Storage of frozen sections in paraffin blocks

E. K. AVES, G. R. N. JONES, and G. J. CUNNINGHAM
*From the Department of Pathology, Royal College
 of Surgeons of England, London*

The development of the techniques for cutting frozen sections, in particular the use of cryostat microtomes (Pearse, 1960; Cunningham, Bitensky, Chayen, and Silcox, 1960 and 1962; Goodbody, 1963 a and b; Cunningham, 1963), has resulted in sections which not only are produced routinely very rapidly but which are completely acceptable histologically. Such sections would be ideal, especially where speed is important, were it not for the problems of storing and recutting the frozen tissue should it become necessary to refer to this material at a later time. The present communication describes a method by which frozen tissue, which has been used for cryostat microtomy, can be fixed and embedded in paraffin wax for indefinite storage.

MATERIALS AND METHODS

Unfixed human tissue, removed at operation and frozen, has been studied: normal breast tissue; breast and lymph node infiltrated by carcinoma; normal stomach; carcinoma of the stomach. Frozen specimens of spleen, kidney, pancreas, and liver of rats have also been investigated.

The tissue was frozen by the method of Cunningham *et al.* (1960, 1962) and sections were cut at 8μ , by the use of a refrigerated microtome. The blocks of frozen tissue were removed from the object-holder and placed at $+4^{\circ}\text{C}$. for one hour. They were then immediately immersed in formal-saline (10% formalin in 0.85% aqueous sodium chloride solution) at room temperature for at least 24 hours. The fixed tissue was then dehydrated, cleared, and embedded either by hand or by the use of an Elliott automatic processor; this procedure did not appear to be critical. Sections were cut at 5μ and, after the wax was removed, they were stained with haematoxylin and eosin.

RESULTS AND DISCUSSION

For each specimen, the results obtained after this whole treatment (Figs. 1, 2, 3) were in every way comparable with those observed with similar tissues which had been fixed directly in formalin, without prior freezing. The complete lack of any sign of damage or altered response to the stains was surprising since other techniques involving simultaneous thawing and fixing of tissue had resulted in gross artefacts. Indeed, it was found that immersing the frozen blocks of tissue in warm formalin-saline at various temperatures, in an attempt to achieve very rapid thawing, proved disastrous to the tissues.

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FIG. 1. *Frozen carcinoma of breast. Haematoxylin and eosin, $\times 100$.*

FIG. 2. *Breast cut after conversion of frozen tissue to paraffin block. Haematoxylin and eosin, $\times 100$.*

FIG. 3. Carcinoma of breast infiltrating into lymph node, cut after conversion of frozen tissue to paraffin block. Haematoxylin and eosin, $\times 300$.

The fact that excellent sections can be easily prepared by the cryostat should do much to encourage the extended use of this method in the routine laboratory. The ability to treat such frozen tissue so that it can be stored and therefore available for future paraffin sectioning should assist in making 'frozen histology' more generally available.

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REFERENCES

- Cunningham, G. J. (1963). *Brit. med. J.*, **2**, 562.
 —, Bitensky, L., Chayen, J., and Silcox, A. A. (1960). First international Congress of Histochemistry and Cytochemistry, Abstracts, p. 5. Pergamon Press, London.
 —, —, — (1962). *Ann. Histochim.*, **6**, 433.
 Goodbody, R. A. (1963a). *Brit. med. J.*, **2**, 53.
 — (1963b). *Ibid.*, **2**, 869.
 Pearse, A. G. E. (1960). *Histochemistry: Theoretical and Applied*, 2nd ed. Churchill, London.

Simple method for the estimation of platelet adhesiveness

E. A. CASPARY *From the Medical Research Council Research Group on Demyelinating Diseases, Royal Victoria Infirmary and University of Newcastle*

Current means of estimating the adhesiveness of blood platelets either utilize special equipment, such as standard glassware (Wright, 1941), complex motor-driven constant flow devices (Hellem, 1960), or else they do not define a constant contact area of glass (Moolten and Vroman, 1949; Hellem, 1960). The method described here is both simple and reliable.

Blood is taken by clean venepuncture from an antecubital vein into sequestrene in plastic containers to give a final concentration of 4.5 mM. This anticoagulant though it reduces the overall adhesiveness (Marx and Derlath, 1957), prevents non-specific clumping and preserves platelet morphology. With heparin results were at times difficult to assess owing to marked platelet clumping. The platelet-free plasma is prepared by centrifugation at 2,000 *g* for 20 minutes. Siliconed bottles are first thoroughly cleaned in 'chromic acid,' washed in tap water followed by distilled water. After oven drying they are given three coats of Repelcote (Hopkin and Williams Ltd.). Cover slips are cleaned in concentrated nitric acid washed clean of acid, and polished with alcohol.

The test is done by mixing 1.0 ml. of blood and 0.5 ml. of platelet-free plasma in each of two siliconed bottles inserting a cover slip into one and shaking both bottles for 90 minutes, either on a reciprocating shaker or a rotating Matburn mixer. Platelet counts are taken both at the end of this period and adhesiveness calculated from the ratio of the control and experimental platelet counts. Ten of the 16 squares of a Neubauer chamber are counted on each side to give an overall cell count of 800 to 1,000 in cases of thrombocytopenia additional counting chambers are used to bring the overall number of cells counted into the stated range. No attempt was made to relate the adhesiveness to the initial blood platelet count as even shaking or mixing in coated bottles for this period of time can cause a drop in platelet count of 10% (Wright 1941) and even greater platelet losses occur with reciprocating mixers. The results obtained for a series of 12 normal individuals, ranging in age from 20 to 49, varied between 91 and 78 (expressed as the percentage of control count). Standard deviation of replicate determinations was 2%.

¹The time of 90 minutes was chosen because adhesion appears to be maximal around that period; however, results can be obtained in 40 minutes or an hour.

REFERENCES

- Hellem, A. J. (1960). *The Adhesiveness of Human Blood Platelets in Vitro*. Oslo University Press.
 Marx, R., and Derlath, S. (1957). *Blut*, **3**, 247.
 Moolten, S. E., and Vroman, L. (1949). *Amer. J. clin. Path.*, **19**, 701.
 Wright, H. P. (1941). *J. Path. Bact.*, **53**, 255.

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