THE GASTROINTESTINAL PATHOLOGY CLUB
NEWSLETTER

VOLUME 3, NUMBER 1

FALL-WINTER, 1984-85

CONTENTS

FORWARD

With this issue, we complete our tenure as co-editors of the Newsletter. Issue #6 will be prepared by Juan Lechago and David Owen, who will guide the Newsletter for the next three years. Our job has been much more enjoyable than we had anticipated when we started back in 1982, chiefly due to the incredible cooperation of the membership in offering advice, suggestions, reviews, notices, and technical sections. To all of you who have helped us, a sincere "Thank you!"

We have included two editorials which we hope will be thought-provoking. They concern subjects which will be on the Executive Committee agenda at our Annual Meeting on March 10, 1985. Please express your ideas as to the future direction of the Club by writing to the members of the Executive Committee before the Annual Meeting.

This issue also contains four excellent contributions from the members. All of our technical sections and reviews in the first five issues have been written by members who volunteered by answering a questionnaire we sent out in 1982. Juan and David will continue to work through that list. However, the membership has increased about 30% in the past three years. If any of you, new members or old, want to help in making the Newsletter a continued success, please write to the new Editors.

Don Antonioli

Henry Appelman

GASTROINTESTINAL PATHOLOGY CLUB OFFICERS AND COMMITTEES

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PRESIDENT'S NOTE

John H. Yardley, M.D.

Many items of interest to members are found in this issue of the Newsletter, but I do wish to highlight a few points.

Two areas of Club business that I believe could be especially critical to its effectiveness in the future will be discussed in Toronto. These concern planning and organization of publication efforts and facilitation of training programs in gastrointestinal pathology. The Club will henceforth have a formal agreement with the American Journal of Surgical Pathology to publish papers and other items, and other publishing proposals will certainly be forthcoming in the future. These developments will require an established means for setting policy and for maintaining high standards in the publishing area. The promotion of training programs in gastrointestinal pathology will, I am sure, also be of growing importance to the Club as the need and desire for specialization in GI pathology increases. Furthermore, if we do not have a means for actively encouraging training programs, I believe it would only be a matter of time before someone else did it for us.

To these ends, proposals for creating new standing committees on publications and on training programs will be placed before the membership during the meeting in Toronto. Some may feel that this means we are moving towards too many standing committees. But I think the greater risk is for the Club not to have an adequate organizational structure for expanding its involvement in gastrointestinal pathology along its natural lines of interest. I hope everyone will think about these questions and participate in the discussions at the business meeting.

I attended the special get-together of GI pathologists organized by Harvey Goldman during the International Congress in Miami Beach last fall, and I can attest to the enthusiasm expressed by the attendees for cooperation among counterpart organizations and persons involved in GI pathology from around the world. This co-operative project is one that the Club should encourage, and I am happy to report that Harvey has agreed to continue his role in organizing these efforts (see elsewhere in the Newsletter for details).

The joint meeting with the Society of Pediatric Pathologists in Toronto will surely be excellent, and I am looking forward to it. I am also looking forward to the meeting of the Gastrointestinal Pathology Club to be held for the second time during Digestive Disease Week, this year in New York City. I hope many members can attend the DDW session, which Klaus Lewin is organizing. It will be on May 14 (Tuesday) at 8:00 PM and consist of case presentations and discussion of esophageal disease (see announcement elsewhere in this Newsletter). There will be videomicroscopy demonstrations of microscopic slides as well as conventional projected material. I see this session, which is kindly co-sponsored by Nikon, Inc. and Glaxo Inc., as both exciting and as another chance to learn if videomicroscopy holds the promise as a conferencing device that it seemed to at the AGA meeting in New Orleans last spring (see my previous Note for soap-box style description).

It has been a great pleasure to serve as your President in 1984-85 and I wish all good luck to my successor, Klaus Lewin!

Editorial

The Annual GIPC Scientific Session: What's in it for Us?

The GIPC Scientific Session and Business Meeting at the IAP have been the major focus of our activity since the inception of the Club, primarily because a large proportion of our membership also belongs to the IAP and attends its annual meeting. However, we are beginning to wonder whether the Scientific Session as presently formulated is a "cost-effective" use of time for our members. This meeting has been organized as a forum to review current topics in GI pathology with an orientation to the general pathologist. There was historical justification for this in that as a fledgling organization, the GIPC wanted to establish and publicize itself. Now, after four years of these sessions, we have succeeded with a vengance: over 300 pathologists attend each meeting, and the Club has over 90 members. However, how much of the content of the talks is new or exciting to those of us working in GI pathology?

We would like to propose that part or all of future sessions be devoted to new, controversial, or more basic science aspects of our specialty. This idea could be implemented in several ways: 1) At least one of the three hours of the annual Scientific Session could be devoted to topics of special interest to the members. Obviously, the general pathologists could also attend, but if they decided to leave it would in no way be a negative criticism of the Club; 2) The program of the Scientific Session could alternate every two years between a teaching program for all pathologists in year one and a specialized program for the Club in year two; 3) the general teaching function could be dropped entirely; or 4) The current format could remain intact and the Club members could meet separately for a specialized program. The latter could occur during IAP week, but it is difficult to visualize cramming any more activities into

that already overcrowded time period. In 1984, the Club inaugurated a two-hour program at the AGA; however, how many of our members regularly attend the AGA? A session in tandem with the ASCP meetings might also be considered.

Given the brainpower within the GIPC, there certainly would be no difficulty in organizing special programs. For example, the impact of new technologies (such as flow cytometry, freezing fracture, and immunocytochemistry) on both diagnostic and basic science GI pathology could be reviewed in depth. Slide seminars could be given, with the study cases having been circulated among the members before the meeting. Technical matters, such as the best ways to evaluate nerves and smooth muscle in the gut, might be the subject of detailed review. If you feel, as we do, that the time has come to gear our activities closer to the needs of the members, please contact the Club officers or members of the Education Committee with your thoughts on the subject.

EDITORIAL: COOPERATIVE STUDIES: DOES EACH MEMBER HAVE A RESPONSIBILITY?

When the Gastrointestinal Pathology Club was organized, a number of objectives were identified which formed the raison d'etre for the Club in the first place. Several are stated clearly in the by-laws, in Article II, and include dissemination of knowledge about gut pathology, increasing this knowledge, and encouraging the development of gut pathology as a subspecialty. Not clearly stated in the by-laws, yet nevertheless part of the original objectives, was the issue of cooperative studies. One of the advantages of a national or international society composed of individuals working in a highly specialized area is the opportunity for data collection on a scale impossible at any single member's institution. Rather than reporting one or two cases of an entity, the possibility exists of reporting a large series. Rather than speculating on diseases based upon obviously inadequate data, the opportunity is presented to report data which are more likely to be adequate because of their volume. Such studies actually help to support the objectives of the Club as stated in the by-laws: they will lead to dissemination and increase of knowledge and will demonstrate that the existence of gastrointestinal pathology as a subspecialty, indeed, benefits the entire pathology community.

It is clear that the conjoint study issue is important. What is not clear is the responsibility of the members of the Club toward furthering such studies. Since it is not specifically stated in the by-laws that such a responsibility exists and that acceptance of membership entails acceptance of this responsibility, everything done in this area must necessarily be a voluntary activity.

What we have never defined is the responsibility that members of the Gastrointestinal Pathology Club have to each other and to our specialty in terms

of assistance when it is requested by one of our fellow members. We, the Editors of the Newsletter, wish to raise this issue for the membership at large. We would like to submit to the membership the position that conjoint studies are a necessary function of the Gastrointestinal Pathology Club, that they foster its objectives, and that they must be supported by all members. We further submit that acceptance of membership confers with it the willingness to participate in such studies and the agreement to do so on a regular basis. Finally, we recommend that the issue of conjoint studies and membership responsibility to them be incorporated in the by-laws under a new category entitled "Responsibilities of Members".

There are additional issues which need to be addressed in the cooperative studies category. For instance, has it ever clearly been stated how such studies should be instituted? Is this an issue that is automatically handled by the Education Committee, the Executive Committee, or ad hoc committees, or should it be defined and stated in the by-laws? Peculiarly enough, the by-laws do not mention such studies at all. We suggest that the Education Committee be responsible for approving such studies and appointing study committees, and that this be stated specifically in the by-laws as a function of the Education Committee.

Undoubtedly, there are other debates centering about the subject of conjoint studies. The Editors of the Newsletter would welcome letters to the editor from the members of the Club detailing their points-of-view.

GASTROINTESTINAL PATHOLOGY CLUB
SYMPOSIUM

DURING DIGESTIVE DISEASE WEEK

TUESDAY, MAY 14, 1985 8-10 PM

Versaille Room, Sheraton Centre, New York City

PATHOLOGIC AND CLINICAL DIAGNOSTIC CHALLENGES IN THE BIOPSY DIAGNOSIS OF ESOPHAGEAL MUCOSAL DISEASE

SPEAKERS

RODGER C. HAGGITT, Dept. of Pathology, Univ. of Washington.
KLAUS J. LEWIN, Dept. of Pathology, UCLA.
CYRUS E. RUBIN, Division of Gastroenterology, Univ. of Washington.
WILFRED M. WEINSTEIN, Division of Gastroenterology, UCLA.

TOPICS

1. Role of Biopsy in Gastroesophageal Reflux.
2. Barrett's esophagus and dysplasia.
3. Opportunistic infections.
4. Miscellaneous lesions (glycogenic acanthosis, squamous papilloma, etc.).

FORMAT

Topics will be case oriented with audience participation. Histologic slides will be examined microscopically and will be projected via television to monitors scattered throughout the conference room.

This Symposium has been made possible through the generous support of NIKON, INC. and GLAXO, INC.

International GI Pathology Group
(Summary to be published in International Pathology Bulletin)

A reception and meeting was sponsored by the American GI Pathology Club and held at the Fountainbleu Hilton Hotel in Miami Beach on September 5, 1984, at the time of the IAP Congress. Present were 50 persons from 22 countries.

Points of Discussion:

The notion that there be an international group of persons and societies interested in gastrointestinal pathology was fully endorsed. It was felt that the structure and governance should be loose and involve minimal costs. All areas of the alimentary tract and its ducts and glands (i.e., the liver, pancreas, and biliary tract) would be included.

Potential goals: For the present we might serve to provide a focus of identity and means of communication, by simply compiling a membership list. Future aims would include the fostering of education, training, and research. It was suggested that we might start by forming a union of existing societies, and this will be explored. Individuals from other countries could consider joining an established group but also might favor being an open member of the parent organization. Eventually, we would hope to promote additional national and regional societies.

Existing Groups:

For those interested in obtaining information and possibly joining an available society, they are listed below. Please let us know if additional societies exist.

Dr. Robin Cooke
President, Gastroenterological Society of Queensland
Pathology Department, Royal Brisbane Hospital
Australia 4029
(contact for Australian Society of Gastroenterology)

Dr. Geraint T. Williams
Secretary, British Society of Gastroenterology Pathologists Group
Department of Pathology, The Welsh National School of Medicine
Heath Park, Cardiff CP4 4XN, United Kingdom

Dr. Claude Degott
Secretary, Club d'Histologie Digestive
Departmente of Pathology, Hopital Beaujon
F 92118; Clichy Cedex, France

Dr. Robert R. Rickert
Secretary-Treasurer, Gastrointestinal Pathology Club
Department of Pathology, St. Barnabas Medical Center
Livingston, New Jersey 07039, USA

Next Steps:

Dr. Goldman will contact the secretaries of existing societies, asking them to discuss with their membership the possibility of starting an international group. To gain further input, we would ask interested persons to discuss the matter with your regional groups and pathologists and would welcome your comments.

If we encounter success, we can envision the start by some form of "Newsletter". Clearly, another organizational meeting will be needed, and this might be at the next large international meeting, possibly at the European Pathology Congress in Athens in September, 1985. Please direct any questions or comments to:

Dr. Harvey Goldman
Department of Pathology
Beth Israel Hospital
Boston, MA 02215, USA

ADENOCARCINOMA IN BARRETT'S OESOPHAGUS

A pathological study of dysplasia preceding and accompanying adenocarcinoma.

The aim of this study is to develop criteria for the rate of surveillance and possibly for prophylactic resection in order to reduce the mortality from this disease.

The method is to study the sequential development of dysplasia in reflux oesophagitis.. The study is to be an evaluation of dysplasia in patients with or without an adenocarcinoma.

Specifically we wish to examine specimens from up to 500 patients with reflux oesophagitis who had two or more biopsies of Barrett's oesophagus at different stated times and up to 150 with adenocarcinomas in Barrett's oesophagus or at the gastro-oesophageal junction, who have at least one histological sample each of mucosa just above and just below the adenocarcinoma. The presence or absence of a hiatus hernia should be noted and tissue samples from it so identified, if possible. These specimens will then be examined for incidence of intestinal metaplasia and for incidence and severity of dysplasia. All sections should have been stained with H&E (or HPS), PAS, Alcian Blue (pH 2.5) and High Iron Diamine stains. The last is optional, to be used if available.

The submitted cases will all be examined independently by the contributors. Criteria will first be established by correspondence, based on previous descriptions such as those of Morson (J. Clin. Path. 33, 711, 1980) or of Riddell (Am. Surg. 198, 554, 1983).

The samples should be submitted to:

Dr. J.V. Frei
Department of Pathology
University Hospital
P.O. Box 5339, Station A
London, Ontario, Canada
N6A 5A5

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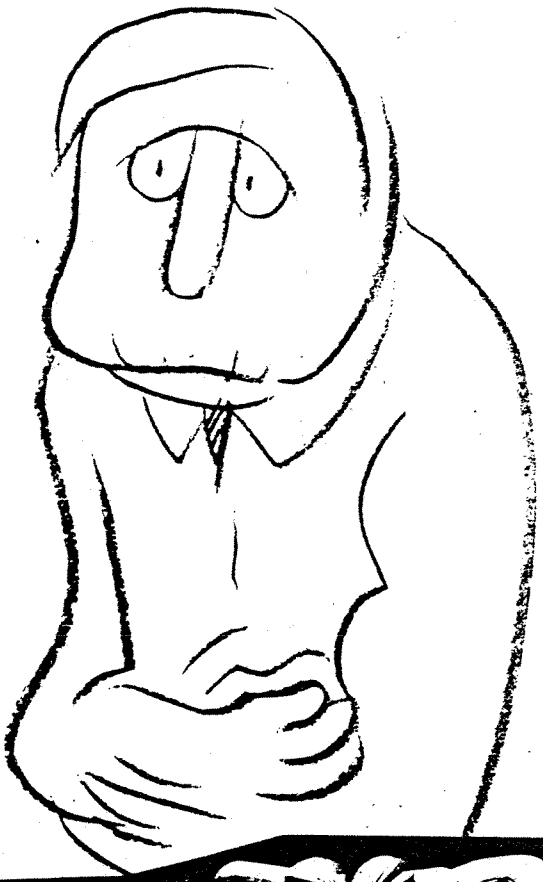

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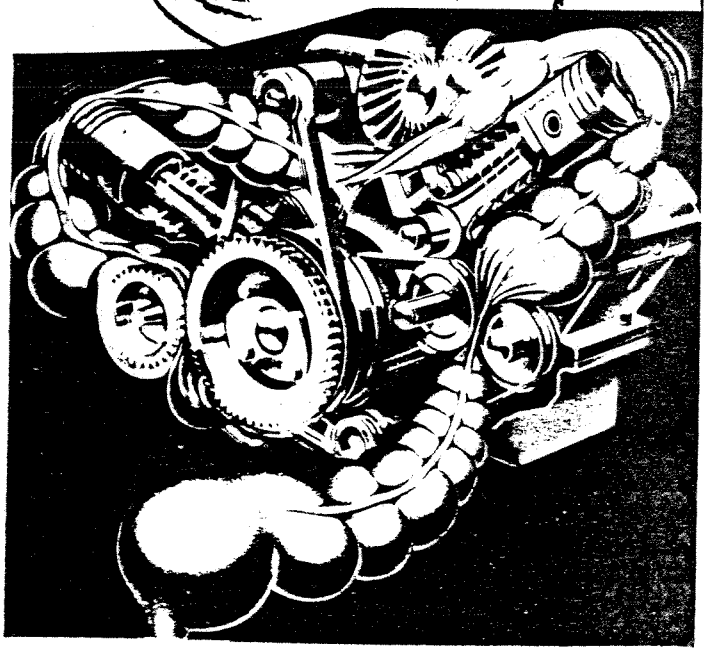

LESIONS OF THE MUSCULARIS PROPRIA

Since Schuffler's elegant description of some striking changes in the smooth muscle of the small bowel in patients with a motility disorder[1], there has been renewed interest on the part of pathologists in the previously much neglected muscularis propria of the gut. The findings that he described, fibrosis replacing smooth muscle elements, often encircling individual swollen smooth muscle cells and imparting a vacuolar appearance, have been seen in a growing number of patients[2]. This fibrosis is most commonly found in the longitudinal layer, although both layers may be involved.

These findings, while striking, can be overlooked if proper care is not exercised in handling specimens of bowel. When we suspect a motility disorder, we handle specimens in the following way. As soon as possible after resection, the specimen is rinsed (tap water is fine) and examined in the standard fashion of surgical pathologists. Fresh material is submitted for electron microscopy; this includes both circular and longitudinal muscular layers, and the myenteric plexus; this is accomplished by cutting multiple one millimeter cubes of tissue from a piece of muscularis propria containing the grossly recognizable junction between the circular and longitudinal layers. A piece of bowel is snap frozen (liquid nitrogen or isopentane) for acetylcholinesterase studies[3]. This should include mucosa as well as submucosal and myenteric ganglion plexus. The specimen is then carefully pinned out on a paraffin board, approximating its "natural" circumference by avoiding stretching. The specimen should be pinned out in the standard serosal surface down fashion, but interposing gauze 4 by 4's between bowel and board will insure proper fixation of the

deeper muscle layers. The object of these maneuvers is to obtain large stretches of flat well-oriented bowel. It is wise not to skimp on the formalin (standard buffered variety); if the bowel is particularly bloody, at least one change of formalin is recommended. After overnight fixation, transmural sections are taken, with great care to maintain proper orientation. At each site examined, one cassette containing two pieces of tissue are submitted. These two pieces of tissue represent one longitudinal and one cross section. This allows for comparison of the density of collagen deposition in the layers. This is most readily identified when the muscle fibers are viewed in cross section. If the specimen is stomach, several sections of body and antrum are submitted; even though this organ does not have the standard distinct circular and longitudinal layers, our sections are still taken parallel to and at right angles to its long axis. With regards to the colon, the minimal sampling should include right, transverse, and left colon. The cross sections here should include one of the taenia in its center; the longitudinal sections are ordinarily taken between the taenia. Special care should be taken throughout the gut when taking cross sections, since in this plane the layers of bowel tend to slide on one another resulting in poor orientation. Fresh sharp blades are recommended!

In addition to standard H&E staining, a trichrome stain is a <u>necessity</u>. We have found the standard Masson's technique to be satisfactory. In addition, we have employed the following stains as needed. Iron staining is employed when chronic ischemic damage is suspected; hemosiderin is much more difficult to pick up on standard H&E sections in bowel than it is in liver. A PAS with diastase is often useful in accentuating the ceroid

pigment seen in brown bowels, and often provides a new perspective on the muscular architecture. Kluver staining is also helpful for demonstrating the ceroid pigment. Amyloid can also infiltrate bowel wall and interfere with motility, and should be sought in the standard fashion (Congo Red, Thioflavine T). We have not found PTAH and reticulin to be of great use.

The chief findings one seeks are replacement of smooth muscle by collagen. In the familial forms of visceral myopathy[2], this finding is diffuse over relatively long segments of bowel (autosomal dominant form) or over almost the entire bowel (autosomal recessive form). Because of this, sampling is not usually a problem. In scleroderma, however, the lesions can occur in smaller segments, and greater care is needed in selecting the sites to be sampled. Because of the vagaries of fixation, it is not uncommon to find small clear areas in the cytoplasm of smooth muscle cells. This is not to be considered the vacuolar change characteristic of visceral myopathy. In order to be considered vacuolar change, the swollen smooth muscle fiber should be encircled by collagen fibers. In the familial forms, the change is usually limited to or is most severe in the longitudinal layer, although we have recently seen a family with an apparent predilection for damage of the circular layer. In the sporadic or non-familial cases, the pattern of distribution of the fibrosis is more erratic, but usually occupies a given location in a particular patient; for example, we have seen one 12 year old with severe motility problems who had fibrosis exclusively of the outer half of her circular muscular layer. To date, we have not identified lesions involving the muscularis mucosae. In all of our cases with muscle damage, we have not identified lesions of the myenteric plexus.

Electron microscopy has not revealed much except nonspecific damage of smooth muscle fibers, and collagen deposition. The contractile filaments are in a state of disarray, and the plasma membrane is discontinuous. These severely damaged cells can be found adjacent to entirely normal cells. The structures of the myenteric plexus are entirely normal ultrastructurally as well as by acetylcholinesterase. We have examined the myenteric plexus by the techniques described by Barbara Smith, and have found no lesions.

The approach described should identify any lesions of smooth muscle in patients with a motility disturbance. It is not yet clear what proportion of these patients have muscular versus neural problems. The latter are very difficult to demonstrate. Care should be taken to save a suitable piece of bowel for possible studies by the Barbara Smith technique. A flat 2 cm piece of bowel can be saved indefinitely in formalin. Folklore has it that prolonged storage improves the reaction with silver stains. One type of specimen seen not infrequently, i.e. colectomy for intractable constipation, does not usually show a defect in muscle. Recent evidence suggests that this is primarily a neural problem[5]. Jejunal diverticulosis can have either a muscle or nerve defect[6]; who knows what may be present in colon diverticulosis if properly studied. A variety of other diseases have been described that have significant motility problems, and have not yet been looked at in great detail. With the simple stratagems described here, you too can become a smooth muscular pathologist!

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HISTOCHEMICAL TECHNIQUES FOR EPITHELIAL MUCINS

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Epithelial mucins are a heterogeneous group of glycoproteins histochemically classified as neutral mucins that lack acidic reactive groups, sialomucins that contain sialic acids, or sulfomucins that contain sulfate radicals. The identification and characterization of mucins in normal, metaplastic, and dysplastic GI epithelium may have pathogenetic, diagnostic, or prognostic value. Since many histochemical techniques are available for detecting mucins, one problem is selecting those methods that are specific, reliable, technically simple, and easily interpreted. The basic procedures I use are the periodic acid - Schiff (PAS) reaction and two basic-dye methods, alcian blue and high iron diamine.

The PAS reaction strongly stains neutral mucins. Since much of the histologic world, including some sialomucins and sulfomucins, is also PAS-positive, staining results need to be critically interpreted. Removing confounding glycogen by diastase digestion often helps. PAS alone is not sensitive as a general epithelial mucin stain and is not specific for neutral mucins. However, the procedure is simple and is easily monitored with basement membranes serving as an internal control.

Alcian blue is an excellent technique for demonstrating acid mucins. By forming electrostatic bonds with acidic reactive groups, alcian blue provides strong and almost specific staining of both sialomucins and sulfomucins. As the pH of the alcian blue solution is changed, the ionization, and hence the intensity of staining, of the different acid mucins will change. At a pH of 2.5, the carboxyl groups of sialic acid in sialomucins and the sulfate esters of sulfomucins will be ionized and thus both mucins will stain. At a pH of 1.0, however, sialomucins will not be ionized and only sulfomucins will be demonstrated. Alcian blue at pH 2.5 is a good general method for acid mucins, as only strongly sulfated sulfomucins will fail to stain. Sections stained at

a pH of 2.5 can be compared with sections stained at a pH of 1.0 to distinguish sialomucins and sulfomucins. Alcian blue methods are technically straightforward and give reliable results. Rinsing sections at the same pH as the alcian blue solution improves the staining.

A combined alcian blue-PAS technique is useful in detecting all epithelial mucins as well as differentiating neutral and acid mucins. First, alcian blue at pH 2.5 is applied to stain the acid mucins, including those that are also PAS-positive, so that the subsequent PAS reaction stains only neutral mucins. The blue acid mucins are clearly and aesthetically distinct from the magenta neutral mucins. As a general mucin technique, alcian blue - PAS is more sensitive and more informative than mucicarmine or metachromatic stains.

The high iron diamine procedure fairly specifically demonstrates sulfomucins. Diamine salts and ferric chloride form a brown-black compound that binds sulfate esters present in sulfomucins. An alcian blue counterstain can be added to demonstrate any resident sialomucins, which will stain blue in contrast to the brown-black sulfomucins. This combined high iron diamine-alcian blue (pH 2.5) method provides, with one preparation, direct tinctorial differentiation of two types of acid mucins. Distinguishing sialomucins from sulfomucins is therefore simpler by this technique than by comparison of two alcian blue sections at differing pH. However, the high iron diamine method is technically more demanding and results can be inconstant so that appropriate positive controls are essential. The diamine salts can be toxic and must be handled with care.

Epithelial mucins can be practically identified and characterized with the two combined methods, alcian blue-PAS and high iron diamine-alcian blue. Neutral mucins, sialomucins, and sulfomucins are distinguished by direct color comparisons, so interpretation is facilitated. The procedures can be performed by any histology laboratory and methodologic details are given in most histochemistry textbooks. Although other techniques are available, these two preparations, in our hands, have provided accurate and reproducible results. The basic procedures can be modified by enzymatic digestion or blocking techniques such as methylation or saponification to change or improve specificity; these embellishments are more difficult to control and thus less

reliable and, because they often require comparison of slides before and after treatment, are more difficult to interpret. One noteworthy modification of the PAS reaction detects O-acetylated sialomucins, a subgroup of sialomucins found in the colon and small intestine, and in intestinal metaplasia of the stomach, Barrett's mucosa, and some carcinomas of these regions. The procedure, described by Culling and coworkers, is a modified PAS reaction that uses borohydrate reduction and potassium hydroxide saponification to specifically render only the O-acetylated sialomucins PAS-positive. The method requires careful attention to technique; proper controls are essential.

References:

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MUCIN CHANGES IN ULCERATIVE COLITIS

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Detection of early changes has been a goal of those seeking to reduce mortality from colorectal carcinoma. Almost two decades ago "pre-cancer" was identified in mucosal biopsies from patients with ulcerative colitis (Morson and Pang, 1967) and it was subsequently suggested that this finding might warrant prophylactic colectomy under certain circumstances. Since this dysplasia represents relatively advanced disease, attempts have been made since that time to detect biochemical changes in more normal mucosae in pre-malignant conditions. Thus, Deschner and Lipkin (1975) found that surface epithelium from areas of normal mucosa in patients with polyposis coli exhibited persisting DNA synthesis in contrast to normal subjects in whom DNA replication was confined to the deeper crypt areas. That group has suggested that investigation of mucosal biopsies or cytological material by means of in vitro radioautography with ^{3}H-thymidine might have value in the screening and management of high risk patients. Similarly, the role of alterations in mucin composition in pre-malignant diseases of the colon has been explored fairly extensively in the past decade. Most of the attention has been directed towards the mucosa adjacent to primary colorectal carcinomas. Filipe and Branfoot (1979) found an increase in the sialomucin/sulfomucin ratio in this mucosa and feel that such changes represent an early and primary event in the course of colonic carcinogenesis. Other investigators, while confirming the histochemical findings, claim that they are secondary to the presence of a nearby tumor mass and can be found adjacent to lesions other than primary colon carcinomas (Isaacson and Attwood, 1979; Listinsky and Riddell, 1981; Lev et al, In Press).

What is the evidence for mucin changes in ulcerative colitis? An early histological observation (Hellstrom and Fischer, 1967), subsequently confirmed by the majority of investigators, was that mucosal mucin was quantitatively reduced in ulcerative colitis but remained normal in amount in Crohn's colitis. These histological findings have been supported by recent biochemical studies demonstrating reduced carbohydrate content in mucins in ulcerative colitis (Clamp et al, 1981); a similar reduction was found in Crohn's colitis, however. Those workers also found that the oligosaccharide side chains were shorter in ulcerative colitis and speculated that this might have resulted from increased activity of endogenous glycosidases or decreased production of activated sugars due to cell damage. Another possibility was that the incomplete glycosylation was due to more rapid turnover of epithelial cells; although this sounds reasonable, one study of epithelial cell kinetics demonstrated prolongation of turnover time in active ulcerative colitis (Shorter et al, 1966). Another biochemical finding is that of a relative decrease in N-acetylation of glucosamine but this was noted in both ulcerative and Crohn's colitis (Burton and Anderson 1983). Histochemical studies have demonstrated increased sialomucin and decreased sulfomucin in ulcerative colitis (Ehsanullah et al, 1982; Culling et al, 1979); since this pattern was found in non-dysplastic as well as dysplastic mucosa the possibility that it might presage morphological dysplasia has been raised.

Other biochemical information has been made available by immunofluorescence studies of a prospective type with lectins (Boland et al, In Press). Using Dolichos biflorus and soybean agglutinins, which recognize N-acetylgalactosamine, and Bauhinia purpurea agglutinin, which recognizes galactose, a pattern of staining of goblet cells in surface epithelium was found in many cases of ulcerative colitis which resembled that found only in deep colonic crypts from

normal individuals. This pattern of "persistently immature" cells was seen in biopsies several years prior to the appearance of morphological dysplasia in those patients implying that it might have predictive value as a screening technique. The same group also noted general reduced binding of those lectins suggestive of underglycosylation and supportive of the biochemical conclusions of Clamp et al (1981) described above.

The most recent work demonstrating alterations in mucins in ulcerative colitis is that of Podolsky et al (1984) who have developed a microchemical method for analyzing mucins in mucosal biopsy samples of 2-3 mg wet weight. The mucins are partially purified, reduced and radiolabeled with NaB^3H_4, separated on a DEAE-cellulose column and eluted with NaCl solutions of increasing molarity. Of the 6 mucin species thereby obtained, one (number IV) was significantly reduced over normal in patients with both quiescent and active ulcerative colitis; only minor changes were found in two other mucin species. Mucosal biopsies from other inflamed mucosae, including patients with Crohn's colitis, did not show these changes and resembled biopsies from normal subjects. Since patients were not examined before they developed the disease, it is not known if the decrease in mucin species IV precedes or results from ulcerative colitis. Moreover, the basis for separation with DEAE-cellulose is poorly understood, although charge density plays a role since the fractions eluted at higher NaCl concentration contain more sialic acid (and presumably sulfate) than those obtained at lower concentrations. Species IV, however, has no distinctive profile, having an intermediate sialic acid concentration and low (but not the lowest) concentrations of galactose and galactosamine. Although glucosamine is one of the five major saccharides in colonic mucins, it was undetected with their method; one wonders if this may be related to the reduced acetylation of glucosamine found by Burton and Anderson (1983). Finally another major mucin

constituent, fucose, was not investigated. It is thus difficult to interpret the significance of their findings and to correlate them with previous biochemical and histochemical work. One apparent contradiction (mentioned by the authors) should be noted: they found no differences between anatomic segments of the colon whereas shifts in sialomucin/sulfomucin ratios have been noted histochemically. Another problem, alluded to by the authors, is that the source of their mucins (deep crypts vs. surface cells) is unknown. Despite these reservations, it is quite possible that future refinements of this micromethod may supply important information about the mucins in such pre-malignant states as inflammatory bowel disease, polyposis coli and the family cancer syndrome and may even provide the predictive value eagerly sought for during the early stages of carcinogenesis. Among such recommended modifications could be included separate analysis of surface and deep crypt mucins, quantitative analysis of all of the major sugars known to occur in colonic mucins and, should the technology become available, a search for structural differences between the various mucin species (e.g. the presence of substituents on the saccharide residues, the amount and location of sulfate esters, etc.).

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CRITIQUE OF A RECENT ARTICLE

Criteria for Gastric Dysplasia

Ming and his colleagues, working as the Pathology Panel of the International Study Group on Gastric Cancer (ISGGC), have collaborated to develop a set of criteria for the diagnosis and grading of gastric dysplasia.[1] Over a two year period the panel examined microslides of 93 gastric lesions demonstrating all gradations of epithelial abnormalities. The published system was arrived at during a three day workshop held in Italy in June, 1982.

The panel reached the following consensus:

a) Immature and proliferating gastric epithelium can be divided into two categories - hyperplastic and dysplastic.

b) The term dysplasia, especially of high-grade type, should be restricted to precancerous lesions, whereas hyperplasia is applied to regenerative changes.

c) Regenerative hyperplasia may be simple or atypical, but dysplasia includes both moderate and severe abnormalities.

Significant disagreement was encountered in about 15% of the cases reviewed, mainly in the differentiation between regenerative hyperplasia and mild dysplasia and between severe dysplasia and malignancy.

Since it is central, a statement from the paper defining dysplasia in the stomach should be quoted:

> "In the context of premalignant potential, the dysplasic gastric epithelium is defined as one showing prominent cellular and structural abnormalities and believed to have high propensity to malignant transformation, irrespective of the presence or absence of metaplastic changes."
>
> "This definition narrows the application of dysplasia, according to currently available information, to only severely abnormal tissue,

which alone has been shown to undergo malignant change after a period of observation.[2] Mild abnormalities commonly seen in benign conditions are not considered inevitably precancerous and are, therefore, not necessarily dysplastic."

At the benign end of the spectrum the term "hyperplasia" is applied to clearly regenerative epithelial proliferation in an area that is inflammed, ulcerated, or otherwise acutely injured. Regenerative hyperplasia is termed "atypical" when cell proliferation is active and prominent and there is much immaturity with hyperchromatism, pseudostratification, mitoses, etc. It is important to stress that the authors view such changes as not dysplastic and therefore not precancerous.

The paper clearly defines and illustrates the criteria used to identify dysplasia and gives a detailed description of the morphologic features that help to differentiate dysplasia from reactive epithelial changes. The authors also provide a helpful tabulation of previous grading systems for gastric dysplasia. Their table has been reproduced here, along with the recently published system for classification of dysplasia in inflammatory bowel disease (Table I).

Comments:

Consideration of Table I should help the reader to understand why the new scheme by Ming, et al. is a significant step forward in characterization and classification of gastric dysplasia. The overview of prior schemes not only makes vivid the wide variation in terminology and the inevitable ambiguities that have prevailed over the years. It also emphasizes how terminology and classification of gastric dysplasia has evolved towards a sharper definition of dysplasia and a greater recognition that there are limits to the pathologist's ability to categorize borderline findings and to predict that a dysplastic lesion is or will become invasive. Thus, whereas in earlier schemes dysplasia was equated more or less with "atypia" and was sometimes used for circumstances where it was a near

certainty that the epithelial changes were not precancerous, the term was applied in later classifications only to circumstances where a relation to malignancy was felt clearly to exist. The current paper strongly reflects these evolving views, as is implicit in the descriptions and quoted statements given above.

We do have concern with regard to use of "hyperplasia" by Ming, et al. to describe non-dysplastic epithelial changes. Strictly speaking hyperplasia simply means increased number of cells, whereas the histopathologic changes seen after mucosal injury often reveals that the number of proliferating cells in the early phases of regeneration is less than is normally found in foveolar epithelium. And if the injurious agent persists, regeneration will sometimes remain incomplete, frequently giving rise to an "atrophic" rather than "hyperplastic" mucosa. On the other hand, if the regenerative process is successful and the epithelium is restored ad integrum, proliferating cells can outnumber those found in normal mucosa, thereby resulting in hyperplasia in its correct sense.

Because of this range of possible outcomes from regeneration, we believe that using the term hyperplasia strictly according to its original meaning not only serves semantic or academic purposes. It also has important practical implications, particularly when one is dealing with endoscopic gastric biopsies. Surface or foveolar hyperplasia, for example, is found as a focal change in inflammatory lesions, hyperplastic polyps, Menetrier's disease, Peutz-Jeghers polyps, and certain forms of hypertrophic hypersecretory gastropathy.[10] In these conditions, the hyperplastic cells closely resemble their normal counterpart. If applied in an indiscriminate way, use of the term hyperplasia in the context of a system for classifying dysplasia could, therefore, also obscure and confuse understanding of diseases that involve "expansions of the gastric mucosa."[10]

The trends in terminology, definition and approach to gastric dysplasia seen in Table I have counterparts in development of the recently published system for classifying dysplasia in inflammatory bowel disease (IBD).[3] That system, however, goes even further towards narrow definitions and acceptance of predictive limitations. For instance, for the IBD/dysplasia system dysplasia is defined as: "... an unequivocal neoplastic epithelial proliferation. It may be non-invasive (i.e. benign), but it may also be or may have the potential for becoming invasive."[3] The IBD/Dysplasia system also gives even stronger recognition that there will inevitably be circumstances where a clear decision cannot be made about presence of dysplasia. It does this by including a central category of "Indefinite" (Table I). The Indefinite category is then further subdivided according to the pathologist's best judgement as to the likelihood that the findings represent dysplasia. The Indefinite category can thereby be used by the pathologist for a committed opinion that is as strong as when declaring that a specimen definitely does or does not show dysplasia. The longstanding and repeated injury and regeneration which is characteristic of inflammatory bowel disease probably intensifies the need for an "Indefinite" category. But in our experience there is a similar need in dealing with dysplasia generally, especially when inflammatory and regenerative changes are noted. (In the scheme of Ming, et al. uncertainty about presence of dysplasia is partly covered by the term "atypical hyperplasia".)

The IBD/Dysplasia system also accepts the fact that cytologic criteria for dysplasia do not permit absolute determination as to whether cancer (invasive disease) is present. This decision was influenced by a practical reality, i.e., that patients with IBD and dysplasia on biopsy will often show invasive disease in their colectomy specimens. But again, similar circumstances can prevail in gastric and other forms of intestinal cancer. In addition, the IBD/dysplasia system has limited the degrees of severity of dysplasia to two: Low grade and High grade.

This, too, was partly contingent on practical considerations. For instance, distinguishing between High grade dysplasia that is merely "severe" and "in situ carcinoma" will vary between pathologists. Making such a distinction is also of no great practical importance in inflammatory bowel disease since it is widely agreed that all cases of High grade dysplasia should be viewed as candidates for colectomy. Similar practical considerations may or may not apply to other circumstances where the the IBD/dysplasia system might be used, but we feel that two degrees of dysplasia could prove satisfactory for most purposes.

No claim can or will be made that the system for IBD/dysplasia is foolproof. Indeed, its limitations as a means of achieving uniformity of opinion among pathologists was clearly evident from data collected during its development.[3] But the terminology is so general that in our experience only slight modification is needed for use in other settings (principally relating to the characterization of "Negative" according to the activity level of the inflammatory bowel disease). Thus the IBD/dysplasia system may well provide a format that has broad applicability for recognizing and classifying dysplasia. We would be interested in learning of the experiences and views of others on this point.

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Table I

Grading Systems for Dysplasia of Stomach and in IBD (colonic)*

Nagayo (1971)[4]	No atypia	Slight atypia	Borderline	Probable cancer	Cancer
Grundman and Schlake (1979)[5]	Inflammatory	Mild dysplasia	Moderate dysplasia	Severe dysplasia	
Oehlert (1979)[6]		Grade I	Grade II	Grade III	
Ming (1979)[7]	Grade 1	Grade 2	Grade 3	Grade 4	
	Hyperplastic dysplasia		Adenomatous dysplasia		
Cuello et al. (1979)[8]	Mild	Severe	Mild	Severe	
Morson et al. (1980)[9]	Inflammatory regenerative	Mild dysplasia	Moderate dysplasia	Severe dysplasia	
	Hyperplasia		Dysplasia		
ISGGC (1982)[1] (Current study)	Simple	Atypical		Possible carcinoma	
Dysplasia in IBD (1983)[3]	Negative (no dysplasia)	Indefinite(?dysplasia)		Positive (dysplasia)	
	Normal Inactive IBD Active IBD	Probably Negative	Unknown Significance	Probably Positive	Low Grade / High Grade

* Reproduced (with references) from Ming, et al.1 except for portion on dysplasia in IBD.