HISTO-LOGIC™

Lee G. Luna, D. Lit., H.T. (ASCP), Editor A Technical Bulletin for Histotechnology Vol. IV, No. 1 - January, 1974

Erratum

Re: October 1973 issue of HISTO-LOGIC
In the announcement about the International Histology Seminar held in Tokyo, it was indicated that Dr. Hirayama is affiliated with Tokyo Women's College. Dr. Hirayama is, in fact, at Tokyo Women's *Medical* College.

National Society for Histotechnology

The *National Society For Histotechnology* is now a reality. On September 29, 1973, in Silver Spring, Maryland, forty-two representatives from the United States and Puerto Rico met with this purpose in mind. After intensive discussions concerning policies, aims, and goals, it was unanimously agreed that incorporation proceedings be started. Pro-tem officers were elected and committee chairmen appointed. It is hoped they will initiate a strong physical structure.

Why is the word hope emphasized? A dream for many has now become a reality. Reality does not mean instant maturity. It is with the advice, suggestions, and participation of *each individual* technologist in this varied and all-encompassing field of Histotechnology that this Society will grow, giving to all the benefits it will receive from the counsel of each individual member.

Become a member! Actively participate in any area that will benefit you and your profession. If you cannot actively participate, don't allow that fact to intimidate you. Sit down and put your feelings and suggestions in letter form and mail them to one of the officers or committee chairmen.

Membership is open to all persons working with tissue. Examples are: hospital histology, histochemistry, immunofluorescence, electron microscopy, marine biology, plant pathology, veterinary medicine, radioautography, etc.

Membership dues (which will not be tax deductible until incorporation) are $10.00 per year. Persons filing applications before October, 1974 will be deemed charter members.

The formation activities were presented to attendees at the "Symposium on Histopathological Techniques." They evoked instant response for membership. Donations were also presented to the Society at that time: Dr. Miles Bouton from South Carolina donated $100.00; Lerner Laboratories donated a $250.00 cash gift to be used for an annual award, or awards; the New York State Histotechnological Society and the Virginia State Society of Histology Technicians each pledged $100.00.

The entire group of representatives instrumental in founding N.S.H. were named the "Founding Board of Directors" with Lee Luna elected Chairman of the Board. Pro-tem officers are: President–Dominic Europa, New York City, New York; Vice President–Elaine Boyd, Thousand Oaks, California; Treasurer–John Koski, Ann Arbor, Michigan; Executive Secretary–Elizabeth Mayle, Rochester, New York; Recording Secretary–Kenneth Urban, Chicago, Illinois. The formed committees with their chairpeople are as follows: Membership–Ruth Elder, Lynchburg, Virginia; By-Laws–Kenneth Urban, Chicago, Illinois; Budget and Finance–Phillip Womack, Lynchburg, Virginia; Education–Barbara Spillan, Jacksonville, Florida; Convention–Lee Luna, Lanham, Maryland; Public Relations–Elizabeth Mayle, Rochester, New York.

October, 1974 is the projected date for the first business meeting of the National Society for Histotechnology. It will be coupled with scientific sessions.

Remember–the keys to the professional growth of our Society are communication and participation. (Help yourselves with the help of your colleagues.)

Demonstration of Lipids in Paraffin-Embedded Tissue Sections

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Introduction

The most commonly used techniques for the demonstration of lipids in tissue sections are probably the oil-red-O and the Sudan methods. The employment of these techniques has aided pathologists in the demonstration and diagnosis of diseases such as Reye's syndrome, fat embolism, and lipidosis. However, due to the fact that lipids are easily extracted from tissues by alcohol, frozen sections are used in all the conventional techniques for the demonstration of lipids.[1,2] Since fresh tissues are recommended for frozen techniques, storage and transportation of the tissue samples, as well as quantitative and qualitative productions of the histological slides, often prove to be difficult or even impossible. In the present report, we would like to suggest a simple technique, which we have successfully used in our laboratories, for the demonstration of lipids in paraffin-embedded tissue sections.

Materials and Methods

Tissue samples obtained from autopsy cases or from experimental animals are allowed to be fixed in neutral buffered 10% formalin, formal-saline, or preferably, formal-calcium. The fixed tissues are then trimmed into 2-3mm thick and post-fixed with 1.0% osmium tetroxide (OsO_4) for 12-24 hours at room temperature. The osmium fixed tissues can then be processed as regular tissues (dehydrated with graded ethanols and cleared with xylene) and embedded in paraffin. Sections are obtained with a conventional microtome, deparaffinized, mounted with Permount and examined with the microscope. No counter stain is necessary.

Results

Lipid droplets in tissues are stained black by the osmium while the general tissue background is only faintly stained.

For illustrative purposes, paraffin sections of a fatty kidney

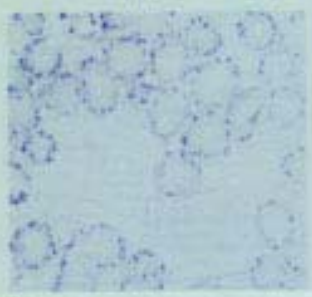
(obtained from a case of Reye's syndrome) stained with osmium tetroxide are shown (Figures 1, 2). Lipid droplets in the proximal tubular cells are nicely preserved and stained. Basement membranes of the renal glomeruli are also demonstrated by the present method.

Discussion

Lipids, particularly oleic acid and triolein, react immediately with osmium tetroxide due to the oxidation of the double bonds of the lipid substances. Osmium tetroxide (OsO_4), on the other hand, is being reduced to osmium dioxide (OsO_2). The demonstration of lipids in frozen sections with osmium has been cited by Mallory.[3] Paraffin sections prestained with potassium dichromate-osmium tetroxide have also been attempted with some success.[4] However, since potassium dichromate blocks the reaction of osmium tetroxide with some lipids,[5] the use of potassium dichromate is avoided in the present technique. Therefore, in the formalin-fixed, non-chromated tissues osmium tetroxide is allowed to be reduced by (and thus will preserve and stain) larger numbers of lipids which include, in addition to oleic acid, the carotenoids, ascorbic acid, aldehydes of palmitin and plasmol.[5]

In the conventional methods, the oil-red-O and Sudan colorants color the lipids by virtue of their solubility in the lipids and not by chemical reactions. For such reason, these colorants may gradually leak out from the lipid rendering permanently stained sections impossible. A true chemical reaction (oxidation-reduction), on the other hand, is established between osmium tetroxide and the lipids. The reaction product of such a reaction is believed to be a permanent one.

The present modified osmium-paraffin technique not only eliminates the many artifacts created in frozen section cutting and staining, it also makes storage of tissues for future studies, transportation of tissue samples for pathological evaluations by other laboratories, and quantitative production of microscopic slides for teaching purposes possible. We find this technique to be an extremely valuable one which, we believe, will also be appreciated by other laboratories.



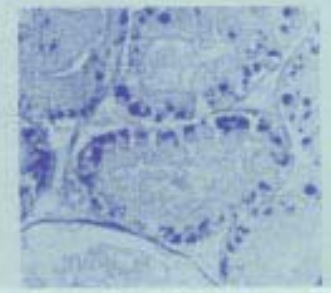


Figure 1:
Reye's syndrome, fatty kidney. Osmium-paraffin technique. All the lipid droplets in the renal tubules are stained black by the osmium. X40

Figure 2:
Reye's syndrome, fatty kidney. Osmium-paraffin technique. Morphological details of the tissue section are well preserved. Lipid droplets and basement membrane of the glomeruli are well demonstrated. X250

References

1. Barka, T. and Anderson, P.J.: *Histochemistry – Theory, Practice, and Bibliography*. Harper & Row Publishers, Inc., New York, Evanston, and London, 1965.
2. Lillie, R.D.: *Histopathologic Technic and Practical Histochemistry*. 3rd ed., McGraw-Hill Book Company, New York, Toronto, Sydney and London, 1965.
3. Mallory, F.B.: *Pathological Technique*, W.B. Saunders Co., Philadelphia, Pennsylvania, pp. 118-119, 1938.
4. Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed., New York, McGraw-Hill Book Co., Blakiston Division, pp. 143-144, 1968.
5. Thompson, S.W. and Hunt, R.D.: *Selected Histochemical and Histopathological Methods*. Charles C. Thomas, Springfield, Illinois, pp. 342-344, 1966.

Hints for Pap Smears and Cell Block Preparations

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Cell blocks and pap smears are often requested on unclotted specimens such as urines and fluids from breast cysts.

Pap Smears: Occasionally, pap smear preparations wash off the slide during the staining process. By smearing the slide with a small amount of commercial egg albumin prior to making the smear, the specimen is more likely to stay on the slide during the staining process.

Cell Block: Some specimens contain an insufficient amount of sediment for a cell block. By adding 0.2ml of Thromboplastin (used by Hematology Department for Prothrombin Tests) and 0.1ml of normal plasma to the sediment, a clot will form. Add fixative as you normally would to a cell block and process as a regular surgical specimen. No cellular changes, due to the formation of the clot, have been noticed during microscopic examination.

Rapid Procedure for Rehydration of Desiccated Tissue

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A frequent laboratory problem is desiccation of tissue owing to malfunctioning automatic tissue processors (ATP) or breakage of specimen containers during mailing. In the past, many methods have been employed for the rehydration of such tissue. These procedures usually involve soaking the tissue in physiological saline and fixatives for several days, or even weeks.[1] The time involved is of particular concern when processing surgical specimens. Also, with these procedures, rehydration is frequently incomplete and the tissue is left brittle and difficult to section. Cells are often shrunken, and as a result the histology is difficult to interpret. In addition, for tissue that has become desiccated during the dehydration phase in the ATP and then continues to the infiltration phase, a great deal of time is required to remove the paraffin before rehydration can take place.

The following is a procedure that I have found to be both workable and rapid. This procedure involves boiling the tissue in a wetting agent for 20 minutes, allowing removal of paraffin and complete rehydration. Processing, cutting, and staining can be accomplished in the same day while excellent microscopic histology is retained. Many common wetting agents, including laboratory detergents, can be used; however, I have found Leconal* most effective.

Procedure for Tissue Infiltrated with Paraffin:

Leave tissue in metal cassette; immerse in a 10% solution of Leconal. Bring to a boil; boil for 20 minutes. Place the container in a pan of ice water. As the solution cools the paraffin will rise to the top and solidify. Remove paraffin; rinse in warm tap water for 10 minutes and reprocess.**

Procedure for Tissue Not Infiltrated with Paraffin:

Place tissue in metal cassette; immerse in a 10% solution of Leconal. Bring to a boil; boil for 20 minutes. Rinse in warm tap water for 10 minutes and reprocess.**

Only one disadvantage of this procedure has been encountered, and that is in the processing of lungs fixed with formaldehyde gas and air dried.[2] In this specialized case, the cellular histology is excellent, but the lung tissue shrinks by approximately one-half. This is only a disadvantage, however, when morphometry is critical.

References:

1. Humason, G.L.: *Animal Tissue Techniques*, 2nd ed., W.H. Freeman & Co., San Francisco, California, 512-514, 1967.

2. Heard, E.G.: *Pathology of Chronic Bronchitis and Emphysema.* Williams & Wilkins Co., Baltimore, Maryland, 2-3, 1969.

* Leconal: Scientific Products, Evanston, Illinois. Use of trade names is for information only and does not constitute endorsement by the U.S. Public Health Service.

** It is recommended that chloroform be used rather than xylene. The xylene tends to harden the tissue making sectioning difficult.

Comments Regarding Articles Published in January, 1973 Issue of HISTO-LOGIC

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Concerning the Histochemical Calcium Techniques, in the past I have done numbers of Von Kossa stains and found that by placing a 100 watt light bulb as close as possible to the top of the coplin jar, excellent results are achieved without exposing the slide to direct sunlight.

In regard to the elimination of wrinkles from paraffin sections, we cut a number of study sets, class sets, and seminar slides in our laboratories. Sections are cut in serial order; 4 or 5 sections at a time are then floated on 30% alcohol with the aid of a 3" x 2" glass slide to remove all wrinkles before placing them on the flotation bath. It takes a little longer but is well worth the time when one must have 100-200 slides from 1 block. This procedure has been used for the last eight years with excellent results.

Problems in Stain Technology

An Editorial

During a review of numerous textbooks and publications on histologic technique, it became evident that the type of water (tap, distilled, demineralized, or de-ionized) to be used in staining procedures is frequently not mentioned. Too little attention has been devoted to this important aspect of preparing stains and solutions.

The concentration of hydrogen ion plays a significant role in staining reactions. Variations in this factor will alter the pH of a solution, especially if the preparation does not require the addition of an acid, metal, or base.

Table I illustrates the range in pH of tap and distilled water obtained during a 90-day period in one laboratory. Control of hydrogen-ion concentration is a requirement for more definitive and differential staining reactions.

TABLE I. Variation in Concentration of Hydrogen Ions in Distilled and Tap Water Over a 90-Day Period

Reading	Type of Water	pH
First Reading	AD*	5.3
	Tap	6.8
2 weeks later	AD	5.1
	Tap	6.9
4 weeks later	AD	4.9
	Tap	7.2
3 months later	AD	4.4
	Tap	7.3
	Over-all comparative variation	
	AD	4.4 - 5.3
	Tap	6.8 - 7.3

*AD = Distilled Water

Dr. Ralph D. Lillie, in his excellent book,† suggests that when purchasing dyes, it is wise to specify the Color Index Number (C.I. No.). He further provides the index number for the dye color for most of the methods he presents, and often the source of the dye. Unfortunately, this essential information has been omitted from some other authoritative texts. It is possible for inconsistent and even negative staining reactions to occur if dyes bearing identical names but different color index numbers are interchanged.

Basic fuchsin of C.I. No. 42510 is required for the preparation of aldehyde-fuchsin to demonstrate Paget cells. Basic fuchsin of C.I. No. 42500 cannot be employed because the possibility of inferior results is markedly increased. In a personal conversation, Dr. Lillie disclosed that basic fuchsin (C.I. No. 52590) is the dye of choice in the methyl green-pyronin Y procedure to demonstrate desoxyribose and ribose nucleic acids.

In many instances dyes of the same name having variations in C.I. number, as well as dyes having the same C.I. number, produce variations in staining reactions. It is also useful to know the dye lot or batch number, in the event the technician encounters a poor dye with the proper C.I. No. These problems can be reduced or eliminated with the establishment of a simple card file of salient information on various stains and C.I. numbers that by experience have proven best for given procedures (Table II). This information will assist in insuring that the laboratory reorders the same item; this in turn provides a means for quality control of the staining method and will help identify the dye with the technique.

TABLE II. Sample File Card Giving Information on Dye.

Dye — Information Required	
1. Dye:	Acid Fuchsin
2. Source:	Harleco (Cat. No. 218)
3. Dye Content:	65%
4. C.I. No.	42685
5. Lot No.	648
6. Technique:	Van Gieson, Masson, etc.

Dye content is also an important aspect of tissue staining since it is possible to obtain a given dye with a wide range of dye contents. For example, acid fuchsin (C.I. No. 42685) ranges from 58 to 75%. Eosin varies from 90 to 94%, and pararosanilin (C.I. No. 42500) may be obtained in concentrations from 94 to 99%. Such differences are not rare. A study was conducted to reveal the importance of dye content. Acid fuchsin C.I. No 42685 was selected for the survey. Lots of the dye from five manufacturers and distributors yielded concentrations of 58, 60, 65, 66, and 75% respectively. The acid fuchsin dyes rated at 75 and 58% concentrations were selected. A one-tenth of 1% aqueous solution was prepared with each. The 75% solution had a pH of 2.9 and the 58% solution a pH of 4.6. The latter dye did not stain tissue sections. The former performed satisfactorily. The conclusion from this elementary study was that the pH is dependent upon dye content and was responsible for unsatisfactory staining when the 58% product was used.

Additional solutions, one-tenth of 1%, were prepared, and 1ml of glacial acetic acid, ACS, was added to each. The solution with an initial dye content of 75% yielded a pH of 2.65. A pH of 2.75 was observed in the diluted, acidified 58% product. The staining results from both solutions were satisfactory and identical. The relationship of pH and dye content is obvious, important, and cannot be overlooked or disregarded. The variation in dye content and the pH of the staining solution may explain the varied staining results obtained in different laboratories even though identical staining procedures are employed.

† Dr. R.D. Lillie: *Histopathologic Technic and Practical Histochemistry*, ed. 3, New York: McGraw-Hill, 1965, p. 36.

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Editor's Corner

Continuing Education Program

Continuing Education Program in Histotechnology will be held March 8th and 9th, at the University of Tennessee Medical Units in Memphis, Tennessee. For information contact: Mr. Wallace Maytar or Miss Gerre Wells, Continuing Education Department, University of Tennessee Medical Units, 800 Madison, Memphis, Tennessee 38163.

Education Conference

The Histology Society of Ohio, Inc., is presenting its Second Annual Continuing Education Conference on May 17-18, 1974, at the Sheraton Inn, Beachwood, 26300 Chagrin Boulevard, Cleveland, Ohio 44122. For information contact: Mrs. Frances Christovich, The Mt. Sinai Hospital, Eye Pathology Laboratory, 1800 E. 105th Street, Cleveland, Ohio 44106.

Letter of Thanks

I would like to take this opportunity to thank all those who have sent letters of congratulation to me as the recipient of Lab-Tek Product's "Golden Forceps Award."

I am deeply moved by all the good wishes sent from far and near. I thank you all from the bottom of my heart.

Now let us move forward in harmony and unity in this – our chosen field. The future is NOW!!!!

Elaine S. Boyd, HT (ASCP)

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.
To receive your own personal copy of HISTO-LOGIC, or to have an associate added to the mailing list, write: Lab-Tek Products, Division Miles Laboratories, Inc., 30W475 N. Aurora Rd., Naperville, Illinois 60540.