The application of lipid-soluble stains in plastic-embedded sections

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Summary. The present study was designed to develop a routine method for direct demonstration and precise localization of lipid substances in tissue sections. A panel of lipid-rich tissues was fixed in 4% buffered formaldehyde, infiltrated, and embedded in the water-soluble plastics Technovit 7100, EFL-67, and JB-4. The use of alcohol containing fluids was avoided. Staining with the lipid-soluble dyes Sudan Black B and Oil Red O revealed excellent preservation of tissue lipids in Technovit 7100 embedded sections when compared with cryostat sections of the same tissue specimens. Lipid preservation in EFL-67 and JB-4 embedded sections was inconsistent, even when infiltration and polymerization procedures were performed at 4°C. Combination of lipid-soluble dyes with the periodic acid Schiff, Jones’ methenamine silver, or Gomori’s reticulin method allowed for an exact localization of lipids in high quality Technovit 7100 embedded sections. The procedure herein is easily applicable in routine histopathology practice.

Introduction

The first stage in tissue processing of fixed material in routine histopathology practice involves the removal of aqueous tissue fluids by a variety of components, many of which are alcohols of varying types (Drury and Wallington 1980; Bancroft and Stevens 1982). In this dehydration process the alcohol soluble tissue lipids are extracted. Thus, in routine paraffin and plastic sections (Ashley and Feder 1966; Rowden et al. 1982) lipids can only be recognized indirectly by the presence of optical lucent areas or vacuoles in which the presence of other tissue constituents such as mucus or glycogen is excluded by additional special stains. For direct demonstration of lipids, cryostat sections of fresh or formalin fixed material with a lower quality of morphology are required. The need for direct localization of lipids in tissue sections prompted us to apply water-soluble plastics as the solidifying medium with avoidance of alcohol containing fluids. Our method allows for the exact direct localization of tissue lipids in high quality plastic sections and is easily applicable in routine histopathology practice.

Material and methods

Tissue processing. Small tissue specimens (4 × 4 × 2 mm) were obtained from atherosclerotic vessels, fatty liver, adrenal cortex, end-stage kidney, renal adenocarcinoma, and liposarcoma. They were fixed by immersion for 12 h to 4 weeks in 4% phosphate buffered formalin at room temperature. Three types of plastics were tested: JB-4 (Polysciences, Inc., Warrington, PA, USA) (Brinn and Pickett 1979; Horton et al. 1980; Poppema 1983), EFL-67 (Serva Feinbiochemica, Heidelberg, Germany) (Nemetschek-Gansler et al. 1972) and Technovit 7100 (Kulzer & Co, GmbH, D-6393 Weinheim, Germany) (Gerrits and Smit 1983). After rinsing in phosphate-buffered saline for 2 h the tissue was infiltrated during a period of 2 h in two changes of 100% catalysed plastic. Infiltration in Technovit 7100 was performed at room temperature, in EFL-67 and JB-4 at 4°C. During this procedure the material was gently agitated in a vertical rotary motion, using a commercial mechanical device (GFL, Gesellschaft für Labortechnik mbH, D-3006 Burgwedel, FR Germany). Specimens processed in Technovit 7100 were embedded in a mixture of 10 parts catalyzed Technovit 7100 infiltration solution (consisting of 40 ml Technovit 7100 (glycol methacrylate, containing Co catalyst XCL), 4 ml of polyethylene glycol 400, and 0.5 g of benzoyl peroxide (moistened with 20% H2O)) and 1 part Technovit 7100 hardener II (the accelerator solution, containing a barbituric acid derivate) (Gerrits and Smit 1983). Tissues processed in EFL-67 were embedded in a mixture of 20 parts catalyzed EFL-67 solution A (consisting of 80 ml glycol methacrylate stabilized with hydroquinone, 16 ml 2-butoxethanol, and 0.5 g benzoyl peroxide) and 1 part of the accelerator EFL-67 solution B (consisting of 15 ml polyethylene glycol – carbowax 200 or 400 – and 1 ml of N,N-dimethylaniline) (EFL-67 Embedding Kit TM 1976 Data sheet 123, Polysciences Inc.). Paraffin was poured around the blockholders placed in EFL-67 and JB-4, because the polymerization of these plastics is more susceptible to inhibition by oxygen than in the case of Technovit 7100 (Gerrits and Zuideveld 1983). Polymerization of Technovit 7100 was accomplished at room temperature in 3 h, of JB-4 and EFL-67 at 4°C in 3 h. Sections were cut at 2 µm on a Sorvall microtome with glass knives, stretched on a waterbath (23°C), transferred to slides, and dried at room temperature. To check tissue lipid preservation in the plastic sections, 2 µm cryostat sections from the same tissue samples were used.

Staining methods. Tissue lipids were demonstrated with the Oil Red O or Sudan Black B method. For staining with Oil Red O the sections were briefly rinsed in 60% isopropanol and stained...
Fig. 1. Hyalinized glomerulus of an end-stage kidney embedded in Technovit 7100 and stained with Oil Red O and methenamine silver. × 500

for 3 min in an Oil Red O solution which was prepared 15 min in advance and filtered just before use. The staining solution contained 4 ml stock solution (i.e. 0.5% Oil Red O in 99% isopropanol) and 6 ml of distilled water. After staining the sections were differentiated in 60% isopropanol until a negative control section appeared colourless, washed in water, counterstained for 5 min with Mayer's haematoxyline, and blued in running tap water for 3 to 5 min. The sections were mounted in glycerin-gelatine (Bancroft and Stevens 1982). For staining with Sudan Black B the sections were briefly rinsed in 70% ethanol and stained in a filtered Sudan Black B solution containing 0.25% Sudan Black B in 70% ethanol. The sections were differentiated in 70% ethanol until a negative control section appears essentially colourless, and washed in destilled water. Counterstaining was performed using 1% neutral red for 5 min and the sections were mounted in glycerin-gelatine. To facilitate detailed light microscopic examination the sections were additionally stained with periodic acid Schiff, Jones' methenamine silver stain for basement membranes, or Gomori's reticulin (Drury and Wallington 1980; Bancroft and Stevens 1982) prior to lipid staining with slight modifications to avoid tissue lipid extraction. The commonly applied alcohol rinse and xylene clearing at the end of the staining procedures were omitted and replaced by careful rinsing with destilled water. The sections were mounted in glycerin-gelatine.

Results

High-quality sections were invariably obtained using our embedding schedule. No differences between JB-4, EFL-67, and Technovit were noted. Tissue lipids were well-preserved in Technovit 7100 and lipid deposits of varying sizes up to very fine grains could be demonstrated and precisely localized with the Oil Red O or Sudan Black B method. Comparison of the plastic sections with cryostat sections did not reveal changes in localization and distribution of lipid substances during tissue processing. Fixation in 4% phosphate buffered formalin for 12 to 24 h produced optimal results. However, longer fixation times up to one month apparently did not affect lipid preservation or quality of the sections. In EFL-67 and JB-4 embedded tissues lipid preservation was inconsistent, even after careful tissue processing at 4°C. Lipid deposits in these sections showed clumping and dissolution to varying extent.

The use of additional staining procedures such as periodic Schiff, reticulin, and methenamine silver considerably improved detailed light microscopic examination of the tissue specimens without detectable tissue extraction of lipids. The Fig. 1 shows a tissue specimen of an end-stage kidney with a hyalinized glomerulus embedded in Technovit 7100 at room temperature and stained with methenamine silver and Oil Red O.

Discussion

In 1979 Brinn and Pickett described a method of preparing surgical pathology bone marrow, lymph node, and kidney biopsies for glycol methacrylate embedding. Cold fixation in 4% buffered formalin followed by dehydration, filtration and embedding in cold glycol methacrylate permitted the demonstration of enzymes and tissue antigens by means of special stains and histochemistry. A comparable glycol methacrylate embedding technique was described by Horton et al. (1980). In the present report this approach was applied for the demonstration of tissue lipid substances in plastic sections. Sections obtained from a variety of tissues embedded in Technovit 7100 provided excellent morphologic detail in the presence of well-preserved tissue lipids as judged from comparison with cryostat sections of the same tissue specimens. However, Technovit 7100 contains glycol methacrylate as a main component and monomers of glycol methacrylate are known as potent phospholipid solvents as demonstrated by Cope and Williams (1969a