled syringe (Shardlow Micrometers Ltd, Sheffield, England). The capillary is laboratory made with an inner diameter slightly larger than that of the beads. By the syringe, the beads are positioned close to the capillary mouth to minimize the amount of water transferred together with the beads. The transfer of the beads is performed in a way appropriate for the purpose of the analyses.

Equilibration between the body fluid and the water of the bead probably occurs within some hours, but this has to be tested for each application. When analysing the secretion of the mouse uterus, the X-ray spectra consequtively obtained displayed a similar configuration after a two-hour stay of the beads in the secretion.

The recovery of the beads are made by flushing with a hydrophobe volatile silicone oil (DC 200, Dow Corning International Ltd., Brussels, Belgium) collecting the beads in a watch-glass. With a preparation microscope, the beads are observed in the oil, and single beads are transferred onto specimen grids which have been submerged into the oil. The grid with the beads is slowly lifted out of the oil bath and left in room temperature for evaporation of the oil. The specimen grids used are nylon grids (Agar Aids, Stansted, England) mounted with a Formvar membrane. Grids prepared with Sephadex beads are carbon coated before the analyses.

Energy dispersive micro-X-ray analyses are performed with an Edax-Edith equipment attached to a Philips 400 electron microscope for 100 s at 40 kV with a fixed intensity and with a take off angle of 30°. The analysing area is slightly smaller than the circular projection of the spherical bead. Before the analyses, the preparations are checked and mapped with a scanning electron microscope (Figs. 1, 2).

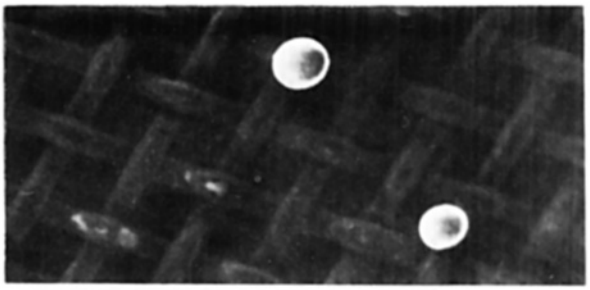

Fig. 1. Sephadex beads prepared on a nylon grid with a Formvar membrane support. X 900.

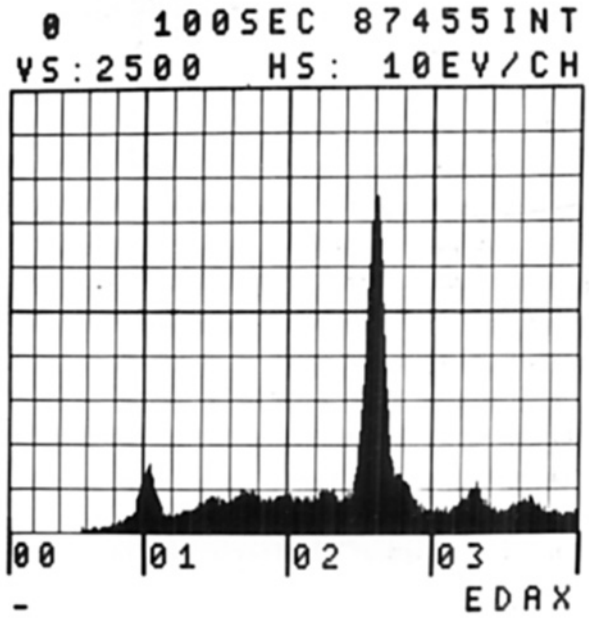

Fig. 2. A representative X-ray spectrum from a Sephadex bead which was recovered from the uterus of a mouse after a 3-hour stay in the secretion. The animal was in an experimental delay of implantation and had been injected with oestrogen 8 h before the preparation.

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