2010

Histological Preparation of Tissue

Kelly Baxter
Parkland College

Histological Preparation of Tissue

An examination of the chemical mechanisms involved in the fixation, chemical preparation and processing of tissue samples

Kelly Baxter
Parkland College
Department of Natural Sciences
Che 102-001

In other words…

How do we get from this:

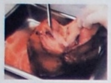


Figure 1: Tissue Sample

…to this:

Figure 2: Microscope Slides

?

Background

- Tissue collection
  - Biopsy
  - Autopsy/Necropsy

Figure 3: The Necropsy Floor

Overview of Tissue Processing

1. Fixation

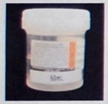


Figure 4: 10% Formalin

2. Specimen accessioning

Figure 5: Receiving Department

3. Gross examination & "trimming"

Figure 6: Cystic Liver
Photo courtesy of Kelly Baxter

4. Chemical preparation of tissues

- *Decalcification (bone)*
- *Keratin softener (nail)*
- *Nitric acid (teeth)*
- *Dissect-Aid (lipids)*

Figure 7: Holding Station
Photo courtesy of Kelly Baxter

5. Automated tissue processing

Figure 8: Tissue-Tek VIP 6 Processor

1) Formalin – (*fixation*)
2) Pen-Fix – (*fixation*)
3) 60% ETOH – (*dehydration*)
4) 80% ETOH - (*dehydration*)
5) 95% ETOH – (*dehydration*)
6) Cleaning Xylene – (*clearing agent, makes infiltration of tissue with parafin possible*)
7) 95% ETOH - (*dehydration*)
8) 100% ETOH - (*dehydration*)
9) 100% ETOH - (*dehydration*)
10) Clearite (Xylene substitute)
11) Clearite - (*clearing agent, makes infiltration of tissue with parafin possible*)
12) Cleaning ETOH

6. Embedding & sectioning

Figure 9: Embedding

Figure 10: Sectioning with a Microtome

7. Staining

Dyes have an affinity to specific structures

3 Classes of Stains[1]
- Stains that differentiate between acidic and basic components of the cell
- Specialized stains that differentiate the fibrous components of the extracellular matrix
- Metallic salts that precipitate on tissues, forming metal deposits on them

Figure 13: Automated Stainer

Figure 14: Hematoxylin and Eosin (H & E) Stain

8. Cover-slipping

Figure 15: Cover-slip Placement

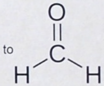
9. Interpretation

Figure 16: Reading the Slides

Slides are "read" by a pathologist to determine if any evidence of disease is present at the cellular level

Now the Chemistry…

Formaldehyde (CH2O)
(simplest aldehyde; features the formyl group)



It is a small molecule that is able to diffuse at a rate of 0.5mm/hour[5]

Aqueous solutions of formaldehyde are commonly referred to as formalin

It is typically available at a 10% concentration and is buffered with a phosphate to control the pH[2]

Formaldehyde is the most commonly used fixative due to its:

-disinfectant properties (toxic to most organisms)

-effectiveness as a preservative (prevents autolysis-"self-digestion" by forming covalent bonds and cross-linking with amino acids, i.e. lysine)

-preserves erythrocytes and the natural color of tissues better than alcohol-based fixatives[3]

The chemistry of formalin fixation is very complex but in the simplest terms it cross links with amine groups found throughout tissues in proteins[2]

$$R\text{-}NH_2 + CH_2O \rightarrow R\text{-}NH\text{-}CH_2OH$$

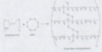


Figure : Example of Cross-linking

Factors affecting fixation[4]:

- Buffering (fixation is optimal at a neutral pH)
- Penetration (thin sectioning required because of 0.5mm/hour diffusion rate)
- Volume (10:1 ratio of fixative to tissue)
- Temperature (kinetics)
- Concentration (equilibrium)
- Time interval (long-term storage of tissues in fixative result in hardening)

Automated Processing of Tissues

(Required because wet tissues can not be directly infiltrated with paraffin)[4]

- Dehydration
  - Graded series of alcohols (hydroxyl reactive group) (since ~60% of the tissue is composed of H_2O)
- Clearing
  - Xylene treatment prepares tissues for embedding because it is miscible with paraffin
- Paraffin infiltration
  - Aided with pressure & vacuum

Conclusion

Histological preparations have evolved significantly since the invention of the first microscope. In the past three decades there has been an explosion of research in immunohistochemistry which uses the localization of specific antigens in tissue samples to mark or 'tag' abnormalities.[4] The development of electron microscopy and its increasing used has also influenced the field of histology and has required a modified method of chemical preparation of the tissue samples.

References

1. Gartner L, Hiatt J. Color textbook of histology. 2nd. ed. New York: W.B. Saunders Company; 2001.
2. Junqueira L, Carneiro J. Basic histology: text & atlas. 11th. ed. New York: McGraw-Hill; 2005.
3. Puchtler H, Meloan SN. On the chemistry of formaldehyde fixation and its effects on immunohistochemical reactions. Histochemistry. 1985; 82: 201-204.
4. The Internet Pathology Laboratory for Medical Education. The University of Utah Eccles Health Sciences Library [Internet]. Savannah, GA: c1994-2010 [cited 2010 Oct 20]. Available from: http://library.med.utah.edu/WebPath/HISTHTML/HISTOTCH/HISTOTCH.html
5. Formaldehyde Fixatives. Southwest Environmental Health Sciences Center & University of Arizona. Tucson, Arizona: c2002-2004 [cited 2010 Nov 24]. Available from: http://swehsc.pharmacy.arizona.edu/exppath/resources/pdf/Formaldehyde.pdf