Topics in pharmacology and biomedical science

Health & Medicine

Choice of Fixatives Background Fixation is a chemical process through which the biological tissues are preserved from decaying , thus preventing autolysis and putrefaction. Fixation helps to terminate any ongoing biological reaction and also increases the mechanical strength or stability of the treated tissues. Normally this fixative works such that the structures are preserved in a state both chemically and structurally and as close to the living tissues as far as practicable. Thus the nature of the chemical fixative should be such that it can stabilize the proteins, nucleic acid and the muco-polysaccharides of the tissues by making them insoluble.(Carson, 2009) Types of Chemical fixations There are generally various types of chemical fixation process: a. Cross linking fixatives: They act by creating chemical bonds between proteins in the tissue, this anchors soluble proteins to the cytoskeleton and thus conforming additional rigidity to the tissues. The commonest is formalin/formaldehyde in buffer solution which fixes the tissue by cross-linking the lysine residues of the protein. It’s effects are reversed by water and avoids formalin pigmentation and can be used in long term storage of the tissue. Glutaraldehyde is another one and is a large molecule so its rate of diffusion across the membrane is slower than formalin. The use can be made by making the sample thin but the main advantage is that it can link distantly placed proteins in a cell as it has two aldehyde groups. It provides best cytoplasmic and nuclear detail and is best suited for electron microscopic analysis. It cannot be used in immunohistochemistry staining. (Carson, 2009) b. Precipitating fixatives: like alcohols act by reducing the solubility of the protein molecules and disrupts the hydrophobic bonds that is essential for the tertiary structure of the proteins. Thus the alcohols precipitates proteins. Common examples are ethanol and methanol. They

are used to preserve blood films but cannot be used to preserve the nuclear and the cytoplasmic details. They are used to preserve glycogen. (Carson, 2009) Acetone: Is used like ethanol and methanol but is used as fixative and dehydrating of hand processed tissues. It is highly lipid soluble but can make the tissues very brittle. (Carson, 2009) c. Frozen Sections –tissues are kept in cryo-preservative and in liquid nitrogen. (Carson, 2009) Case study 1: Whole rat heart for non-fluorescent antibody based immunohistochemistry of a soluble cytoplasmic protein. Formalin: fixes the tissue by cross-linking the lysine residues of the protein. It's effects are reversed by water and avoids formalin pigmentation and can be used in long term storage of the tissue may not link distant proteins and not precipitate Glutaraldehyde: Cannot be used to penetrate the whole rat heart as the tissue will be thick but making it thin will able to link distant protein residues Methanol: Will be used to precipitate the proteins but the cytoplasm will be distorted. Frozen Sections – tissues are kept in cryo-preservative and in liquid nitrogen and maintain the integrity of the tissue and will be used for this case.(Bezolla, 1992) Case study 2: Growth plate cartilage for fluorescent based cell volume analysis Methanol will be best suited because there is no necessity to preserve the cytoplasmic or nuclear details and at the same time they will precipitate the proteins and preserve the glycogen for analysis of cell volume.(Eltoum, 20010(Lowe, 1996) Formalin and Glutaraldehyde cannot be used in immunohistochemistry analysis Frozen Sections will cause dehydration and compromise the cell volume Acetone will also cause the dehydration. Case study 3: Fibroblast monolayer for standard colorimetric (non-fluorescent) histology. Glutaraldehyde is the fixative of choice as it can link the distant proteins in the fibroblast monolayer but methanol will lead to cytoplasmic

distortion and compromise colorimetric detection but frozen sections can be used. Case study 4: Human kidney to be bisected and used as an anatomical demonstration Model Formalin and Glutaraldehyde would be ideally suited to keep the integrity of the tissue more than acetone or methanol which will make the tissue brittle and frozen sections can be used because there is no quantitative analysis of the cell volume. (Carson, 2009) Reference Bozzola JJ, Russell LD.(1992) Electron microscopy: principles and techniques for biologists. Boston: Jones and Bartlett Carson, Freida L; Christa Hladik (2009). Histotechnology: A Self-Instructional Text (3 ed.). Hong Kong: American Society for Clinical Pathology Eltoum I, Fredenburgh J, Myers RB, Grizzle WE. (2001) Introduction to the theory and practice of fixation of tissues. J Histotechnol; 24; 173 -190. Eltoum I, Fredenburgh J, Grizzle WE.(2001) Advanced concepts in fixation: 1. Effects of fixation on immunohistochemistry, reversibility of fixation and recovery of proteins, nucleic acids, and other molecules from fixed and processed tissues. 2. Developmental methods of fixation. J Histotechnol; 24; 201-210. Lowe J. (1996) Techniques in neuropathology. In Bancroft JD and Stevens A eds. Theory and practice of histological techniques. New York: Churchill Livingstone, 1996; 373.