A reliable method for obtaining longitudinal sections of sperms for electron microscopy

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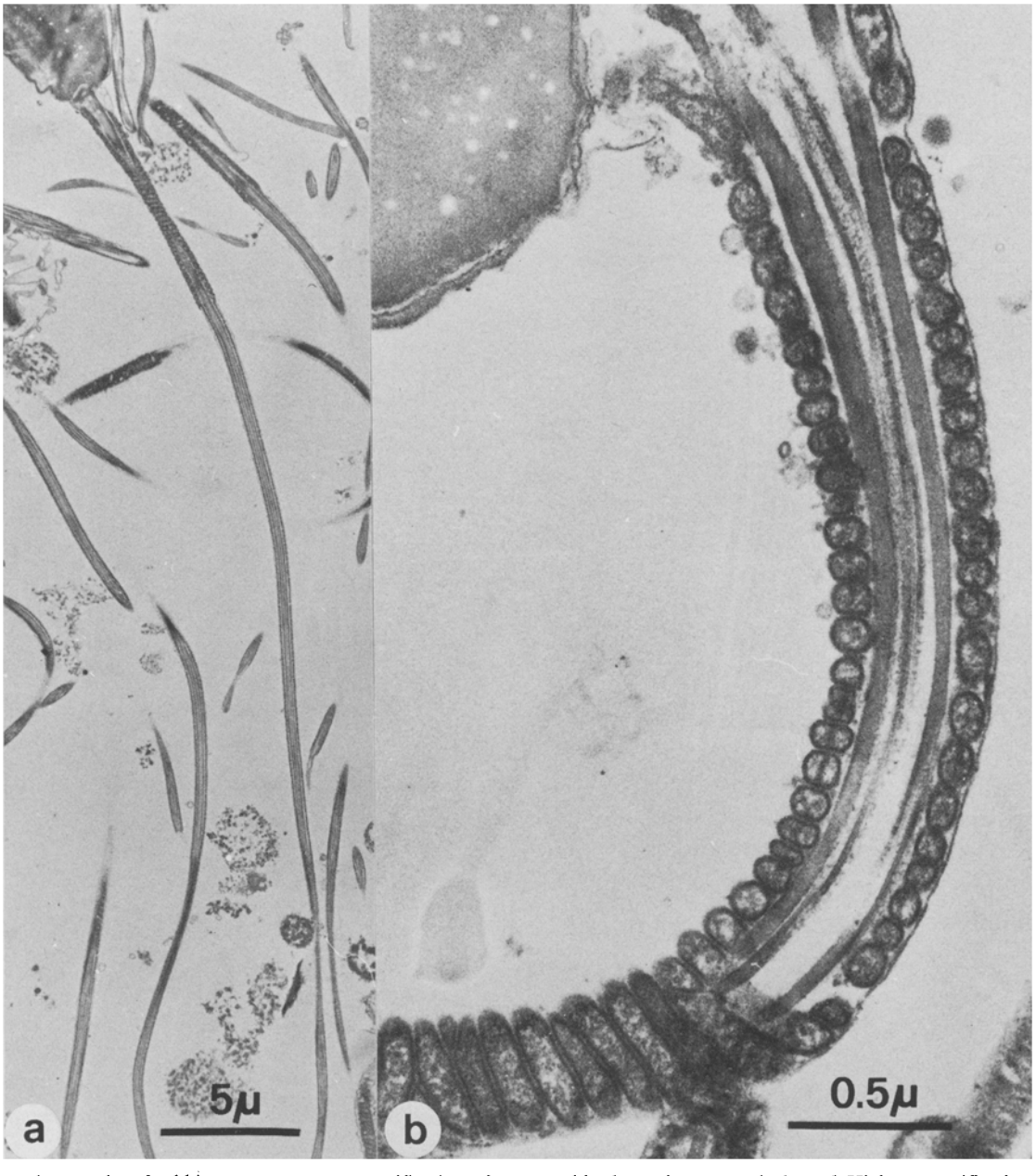
Summary. A method is presented by which it is possible to obtain a high percentage of longitudinal sections through sperms for electron microscopy. The procedure is described in detail in order to permit easy reproduction.

For morphological, morphometrical, ultrahistochemical and autoradiographical investigations of sperms, it is often desirable to have good longitudinal sections for electron microscopy. The preparation methods for sperms used up to now include pelleting and subsequent treating as normal tissue blocks[2], freezing followed by thawing into a fixative[3] or lyophilization[4], centrifugation onto agar[5], collection on filter paper[6]. The ultrathin sections resulting from these preparations present a wide variety of oblique and transverse profiles through random parts of the sperms, but only few truely longitudinal sections. Only the method of Phillips[7,8], using poly-L-lysine-coated coverslips, can overcome the problem of orientation.

In this note, another relatively straightforward method is presented, by which a large percentage of the sperms are sectioned longitudinally. To this end the sperms are forced



Electron micrographs of rabbit sperms. *a* Low magnification of a sperm hit almost in a central plane. *b* Higher magnification, showing good resolution of ultrastructural detail.

into one plane on the block face. The procedure is, step by step, the following:

1. Bake normal light microscope slides, strongly siliconized with Rhodorsil® (Rhône-Poulenc SA) or Silyl-8® (Pierce Chemical Comp., Rockford, Ill.) in an oven at 150 °C for 12–16 h.
2. Place the longer halves of number 1 gelatin capsules upright in a stand of plastilin and slightly overfill them with embedding medium. Not recommended is methacrylate, since it will shrink and evaporate considerably during polymerization. Rapidly turn the capsules upside down and place 6–8 of them on a siliconized slide. Weigh them down by making a 'sandwich' with another slide, held down with sticking tape. Polymerize thoroughly.
3. Remove the blocks from the slides by immersing just the slides into a shallow LN_2 bath. Do not submerge the whole blocks into the LN_2, since they will crack easily. Never touch with fingers the absolutely flat and clean surface thus produced.
4. For the following preparation, the sperms are kept in suspension in a 2-ml centrifuge tube; only for exchange of solutions they are spun down mildly for about 15 min with a tabletop centrifuge. a) Wash 3× with phosphate buffered saline (PBS), pH 7.4[9]. b) Fix for 30 min with 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer pH 7.4. c) Wash with PBS, 3×3 min. d) Postfix for 1 h with 1% (w/v) OsO_4 in 0.1 M cacodylate buffer. e) Wash with distilled water, at least 3×3 min. f) Dehydrate with 2,2-dimethoxypropane, 15 min[10]. g) Complete dehydration with acetone, 15 min (optional). h) Replace acetone successively by 30% and 70% (v/v) embedding medium in acetone, 1 h each, and 100% embedding medium, at least 1 h. i) Spin down until sperms are clearly sedimented. j) Resuspend in a small amount of a fresh batch of 100% embedding medium, yielding a high cell concentration. k) Place 3 small drops of this suspension on a siliconized slide. Squash them each with the flat surface of a previously polymerized block (from step 3). Place another slide on the blocks and fasten as in step 2. Several of these sandwiches are put under a lead block of approximately 2.5 kg. Leave overnight, then polymerize under weight in the oven at 60–70 °C. 1) Separate the blocks from the slides as in step 3.

It is now possible to trim the pyramid without inspection of the block face. For sectioning it should be kept in mind that, with sections of silver interference color, there is enough material in the block for only about 20 sections, and this only if the 1st section is not thicker than gold interference color. It is thus of importance to align very carefully the block face with the knife before sectioning.

The figure, a and b, shows that it is possible with the method described to obtain longitudinal sections of sperms permitting satisfactory representation of ultrastructural detail. The midpiece is normally hit in a more or less longitudinal direction (figure, b). We therefore believe that the method is particularly suitable for morphological, morphometrical and autoradiographical investigations of the midpiece region of sperms. The procedure is of course not restricted to sperms, but may be useful for the investigation of other objects for which sectioning in a defined plane is required.

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Penetration of cells membrane by the piezoelectric driver[1]

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Summary. For better penetration of elastic cell membranes, a simple piezoelectric device is described.

Investigation of intracellular space by electrophysiological methods is often difficult not only for its size, but also because of the elasticity and ridigity of the cells membrane. To overcome second obstacle, many authors are using beveled microelectrodes or stepping micridrive manipulators. To minimize the possibility of indentation, distortion or dimpling of the membrane at microelectrode tips prior to puncturing, we have used the vibrating microelectrodes. The extra fine vibration was achieved with the flexure responsive piezoelectric elements, known as Bimorphs (Vernitron, Piezoelectric Division, Bedford, Ohio). The advantage of using these piezoelectric crystals is that vibration frequency and amplitude of displacement is easily controlled by any audio or puls generator. The vibrating microelectrodes have been used in the past by several investigators, Chowdhury and Snell[2] (microelectrodes driven by piezoelectric device shaped into tube), Lassen et al.[3] and Prazma[4] (microelectrodes driven by Bimorphs beams). The major advantage of beams over the cylinder shaped crystals is larger displacement, controlled by polarization voltage. The displacement of Bimorph beam PZT-5 mounted as cautilever in nonresonant frequency is about 0.0254 mm/15.0 V and about 0.0245 mm/7.5 V for DC depoling. The best results were accomplished with beams arranged as shown in the diagram (figure). This arrangement was chosen to facilitate axial movement of the electrodes. 3 pairs of beams were mounted in cautilever position to the lucite or isolated brass ring at one end, and the other end (at the middle) was attached to the electrode holder (W.P. Instruments, Inc.), or to the simple microelectrode holder made from lucite rod with nylon screw on side for fastening of the microelectrode (figure, middle portion). During construction, attention was taken to orient and connect all of the beams in the same polarity to ensure