HISTO-LOGIC®

No reader should utilize materials and/or undertake procedures discussed in HISTO-LOGIC articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

Editor, Lee G. Luna, D. Lit., H.T. (ASCP) | Technical Bulletin for Histotechnology Published: January, April, July, October | Vol. IX, No. 3 - July, 1979

Golden Forceps Award Winner

We are pleased to announce that Cathy Adler has been selected as the recipient of the Golden Forceps Award for 1979. Her paper, "Gelatin-Chrome Alum: A Better Section Adhesive," was selected from articles submitted to **Histo-Logic** during the past year. Criteria for selection are clarity, originality and scientific contribution. The Golden Forceps Award will be presented at the Symposium/Convention of the National Society for Histotechnology to be held in Dearborn, Michigan, October 8-12, 1979. Reprints of the winning article which appeared in the July, 1978 issue of **Histo-Logic** are available from Lab-Tek Division, Miles Laboratories, Inc., 30W475 North Aurora Road, Naperville, Illinois 60540.

pH Simplified

Robert J. Joyce
Markson Science, Inc.*

Editor's Note: This is the second of a three-part article on the fundamentals of pH. Part one appeared in **Histo-Logic**, Vol. IX, No. 2, April, 1979. Part three will appear in Vol. IX, No. 4, October, 1979.

How pH is Measured:

Today the pH of a solution is measured either by an indicator dye or by a pH meter and an electrode system whose voltage output is proportional to the active acid (H_3O^+) concentration in solution.

Certain organic dye solutions change color over a relatively small pH range. These are called indicator solutions. They can be used to indicate the approximate pH of a solution. By adding a few drops of a phenolphthalein indicator to a solution one can tell if the pH of the solution has a pH greater than 9 by the red color present, or a pH less than 9 by the lack of color. Other dye materials can be chosen whose color changes indicate other pH ranges. For example, phenol red changes at pH 8, bromthymol blue at pH 7, and bromphenol blue at pH 4.

For convenience, these dyes are often deposited on a strip of paper. When a drop of solution to be tested is placed on the paper, the resulting color change is indicative of the approximate pH of the test solution.

Dye indicator solutions or paper have the advantage of being quite inexpensive, very portable, and often suitable where only an approximate pH measurement is needed. On the other hand, where precise measurements are needed and/or the solution to be measured is colored, a pH meter is required. Accordingly, pH meter and electrode systems have been developed which respond in a precise manner to the pH of a solution.

The pH Electrode System:

pH electrode systems are always composed of two electrodes, a sensing electrode and a reference electrode. For convenience, these two electrodes can be constructed in one common body which is called a combination electrode. This is the most popular form of the pH electrode system. The sensing electrode contains the specially-designed surface whose voltage changes with the pH of the test solution. The reference electrode is used to complete the electrical measuring circuit. Its only function is to give a stable (unchanging) voltage to which the sensing electrode voltage can be compared.

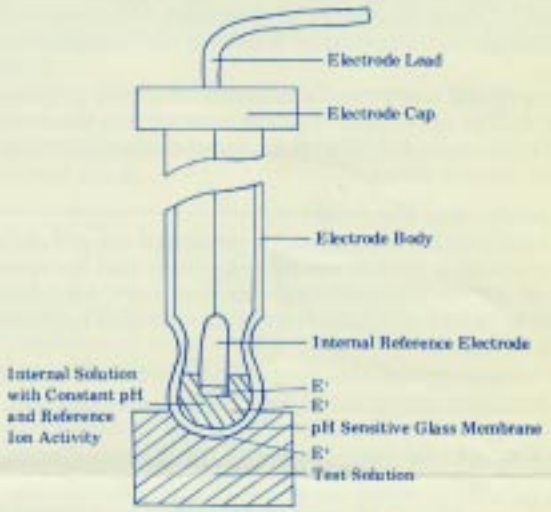

FIGURE 3:
Sensing Electrode

The pH Sensing Electrode:

In 1901 a German chemist named Fritz Haber discovered that the voltage of certain glass surfaces changed in a regular manner with the acidity of a solution. Modern pH sensing electrodes are a refinement of this fundamental discovery.

The essential features of a pH sensing electrode are shown in Figure 3. The important requirements of this electrode are that (1) the voltage at the internal reference/filling solution surface (E) remain constant; (2) the voltage at the internal solution/glass membrane surface (E^2)remain constant; and (3) the voltage at the glass membrane/test solution surface (E^3) changes proportional to the pH of the test solution. It should be noted that the electrical resistance of the glass membrane is extremely high. Thus, a specialized voltmeter is required to measure the voltage from a pH sensing electrode.

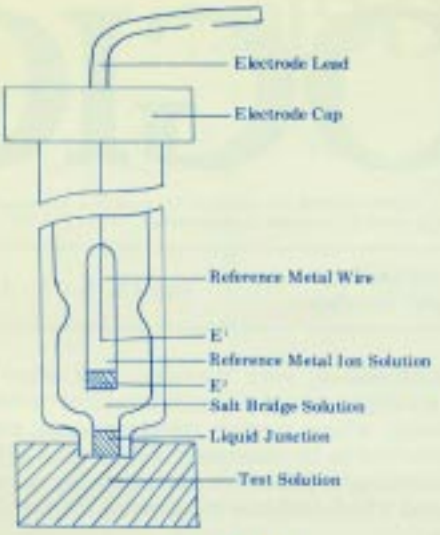

FIGURE 4:
Reference Electrode

The Reference Electrode:

When using a voltmeter to measure the voltage at the pH sensing electrode, the electrical circuit must be completed. The reference electrode, Figure 4, performs this function. Just a piece of bare wire could be used to complete the circuit. However, the voltage at its surface would change in an unpredictable fashion with time and test sample composition. Accordingly, a reference electrode is a wire which has been terminated with the proper choice of metal and surrounded by the proper metal ion solution, so as to give a constant voltage independent of time and test sample composition.

The essential features of a reference electrode are shown in Figure 4. The important requirements of this electrode are that the voltages E^1, E^2 and E^3 remain constant with time and test sample composition.

The Combination Electrode:

The combination electrode is a version of the pH electrode system in which the pH sensing electrode and the reference electrode are combined into one common body. All comments applicable to the individual electrodes are also applicable to their combination.

The advantages of this form of the electrode system include handling convenience and rugged construction. The single body construction also allows one to measure the pH of small sample volumes, as well as the pH of surfaces, such as soil and skin.

Morphologic Alterations Produced in Tissue by Histopathologic Techniques

An Editorial

Alterations Caused by Fixation:

The cellular changes that are possible with the use of different fixatives and/or ingredients are dramatically illustrated by Bloom and Fawcett[1] in *A Textbook of Histology*, 10th Edition. These authors employed epithelium of the small intestine of a guinea pig. Multiple fixatives and stains were used to emphasize the extreme importance of selecting a proper fixative for preservation, staining, and study of cytoplasmic and nuclear structures.

Luna[2] reported the microscopic findings and staining results produced by 26 different fixatives on human and guinea pig skin. He stated that staining effects varied with different fixatives, and that phosphate-buffered 10% neutral formalin is the preferable fixative for general use. Some of the observations are illustrated in Figures 1, 2 and 3. Skin was fixed in phosphate-buffered 10% neutral formalin, formol-saline, and Bouin's fluid, respectively. The types of cellular changes related to a specific fixative must be identified and known prior to initiating a study on a specific organ.

Effects of Unbuffered Formalin:

It must be recognized that staining reactions and tissue elements are altered and others obliterated by the deleterious and insidious effects of unbuffered formalin. Formalin is oxidized to formic acid, and some of the following ruinous effects are produced: (A) The so-called formalin pigment will frequently react during the staining procedure to mask or, in some cases, simulate microorganisms, pigments, or other elements suggesting disease. Formalin pigment has a notorious reputation for reducing silver in procedures for staining fungi (Grocott's methenamine-silver), melanin (Fontana-Masson), spirochetes (Warthin-Starry), and many other silver procedures. (B) Unbuffered formalin gradually destroys nuclear staining basophilia if tissue is exposed longer than three weeks. (C) Unbuffered formalin dissolves copper, iron, and calcium during the process of fixation. (D) It alters the staining of some cell products. For example, it will inhibit the aldehyde fuchsin reaction for demonstrating Paget's cells. These are only a few of many examples but should be convincing evidence that unbuffered formalin should not be used.

Neutralized formalin (not to be confused with buffered formalin) creates a false sense of security. When tissue is placed in a neutralized fixative, the pH is lowered rapidly from the formation of formic acid, and some of the irreversible alterations mentioned above may occur.

Unbuffered or neutralized formalins should not be used for routine fixation and storage of tissue. The number and magnitude of alterations cannot be predicted or estimated.

Morphologic Differences in Peripheral Versus Central Areas:

A study conducted in our laboratories demonstrated marked differences in cellular form and structure between the central and the peripheral areas of tissue fixed for a specific length of time. Changes are also produced by postfixation processing. Sections were obtained from canine liver, sliced 3mm thick, fixed, processed as described below, and sectioned at 6 microns. The section shown in Figure 4 was fixed in Bouin's solution for 24 hours, washed for 3 hours, and processed. The tissue in Figure 5 was fixed in Bouin's for 48 hours and processed. Tissue in Figure 6 was fixed in Bouin's for 48 hours, washed for 3 hours in running tap water, and exposed to 70% ethanol for 4 hours. Specimens were processed according to the schedule on page 16 of the AFIP Staining Manual.[3] Results of treatment after fixation as noted in Figures 4-6 are readily overlooked but cannot be

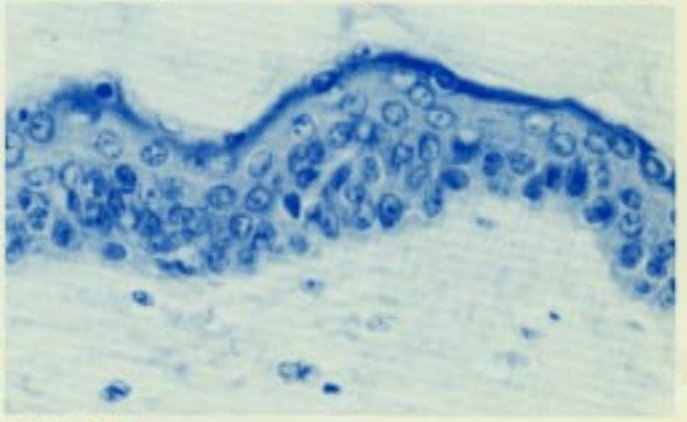

FIGURE 1:
Fixation in phosphate-buffered 10% neutral formalin. Excellent over-all cellular detail. (H&E x575)

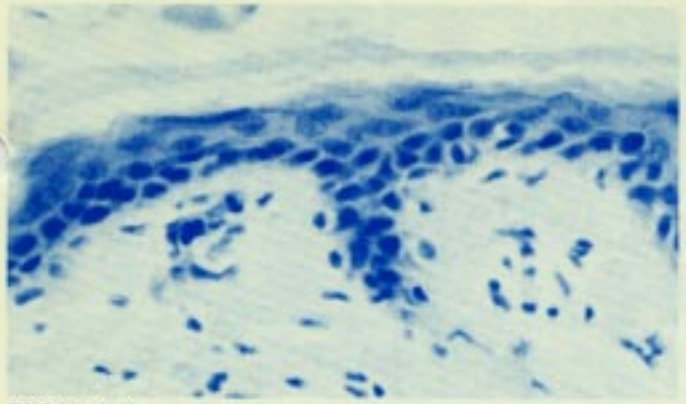

FIGURE 2:
Fixation in formol-saline. Nuclei are noticeably shrunken and compacted, and nucleoli cannot be discerned. (H&E x575)

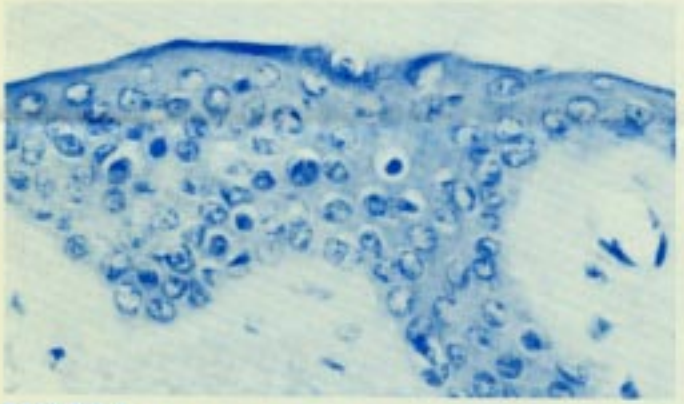

FIGURE 3:
Fixation in Bouin's fluid. Crisp nuclear detail. (H&E x575)

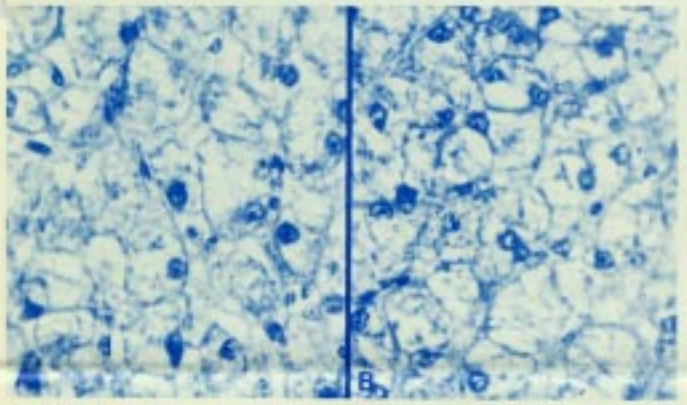

FIGURE 4:
A. Fixation in Bouin's fluid. Periphery: Marginal quality with poor nuclear detail. B. Center: Cytoplasmic vacuolization and poor over-all detail (H&E x575).

dismissed. It is necessary and important for the pathologist and histotechnologist to be aware of these cellular changes for proper interpretation of pathologic conditions.

References:

1. Bloom, W. and Fawcett, D.W.: *A Textbook of Histology*, 10th Edition, W. B. Saunders, Philadelphia, pp. 14-15, 1975.
2. Luna, L.G.: *Effects of Various Staining Reactions in the Skin.* International Academy of Pathology Monograph, The Williams and Wilkins Co., 1971.
3. Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, Third Edition, McGraw-Hill, New York, 1968.

Photos furnished by Armed Forces Institute of Pathology.

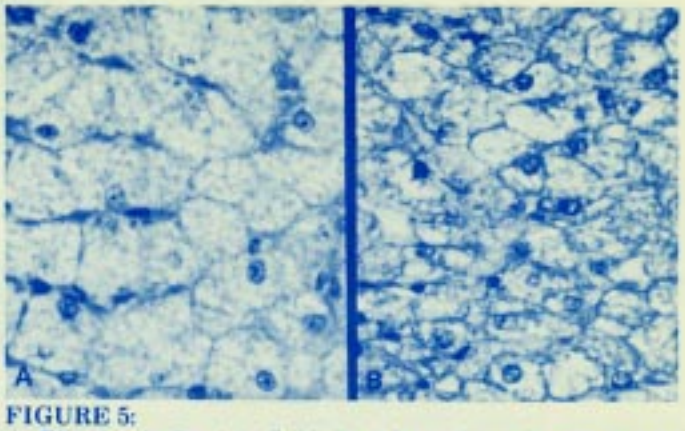

FIGURE 5:
A. Fixation in Bouin's fluid. Periphery: Cytoplasmic distortion and well-defined nuclei.
B. Center: Satisfactory structural detail. (H&E x575)

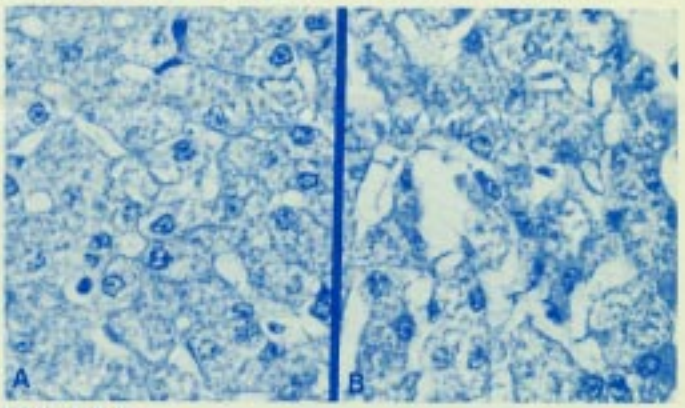

FIGURE 6:
A. Fixation in Bouin's fluid. Periphery: Excellent preservation and structural detail.
B. Center: Widened sinusoids and unsatisfactory structural detail. (H&E x575)

ANNOUNCEMENT

Plan to attend the NSH Symposium/Convention in Dearborn, Michigan, October 8-12, 1979. For program and registration information, write: NSH, P.O. Box 36, Lanham, Maryland 20801.

A New Polychrome Stain

Janet Campbell and Kenneth Saeger, M.D.
The Hospital of the Good Samaritan
Los Angeles, California 90017

In view of the recent unavailability of an acceptable polychrome stain for frozen stat sections from commercial sources, we have experimented with various stains, dyes and dye combinations along with standard reagents. We found that the following stain is fast, reliable, produces uniform polychromatic staining results with good nuclear detail. The stain is easy to prepare from standard dyes and reagents and the coverslip may be mounted either after water (non permanent) or after xylene (permanent mount).

Solutions:

Campbell's Polychrome Stain

Toludine blue	1.0 gm
Methylene blue	1.0 gm
Azure A	0.25 gm
Giemsa powder	0.25 gm
80% ethyl alcohol	650.0 ml

(Continued on next page)

A Technical Bulletin for Histotechnology

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Combine the dyes with the 80% alcohol; dissolve with the aid of gentle heat; allow to cool and filter. Stain is now ready for use. Stain is stable at room temperature and keeps indefinitely.

Brun's Media

Glucose	24.0	gm
Glycerin	6.0	ml
Spirits of camphor	6.0	ml
Distilled water	84.0	ml

Combine in a bottle, shake well and filter. Keep solution stoppered. Keeps indefinitely.

Procedure for Water Mount:

1. Cut frozen section at thickness desired.
2. Adhere section to glass slide as usual.
3. Fix tissue with absolute alcohol for 5 seconds.
4. Drain slide
5. Stain slide with Campbell's polychrome stain for 5 seconds.
6. Rinse slide with tap water.
7. Mount with Brun's media.

Procedure for Standard Mount:

1. Cut frozen section at desired thickness.
2. Adhere section to glass slides as usual.
3. Fix tissue with absolute alcohol for 5 seconds.
4. Drain slide.
5. Stain slide with Campbell's polychrome stain for 5 seconds.
6. Rinse slide with tap water.
7. Clear slide with dioxane, several dips.
8. Clear dioxane with xylene.
9. Mount with resinous media.

Results:

Various tinctorial shades characteristic of polychrome staining reactions.

The above stain is also useful as a rapid cytologic stain for direct smears, aspirates, etc.

To receive your own personal copy of HISTO-LOGIC, or to have an associate added to the mailing list, submit home address to: Lab-Tek Division, Miles Laboratories, Inc., 30W475 North Aurora Rd., Naperville, Illinois 60540.
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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.