Special Fixatives

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Why do we use tissue fixatives in pathology?

The main reason we use a fixative for pathologic specimens is to stabilize tissue to prevent it from rotting or autolyzing after it is received, which is what would happen if it were stored unfixed. However there are several additional functions of fixatives in the pathology laboratory that are also important. Tissue fixation helps preserve microscopic structure through subsequent processing and staining procedures, etc, so we can make diagnoses with a microscope. It also stabilizes antigens, so they do not diffuse away from their site of localization during life. There are two main mechanisms of action of fixatives. Generally they either cross-link proteins, or they coagulate proteins. Let's look at a few common fixatives.

Formaldehyde, the all-purpose fixative

Formaldehyde works chemically by forming chemical cross-links between proteins, facilitated by the aldehyde bond in formaldehyde, that stabilize the proteins and keep them from being autodigested or broken down. Formaldehyde is actually a gas that is dissolved in water. The maximum amount of formaldedhyde that can be dissolved in water is about 37% (by weight), so what is sold as concentrated formaldehyde solution is 37% formaldehyde. The working concentration of formaldehyde is 10% formalin (meaning 1:10 dilution of the concentrate), or about 4% formaldehyde. This formalin solution is buffered, to try and maintain a near neutral pH. If formalin is not buffered, or if the buffering capacity is overwhelmed (which most typically happens in very bloody specimens), the formaldehyde may be transformed into formic acid, which then reacts with hemoglobin to produce a dark brown to black spicular precipitate in the tissue known as formal-hemoglobin or formalin-hematin pigment.

Formaldehyde/formalin is a good all-purpose fixative that works for most types of tissues and most special stains. Formalin penetrates most tissues at about the rate of 1 mm per hour on average, although this will be slower in very fatty or very bloody tissues. So this means that if you are cutting a mass into slices, the slices should be 8 mm or less in thickness if you want them to fix in about 4 hours (penetrating from both sides of the slice). And if place a 10 cm mass in fixative overnight without slicing it, only the outer 1 cm or so will be fixed in the morning--the rest of the tissue on the inside will still be raw. The volume of fixative to be used has traditionally been stated to be 10 times the volume of the tissue, for optimal fixation. In practice, we can often get adequate fixation for routine pathology work with a volume of fixative 2-3 times that of the tissue.

Glutaraldehyde, useful for EM

Glutaraldehyde is an aldehyde fixative similar to formaldehyde, but with a longer side chain. Is is a cross-linking fixative used almost exclusively for electron microscopy. It results in better preservation of cell organelles and membranes for electron microscopy, but only penetrates a maximum of about 0.5 mm into the tissue within a reasonable time frame for preservation of subcellular structures. Therefore, any tissue put in glutaraldehyde should be no more than 1 mm thick in it's thinnest dimension. Which means it doesn't have to be tiny cubes, as you can put in a thin slice of tissue, and as long as it is less than 1 mm thick the fixative can penetrate adequately from both sides and fix all the way to the middle. Our glutaraldehyde is a 2% aqueous solution with cacodylate buffer, stored in the refrigerator in the accession room in small, labeled screw-cap vials. It is stored in the refrigerator to prolong its shelf life, but does not require refrigeration after the tissue has been placed in it. In current practice, its usage is restricted almost exclusively to renal biopsies for medical renal disease. Other tissues on which electron microscopy is occasionally performed include tracheal or nasal biopsies for possible ciliary dysfunction, and liver or occasionally conjunctival biopsies for the evaluation of possible metabolic storage disease.

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Zeus or Michel's media, for immunofluorescence

Zeus solution and Michel's media are similar, and both are very weak fixatives that serve to stabilize antigens for immunofluorescence study, without significantly altering them by cross-linking. They allow us to use antibodies to detect immunoglobulin and complement bound to tissue frozen sections, that would otherwise be destroyed by conventional formalin fixation and processing into paraffin blocks. Zeus or Michel's media will preserve these antigens for approximately **5-7 days maximum**, by which time the tissue must be retrieved from the solution, washed in buffer, and frozen in OCT as preparation for section for IF study. It is stored in the refrigerator to prolong its shelf life, but does not require refrigeration after the tissue has been placed in it.

It is used primarily for renal biopsies, but also has a role in the evaluation of immune-mediated blistering diseases of the skin, oral mucosa and conjunctiva. Although it works well for preserving and stabilizing immunoglobulin and complement in tissues, it is a poor fixative for preserving tissue structure. If you attempt light microscopic study or electron microscopy on tissue preserved in Zeus or Michel's media, the morphology is very poor and difficult to evaluate.

Take care to read the label and not confuse Michel's media with glutaraldehyde solution, as the screwcap bottles are very similar. If immunofluorescence study is wanted, the tissue is put in glutaraldehyde fixative, it will be worthless. Likewise, submission of tissue for EM in Michel's media will pretty much render it useless.

Bouin's fixative

Bouin's fixative or solution is a mixed or compound fixative that is bright yellow and composed primarily of formaldehyde, picric acid and acetic acid, that was invented by a French biologist named Pol Bouin. It has a bright yellow color, imparted by the picric acid component. Aqueous Bouin's fixative was used for many years here for fixation of GI and liver biopsies, as it has both a cross-linking and a coagulative component to its fixation chemistry, that results in crisp cellular detail, and enhances some of its staining properties. However it is no longer used for those biopsies, as the coagulative component of its fixation makes it difficult to impossible to perform subsequent molecular genetic studies on the tissue block, if they turn out to be needed. It also makes EM impossible. The bright yellow color comes from the picric acid component.

Alcoholic Bouin's solution (in which the reagents are primarily dissolved in alcohol, rather than water) is still in use for fixation of medical renal biopsies, i.e., renal biopsies performed to evaluate for renal dysfunction rather than for tumors of the kidney. In this context it facilitates recognition of the finer details of glomerular and tubular structure in the kidney on the special stains used for evaluation of medical renal disease.

Alcohol

Alcohol fixes tissue by dehydration and coagulation. It works fairly well for morphologic study of cytology specimens. Rarely it may be used for actual tissue biopsies if there is concern for preservation of glycogen or come crystals, but the tissue morphology is less well preserved than with formalin. Antigenic reactivity for immunohistochemical (IHC) staining is also different than that of formalin fixed tissue, so that one cannot use IHC protocols for alcohol fixed tissue without first validating them on alcohol fixed tissue. Alcohol should generally be avoided for tissue fixation, and even on cell blocks from cytology procedures, formalin is the preferred fixative.

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RPMI

This bright red solution is a tissue culture media designed to keep cells alive so that they can be transported either for flow cytometry, or for cytogenetic study. It is not a fixative, and tissue pulled out of RPMI on the following day typically looks horrible if you try and process it for light microscopy.