Cryostat Sectioning of Formalin-Fixed Brain: Further Attempt to Improve Section Quality by Previous Infiltration with O.C.T. Compound

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We previously reported that in non-brain and formalin-fixed specimens, one can obtain frozen sections of markedly improved quality not only by supporting specimens with O.C.T. compound as usually done, but by infiltrating them with the medium prior to embedding. In the present paper we show that this technic also proved to be quite effective in the cryosectioning of brain tissues, which so far has been difficult because of the liability of sections to shattering, particularly when one attempts to obtain a thick section. The present paper also discusses about the reason why sectioning is improved by the procedure, and aims to establish the optimal temperature for cryostat sectioning in relation to section thickness.

histological technics; frozen sections; formalin-fixation; water-soluble embedding media; brain tissues

O.C.T. compound (OCT; Tissue Tek Products, Ames Division, Miles Laboratories, Inc., Elkhart, IN, USA) is a water-soluble medium widely used to support tissue specimens in cryostat sectioning. Previously, we reported that in preparing frozen sections from formalin-fixed materials such as the liver, spleen and adipose tissue, far better results are obtained if specimens, instead of being merely surrounded by OCT as usually done, are infiltrated with the medium prior to embedding (Ishii et al. 1990). Recently, we found that the procedure, as applied under an optimal temperature for cryostat sectioning, is also fruitful in frozen sectioning from formalin-fixed brain, whose thick sections are quite liable to shatter. In this paper we report the optimal conditions for cryosectioning of brain and, in addition, discuss about the technical background for applying
water-soluble media to embedding formalin-fixed tissue specimens.

**Procedure and Results**

1) Take specimens about 5 mm thick from formalin-fixed human brain, wash in running water, rinse in distilled water and infiltrate with undiluted OCT, after slightly wiping the specimen with filter paper. A volume of OCT 15 to 30

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Fig. 1. Human cerebellum. 10 μm thick section, Bodian stain, 220x.

Fig. 2. Human cerebellum. 10 μm thick section, Bodian stain, 220x.

Fig. 3. Human cerebrum. 10 μm thick section, Klüver-Barrera stain, 220x.
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Times that of specimen is preferable. Using wide-necked stoppered bottles (I, II and III) is convenient. Sufficient infiltration is reached after changing the medium three times (5, 4, and 15 hr at room temperature). Infiltration appears to be better if bottles, particularly the first two ones, are moved well from time to time. The longer the infiltration (within several days), the easier the cutting, although the bathing can be shortened for small specimens.

After several uses, renew the OCT: Abandon Bottle I together with its medium, put the label of I to Bottle II, that of II to III and that of III to a new bottle containing fresh OCT, respectively.

2) Place an OCT-infiltrated specimen in an appropriate mold containing fresh OCT, and keep in a -80°C freezer for hardening.

3) The optimal cutting temperature for cryostat sectioning is -5 to -6°C for sections thicker than 15 μm. The thinner the sections desired, the lower the temperature required (see Discussion).

Figs. 1, 2 and 3 are micrographs of frozen sections from human brain specimens infiltrated with OCT. The tissue structure is splendidly preserved. Fig. 3 presents a section of cerebrum stained with Klüver-Barrera method; one can see the staining quality of sections not having been affected by the procedure.

DISCUSSION

The tissue infiltration with OCT, we previously applied to non-brain specimens, also proved to help prepare quality frozen sections of brain. This is significant because otherwise, frozen sections of brain are quite liable to shatter, particularly when one attempts to obtain a thick section. We consider that the high quality of sections from OCT-infiltrated tissue is attributable, first of all, to the presence of the medium diffused in the tissue, which, by hardening at a low temperature, supports the tissue constituents during sectioning. This essentially differs from the usual technic of cryosectioning where a specimen is hardened by freezing of water in the tissue, while OCT is used only as a medium bracing the specimen from outside.

However, OCT infiltration does not ensure good results unless several requirements are satisfied. In dealing with a cerebral tissue rich in cortex, 5 μm thick sections were cut without shattering at a temperature of -15°C. With increasing thickness, coherent sections became more and more difficult to obtain, and 30 μm thick sections almost always shatter at this temperature. At -10°C, sections are coherent at a thickness of 10 μm (Fig. 4b), while at 30 μm they shatter and at 5 μm shrink, respectively. On the other hand, a cryostat temperature of -5 to -6°C ensures to obtain quality sections thicker than 15 μm (Ishii et al. 1991). Thus, one is required to change the hardness of OCT block by controlling the cryostat temperature according to section thickness. This is quite similar to the well-known maneuver for the sectioning of paraffin blocks where the temperature has also to be controlled in various ways. In this sense, the trade name “Opti-
mum Cutting Temperature (O.C.T.)” compound is very apt.

In contrast to the cortex, the medullary substance of the cerebrum is indifferent in this respect. No matter whether infiltrated with OCT or not, it allows one to obtain coherent sections (Fig. 4a).

Previously we assumed that we can expect more or less the same effect of infiltration for water-soluble compounds other than OCT so long as they may assist cryostat sectioning (Ishii et al. 1990). This assumption was based on our unpublished experience using polyethylene glycol 600. Although this water-soluble compound, as used without any additive, proved to be inferior to OCT as an embedding medium for cryostat sectioning in many respects, there was one advantage: its infiltration made it possible to cut thick frozen sections of far better quality than from tissue specimens surrounded, but not infiltrated, with OCT (Ishii et al. 1991). In an attempt to verify this, Konno (personal communication 1990) obtained excellent frozen sections of brain tissue by infiltrating with a new, less viscous water-soluble compound “Tissu Mount® (manufactured by
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Chiba Medical Co., Ltd., Sohka, Saitama; available through Shiraimatsu & Co., Ltd., Hongo, Tokyo); the result was comparable to the OCT infiltration we presented above.

If, in the OCT infiltration, a tissue block has to be stocked for future use, one can keep it in a freezer after coating the cut surface with OCT.

CONCLUSION

In order to obtain frozen sections of good quality from formalin-fixed brain tissue we recommend once again:

1) to use OCT (or other water-soluble embedding media for assisting cryostat sectioning) not only to surround the specimens and bind them to the cryostat chuck, but also, before that, to infiltrate them with OCT, and

2) to adjust the cryostat temperature to that optimal for cutting the embedding medium.

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References
