DIDACTIC SEMINARS FOR FIRST YEAR RESIDENTS IN PATHOLOGY

"FIXATION"

Histologic techniques deal with the preparation of tissue for microscopic examination. This is accomplished by submitting the total or part of the tissue to a series of processes as follows:

(1) FIXATION
(2) DEHYDRATION
(3) CLEARING
(4) IMPREGNATION
(5) EMBEDDING
(6) CUTTING
(7) STAINING

Today's seminar will focus on the first aspect of tissue processing - "Fixation".

INTRODUCTION

In histopathology, most tissues are fixed before they are examined microscopically. It follows that fixation is the foundation for the subsequent stages in the preparation of the sections, through to the making of a diagnosis. For ease of carrying out these subsequent techniques, it is essential that fixation is effective and that the appropriate fixative is used. One is therefore faced with the problem of selecting a fixative that will preserve.

(A) The gross and microscopical anatomy of the tissue
(B) Those chemical compounds to be investigated and more particularly their histochemical reactive groups
(C) Other classes of compounds which may subsequently become important during the course of investigation.

It is essential that tissue be fixed as soon as possible after death or removal from the body so as to prevent the onset of autolysis and putrefaction. The amount of fixative should be about 15 times the volume of tissue to be fixed and tissues should never be more than 3-5 mm in thickness. As this determines the speed and penetration of the fixative.

Small biopsies must be wrapped in lens paper to ensure they do not get lost during fixation and processing. Large specimens such as bowels and stomachs should be opened to allow fixatives to enter the lumen rather than wait for penetration and spleens, breasts etc. should be serially sectioned and allowed to fix overnight.

Remember, the foundation of all good histological preparations is adequate and complete fixation. Faults in fixation cannot be remedied at any later stage and the finished section can only be as good as its primary fixation.

EFFECTS OF FIXATION

The aim of fixation is to preserve tissue in as life-like a manner as possible. This preservative must also allow a variety of techniques to be performed upon it without destroying its structure. The more common effects of fixation are listed below.

(1) **INHIBITION OF AUTOLYSIS AND PUTREFACTION**

(A) Autolysis - The lysis or dissolving of cells by enzymic action due to rupture of the lysosome

(B) Putrefaction - The breakdown of tissue by bacterial action

Tissues placed in the refrigerator without fixing slows down the above processes.

(2) **PRESERVATION**

The preservation of cells and tissue constituents in as life-like a manner as possible.

(3) **HARDENING**

The hardening effect of fixatives will allow easy manipulation of naturally soft tissues (for example, brain)

(4) **SOLIDIFICATION OF COLLOID MATERIAL**

Fixation has the effect of converting the normal semi-fluid consistency of cells (Sol) to an irreversible semi-solid consistency (Gel).

(5) **OPTICAL DIFFERENTIATION**

Fixation alters to varying degrees the refractive indices of the various cell and tissue components, which enables unstained components to be more easily seen than when unfixed.

(6) TISSUE SHRINKAGE

Studies show that the combined effect of fixation and processing can cause a total tissue shrinkage of up to 30-40 percent.

COMMON FIXATIVES USED IN OUR LAB

(1) FORMALIN

Generally we use neutral buffered formalin for all types of tissue: Formalin is cheap, easily prepared, lacks the toxic components of other fixatives and requires no extra preparation of sections before staining. 10% buffered formalin can be called the "all purpose fixative" as you get generally good results with most staining techniques including immunoperoxidase procedures although prolonged fixation is known to decrease or destroy the number of antigen sites.

(2) GLUTERALDEHYDE - KARNOVSKY

This is used for all EM specimens in our laboratory and is kept refrigerated. Fresh tissue should be placed in this fixative as soon as possible and delivered to the EM lab for studies.

(3) B-5

A commercially prepared fixative containing mercuric chloride and an acetate buffer with which bone marrows and bone biopsies are fixed. This is a good micro-anatomical fixative. The use of mercuric chloride leaves a brown deposit in the tissue after fixation and therefore requires a special treatment to remove it before staining.

Further reading on fixation and fixatives can be found in any histology textbook. A good example is Culling's handbook of histopathological and histochemical techniques.

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"PROCESSING SPECIMENS - FROM TISSUE TO SLIDE"

Histologic techniques deal with the preparation of tissue for microscopic examination. This is accomplished by submitting the total or part of the tissue to a series of processes as follows:

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(2) DEHYDRATION
(3) CLEARING
(4) IMPREGNATION
(5) EMBEDDING
(6) CUTTING
(7) STAINING

Today's seminar will focus on the remaining aspects of tissue processing dehydration to staining. As last time we did fixation.

PRIOR TO PROCESSING

As a review, remember tissues have to be adequately fixed prior to processing as faults in fixation cannot be remedied. Also, tissues such as bone, discs etc. that contain calcium have to be decalcified prior to processing or it would be very difficult to obtain adequate sections. Decalcification takes place by putting the tissue in a commercially prepared solution called CAL-EX which removes the calcium. This process can take up to several days.

Proper identification of tissue is a must prior to processing and the specimens must be assigned a surgical pathology number and recorded on the laboratory information system by one of the technologists. Labels and requisitions must be checked and care taken to insure that the proper surgical number is written on the capsule. Use graphite pencil for writing on the capsule as ink will wash during processing.

(2) **DEHYDRATION**

Tissue contains large amounts of water. This must be removed so that it may be replaced by wax. This process of water removal is called dehydration. The best reagent for this is ethyl alcohol. Dehydration is best accomplished by the use of graded alcohols and continuing through to absolute alcohol.

(3) **CLEARING**

This has to be done so that the alcohol in the tissue is replaced by a fluid which will dissolve the wax with which the tissue must be impregnated. The two most common reagents used are chloroform and xylene.

(4) **IMPREGNATING**

Tissues having been completely dehydrated and cleared are then impregnated with paraffin wax by immersion in a succession of moulton wax baths.

(5) **EMBEDDING**

Embedding takes place on the Tissue Tek machine. The tissue is placed in a metal mold and filled with molten paraffin wax. The white capsule (used in gross sectioning and containing the surgical or autopsy number) is placed on top and again filled with wax. The block is allowed to cool and base mold released.

(6) **CUTTING**

Sections are cut using the rotary microtome. The sections are usually cut at 5 microns, except where otherwise specified. The cut sections are floated unto a water bath, wrinkles and folds removed and then picked up on albuminized slides. The slides must be drained and then thoroughly dried. This process insures the evaporation of all moisture between the section and the slide to adhere the section to the slide. If this is not properly done the section will probably detach from the slide during staining. The slides are usually dried in a 56°C oven.

(7) **STAINING**

Since most stains are aqueous solutions, the sections must be so treated so that the wax must first be removed by xylene which is in turn removed by absolute alcohol. The sections are then brought down through graded alcohols to water. The most common routine stain is the H & E: