

This chapter discusses the basics of fixation, alongside the advantages and disadvantages of specific fixatives. The advent of new biological methods, increased understanding of the human genome and the need to rapidly evaluate the biology of disease processes means that fixatives should also permit the recovery of macromolecules including proteins, mRNA, and DNA from fixed and paraffin-embedded tissues without extensive biochemical modifications. Almost any method of fixation induces shrinkage or swelling, hardening of tissues and color variations in various histochemical stains (Sheehan & Hrapchak, 1980; Horobin, 1982; Fox et al., 1985; Carson, 1990; Kiernan, 1999; O'Leary & Mason, 2004). Minimizing the loss of cellular components which include large proteins, small peptides, mRNA, DNA and lipids, prevents the destruction of macromolecular structures such as cytoplasmic membranes, smooth endoplasmic reticulum, rough endoplasmic reticulum, nuclear membranes, lysosomes and mitochondria. In order to visualize the microanatomy of stained tissue sections, the original microscopic relationships between cells, cellular components (e.g. the cytoplasm and nuclei) and the extracellular material must be maintained with little disruption to the organization of the tissue. Fixation interacts with all phases of processing and staining from dehydration to staining of tissue sections using histochemical, enzymatic or immunohistochemical stains (Eltoum et al., 2001b; Rait et al., 2004). If soluble components are lost from the cytoplasm of cells, the color of the cytoplasm on hematoxylin and eosin (H&E) staining will be reduced or modified and aspects of the appearance of the microanatomy of the tissue, e.g. mitochondria, will be lost or damaged. The fixative must have the ability to prevent short and long term destruction of the micro-architecture of the tissue by stopping the activity of catabolic enzymes and hence autolysis, minimizing the diffusion of soluble molecules from their original locations. These include molecular changes or losses from 'fixed' tissues, swelling or shrinkage of tissues, variations in the quality of histochemical and immunohistochemical staining, the effect on biochemical analysis and the ability to maintain the structure of cellular organelles. Many tissue components are soluble in aqueous acid or other liquid environments and to reliably view the microanatomy and microenvironment of these tissues the soluble components must not be lost during fixation and tissue processing. Similarly, the analysis of mRNA and DNA from formalin-fixed, paraffin-embedded tissue has been problematic (Grizzle et al., 2001; Jewell et al., 2002; Steg et al., 2006; Lykidis et al., 2007). The chosen fixative acts by minimizing the loss or enzymatic destruction of cellular and extracellular molecules, maintaining macromolecular structures and protecting tissues from destruction by microorganisms. A fixative not only interacts initially with the tissue in its aqueous environment but it also has ongoing reactivity with any unreacted fixative and the chemically altered tissues. The most important characteristic of a fixative is to support high quality and consistent staining with H&E, both initially and after storage of the paraffin blocks for at least a decade, although new guidelines within the United Kingdom recommend that paraffin processed blocks are now kept for 30 years. It follows that any stained tissue section, produced after specific fixation combined with tissue processing, is a compromise of fixed tissue changes formed from the natural living tissue. An important constraint in using formaldehyde has been the loss of antigen immunorecognition due to this type of fixation combined with processing the tissue to paraffin wax (Eltoum et al., 2001a, 2001b).