A simple and reliable procedure for preparing frozen sections of free mouse blastocysts

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Certain histochemical and radioautographic procedures require frozen tissue sections for maximum efficiency. The procurement of frozen sections of mammalian blastocysts has been limited to the sectioning of frozen uteri at the proper stage of pregnancy. When fresh or frozen thin mounts of preimplantation embryos have been needed, squash techniques of the whole embryos have been used (Prasad, Dass & Mohla, 1968; Sanyal & Meyer, 1969). This communication describes a method whereby free blastocysts can easily and quickly be embedded and cryostat sectioned in the absence of all organic solvents with minimum disruption of the embryos.

Groups of 25 Swiss-Webster mouse blastocysts were fixed in 2 ml neutral buffered formalin at 4°C for 1 hr. They were then transferred with a minimum of fixative into 1 ml 10% gelatin in a trans-illuminated watch glass warmed to 37°C. The watch glass was warmed by fitting it to the open end of a hollowed Perspex block through which water at 55°C was passed (Text-fig. 1). The

Text-fig. 1. Apparatus used to keep the gelatin solution warm for blastocyst transfer. Slowly flowing 55°C water is passed into (in) and out of (out) the hollowed chamber of the Perspex block (Pb), keeping the solution in the watch glass (wg) at a constant 37°C. The watch glass was held tightly to the open end of the water chamber by the watch glass clamp (wc) and a rubber 'O' ring (ro). $\times 0.75$.

transfer of the blastocysts was precisely controlled by means of a 0.2-ml micrometer syringe attached by plastic tubing to a melting-point capillary tube drawn out to a diameter of 150 μ m at one end. Subsequent location of the blastocysts in the blocks and sections was aided by the addition of a few grains of water-insoluble carmine red powder (Carmin-Alum Lake: Hartman-Leddon Company) to the 10% gelatin. Before transfer of the blastocysts, approximately 0.1 ml 10% gelatin without the carmine red was drawn into the transfer pipette to prevent the later expulsion of air bubbles. Then the blastocysts in the 10% gelatin and carmine red were injected and briefly stirred into a small drop of previously warmed 25% gelatin that was just beginning to solidify on a 1 $\times$ 3 in microscope slide. The drop of gelatin with the blastocysts was solidified by cooling the slide on an ice cube before quickly freezing it in a solid CO₂-isopentane slush.

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The frozen drops of gelatin containing the embryos were trimmed to 3-mm² blocks. After carefully removing these blocks from the slides, they were frozen to cryostat specimen holders with a small amount of cryostat embedding matrix. Serial sections were cut at 8 µm from the blocks with a cryostat (International Equipment Company) and mounted on acid-cleaned 22-mm² cover slips. The cover slips were immediately placed on a 35°C slide-warming tray to dry and then soaked in distilled water for 5 min to remove the gelatin matrix, thus minimizing background staining. The soaking does not remove the blastocyst sections from the cover slips as long as the latter are very clean.

The technique is not difficult or time consuming. Each group of up to 25 blastocysts takes from 15 to 30 min to embed and much of the time is spent concentrating the embryos and carmine red grains at each transfer. The most difficult aspect is transferring the blastocysts into the 25% gelatin drop before it gels.

Fixation is not absolutely necessary in preparing the blastocysts for sectioning. However, the unfixed blastocyst is more likely to collapse away from the zona pellucida with the subsequent loss of morphological detail. The presence or absence of fixation has no effect on the ease of sectioning.

Most embedding and sectioning methods entail a high probability of the loss of small tissues such as isolated blastocysts. To circumvent this problem various procedures have been described (Metz, 1921; Samuel, 1942; Mintz, 1971). However, all have been applied to paraffin wax-embedding techniques and are not applicable when frozen sections are required since the tissues have no supporting matrix until embedded. In the method described here, not only can frozen sections be made without organic solvents and time-consuming rinses, but the problem of loss is kept to a minimum by handling only a few embryos at a time in small quantities of solutions and by using the carmine red powder as a marker. The problem of inadequate support has been overcome by the use of 25% gelatin. The 25% gelatin solution is well suited to this application. Besides providing the necessary support, it is workably fluid at 37°C and sections nicely at -15°C.

The procedure has useful applications whenever sections are needed of free embryos without the use of any organic solvents or extended periods in aqueous solutions. Such applications include studies on lipids, steroids, and prostaglandins, certain histochemical reactions, and any radioautographic localization studies in which translocation of the label may be a problem. Although only the use of blastocysts is described here, the technique is equally applicable to embryos from the one-cell to the blastocyst stages.

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