Scanning Microscopy

Volume 3 | Number 3 Article 24

10-9-1989

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Scanning Microscopy, Vol. 3, No. 3, 1989 (Pages 929-935)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/89$3.00+.00

GASTROINTESTINAL SURFACE CHANGES: INTERPRETATION PROBLEMS AND INDEXING POSSIBILITIES (A REVIEW)

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(Received for publication July 1, 1989, and in revised form October 9, 1989)

Abstract

The purpose of this review on state-of-the-art and new perspectives on the use of scanning electron microscopy (SEM) in gastrointestinal pathology is to discuss the possibility of developing an index for quantitatively grading mucosal epithelial injury. This topic is reviewed within the framework of ulcer indices previously developed for gross lesions, where analogous problems exist, and in relation to the transmission electron microscope staging of epithelial cell pathology. If such an index could be developed it would increase objectivity and standardization of data analysis from laboratory to laboratory, and would allow for quantitative and statistical analysis of morphometric data. It is concluded that an index is possible based upon fields of injured cells rather than upon the grading of individual cell injury progression. An example of a useful SEM lesion index is presented. There are definite limitations to development of such an index, and guidelines are provided to help minimize some of the numerous complicating factors. These guidelines include comments on magnification, tissue contour, cell versus tissue analysis, morphometric considerations, sources of error, and other factors.

Key Words: Stomach, intestines, mucosa, pathology, duodenum, ulcer, erosion, gastritis, index, gastrointestinal.

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Introduction

The ultrastructural analysis of normal and diseased gastrointestinal tissues has for many years contributed to enhancing understanding of digestive tract functions. Scanning electron microscopy (SEM) of the gut surfaces has played a supportive role in providing surface perspectives to the more detailed information provided by transmission electron microscopy. Early scanning electron micrographs of the gastric mucosal surfaces of human and animal tissues (Ogata and Murata, 1969; Pfeiffer, 1970a, b, Pfeiffer and Weibel, 1973) and of intestinal tissues (Toner and Carr, 1969; Marsh and Swift, 1969; Balcerzak et al., 1970) have been available for the past two decades, and three gastrointestinal atlases have now been published presenting considerable SEM information, including texts by Toner et al. (1971), Pfeiffer et al. (1974), and Motta and Fujita (1988). Generally, SEM analyses have focused on mucosal surfaces which are the main site of disease and normal absorptive functions; however, SEM of the serosal surface (Furubayashi et al., 1984; Pfeiffer et al., 1987a) has also revealed complex morphology suggestive of active transport or other functions.

Since mucosal disease processes often begin within or significantly involve the surface epithelium of the mucosal layer in response to drugs, bacterial, viral, or other infectious agents, or other lumen-derived factors, the pathogenesis of mucosal disease can best be studied by ultrastructural analysis of the epithelial lining. Such fine structural responses of the epithelium, for example, to common drugs, have earlier been reviewed by the present author (Pfeiffer, 1975). Acute and chronic cell injury progresses through a number of predictable stages which have been delineated by transmission electron microscopy (TEM) and involves the regulation of cytosolic calcium (Trump et al., 1989). This staging has been characterized recently by TEM for acute cell injury induced by cysteamine in rat duodenal epithelium (Pfeiffer et al, 1987b), but will not be reviewed here. Until now, the important use of SEM in pathology of the gastrointestinal mucosa has largely been devoted to qualitative analysis of structural changes with little use of morphometric study, or of any form of indexing of the extent of pathologic change. It is therefore appropriate in a workshop on state-of-the-art level of attainment, and future perspectives of SEM in pathology, to consider the possibilities for quantitative indexing

of mucosal surface damage. The present brief review will generally address, after a brief historical background, the questions: 1) Is it possible to devise a quantitative index for SEM pathology of the mucosal surface? 2) If it is possible, can such a method be practical and useful for routine use? and 3) What specific guidelines can be recommended for such an SEM index?

Historical Background

Although a wide variety of adverse stimuli can induce pathologic changes in the gut mucosa, and numerous cell types are present within the epithelium, the range of acute and chronic histopathologic changes which have long been characterized for the mucosa is not great, and ranges between hyperemia and superficial gastroenteritis to severe, penetrating ulceration along with a variety of connective tissue and immunologic responses. These changes have frequently been reviewed in the ulcer field for both experimental animal and human clinical situations (Pfeiffer, 1971a; Morson and Dawson, 1973; Pfeiffer, 1982; Szabo and Pfeiffer, 1989). Accordingly, for many years indexing systems which attempt to semi-quantitatively grade the severity of ulcers and superficial erosions (Fig. 1) have been used in animal experimentation. Representative indices, which usually are based upon visible, gross assessment of lesions, are shown in Table 1. Most who have used these indices in grading pathologic responses will concur that all indices are imperfect. Many of the problems encountered in their implementation can be anticipated to be analogous to those which will be encountered in indices for assessing gastrointestinal lesions as viewed by SEM.

SEM Lesion Index: Rationale and Orientation

As mentioned above, the SEM analysis of gastrointestinal mucosa damage has usually depended upon qualitative evaluation of the nature and degree of pathologic change. However, the scientific value of such determinations would be enhanced if a meaningful numerical system could be used to classify the responses. Quantification of the data would also allow statistical testing of the response variability and differences between treatment groups or different populations. Assuming that the criteria selected to be enumerated can be discriminated with minimal error, the objectivity of the assessment would also increase, thereby allowing greater comparability in judging the pathologic response from laboratory to laboratory, and from viewer to viewer within the same laboratory. Thus, the ultimate goal would be to eliminate all subjectivity. In reality this ideal will never be achieved but nevertheless the rationale of developing a suitable SEM index is to approach this goal.

Lesions upon the mucosal surface, depending upon their size and stage of development, range from the smallest sign of damage to the individual cell such as distortion of the apical surface or single minute cavitations on the cell surface to severe ulceration where the epithelium has disappeared and villi, if normally present, are denuded. All of these changes can be observed by scanning electron microscopy, but only significant tissue damage can be visualized grossly for calculation of an ulcer index. Light microscopic analysis also generally can only appraise damage to tissue rather than cells. By scanning electron microscopy early damage to multiple cells, i.e., constituting tissue damage, can be detected, as well as damage which may be limited to a single cell. Since the term "ulcer" denotes cavitation into the tissue, the term "ulcer index" should be restricted to grossly observed tissue damage, and the term "lesion index" (=LI) is more appropriate for the cellular damage observed by SEM. This SEM cellular damage may reflect cell lesions or tissue lesions. As described below, a lesion indexing system is more suitable for tissue lesion evaluation by SEM than for cell lesion evaluation.

Table 1: Examples of Ulcer Indices for Assessing Gross Pathology

Ulcer Index =
1. Average sum of lesion lengths
2. Average sum of elliptical areas (L x W x $\pi/4$)
3. Average percent of total area ulcerated (morphometric analysis of photographs)
4. Average of severity rating x number rating
5. Average severity rating (Graded 1-5)

Pathologic Changes to Individual Cells

Pathologic surface changes on individual epithelial cells of the gastric or intestinal mucosa present an interesting reflection of cellular vitality, and indeed illustrate dynamic changes which may have either luminal or intracellular origin. These changes show a progression of cell injury and eventually total destruction of the cell. In interpreting such changes as they might be induced by disease or chemical (or drug) intoxification, it must always be remembered that the normal rate of epithelial cell replacement is extraordinarily high in the gastrointestinal tract (100% turnover rate in approximately 2-6 days), so that a few moribund or dead cells are normally observed on the gastric mucosal surface or near tips of intestinal villi. The degree and type of SEM detectable changes must be differentiated with respect to normal or induced cell damage. There is much morphologic similarity at the cell level between normal death due to senescence and induced injury and death due to exogenous factors. However, differences can be discerned in part by quantity of cells showing injury, the abnormal clustering of damaged cells, and in some cases the location of cells showing damage. Early workers (Grant, 1944; Grant et al., 1953) outlined the normal shedding of epithelial cells by light microscopic criteria, and most cells seem to be normally sloughed in a controlled manner with rapid restitution of the epithelial surface. Some individual epithelial cells also undergo as a normal process an in situ degeneration, in contrast to sloughing of the entire moribund cell, as we have documented by SEM in human, ferret, and monkey tissues (Pfeiffer, 1970b). This latter process, in situ degeneration, is present in healthy tissues but likely accounts for only a small percentage of the epithelial cell loss. Thus, in determining an SEM lesion index, it must be remembered that normal, healthy tissue (Figs. 2A, B) will demonstrate some, but a very low degree of cellular damage.

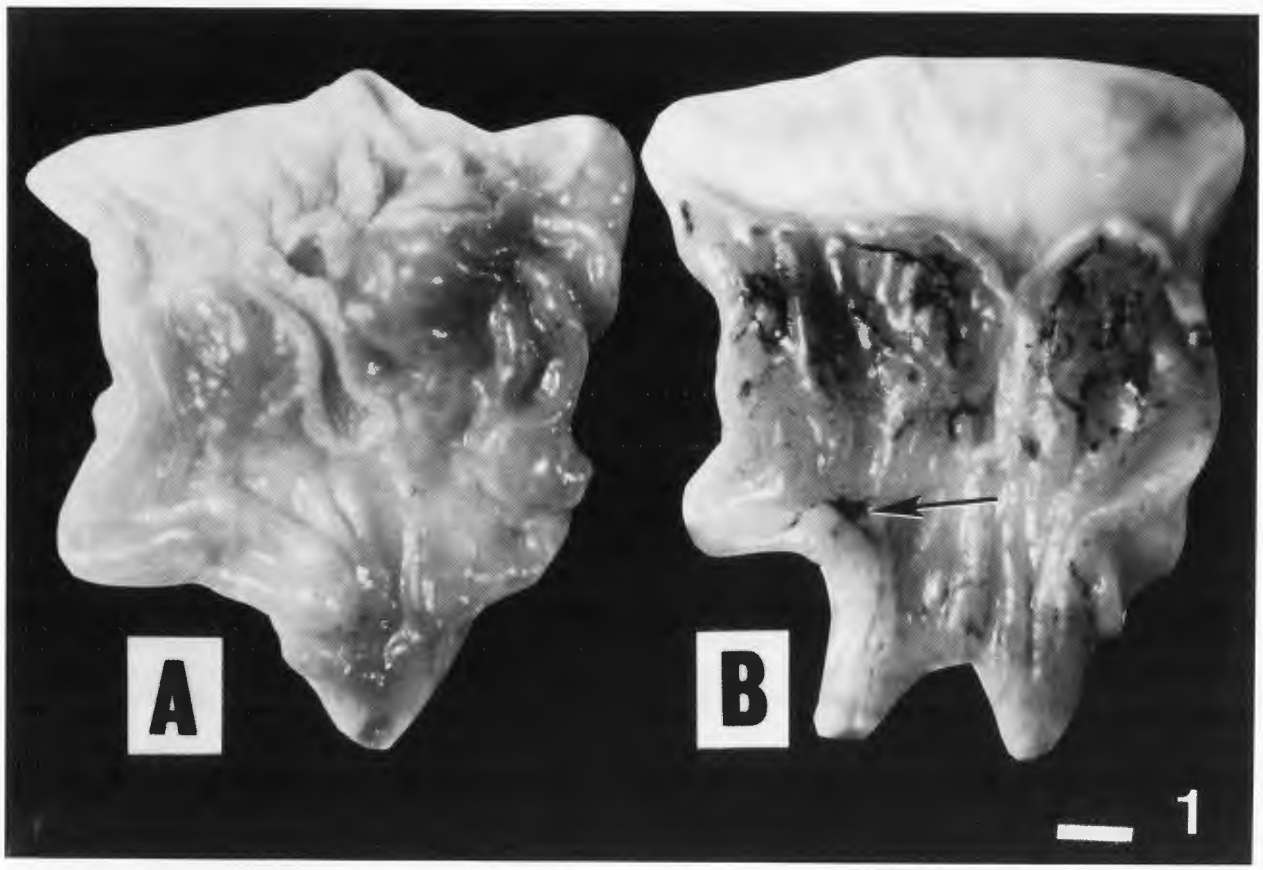
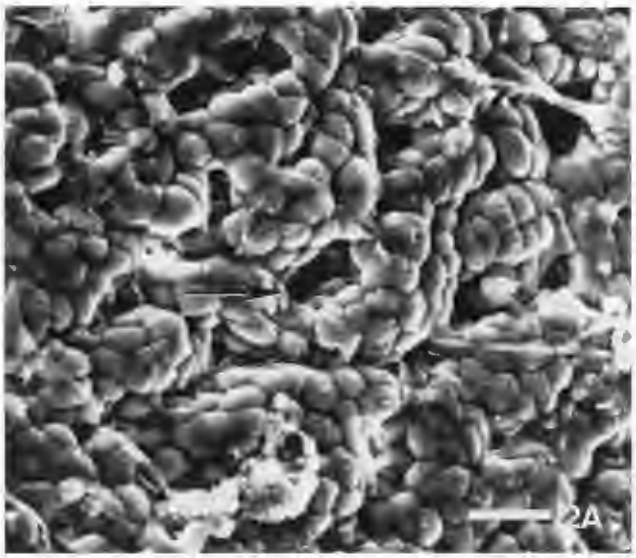
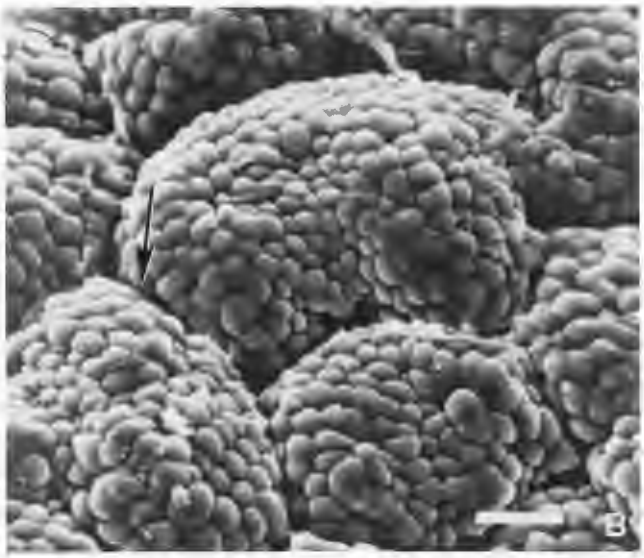


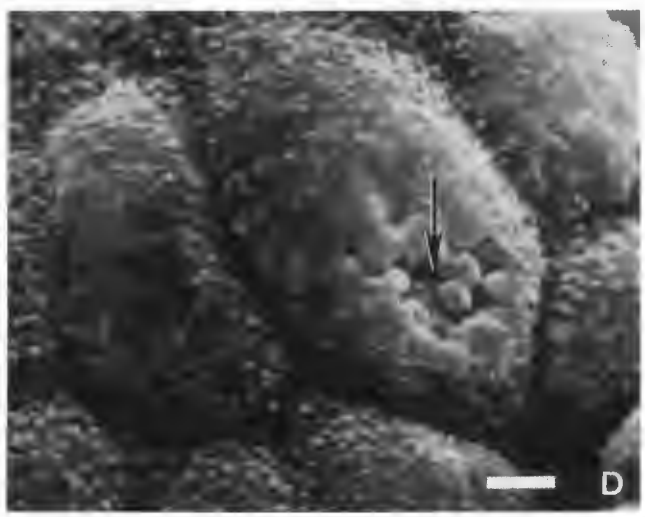
Fig. 1. Many types of conventional ulcer indices have been developed (see Table 1) to score gastric lesions such as shown here in the mouse stomach (arrow) B, which were induced by cold water immersion. A control stomach, A, is shown on the left. Such ulcer indices are used mainly for assessing gross lesions in the rat stomach. Bar = 0.25 cm.

At the level of the individual cell it is possible to create a semi-quantitative index which characterizes the extent of cell injury for either TEM or SEM analysis. The present review is not directed toward TEM analysis but as mentioned in the Introduction, cell injury viewed by TEM progresses through regular stages. They begin with cellular swelling and intracellular edema related in part to ionic shifts and changes in membrane permeability, to vacuolation and swelling of endoplasmic reticula, through mitochondrial swelling and damage, distortion of microvilli of gut cells, etc... Indices at the TEM level can be meaningful because the organelle-related and membrane-related changes have important functional implications. This staging of gastrointestinal cell injury, though not its index, has been done at the TEM level during the study of the duodenal ulcerogen, cysteamine (Pfeiffer et al., 1987b).

At the SEM level cell injury of individual gastrointestinal epithelial cells can be seen to progress from pleomorphic changes (Fig. 2C) and minute single cavitations (Fig. 2D) to larger cavitations which ultimately may include the entire apical surface. This progression of damage has also been shown after cysteamine administration and correlated with the TEM changes, the latter which show that intracellular injury begins earlier (within minutes after drug administration) than detectable surface changes (Pfeiffer et al., 1987b, c). From SEM perspective, and at the level of the single cell, this morphologic transition closely resembles the *in situ* degeneration which can take place normally (Pfeiffer, 1970b). Other manifestations of cell injury can also occasionally be seen by SEM, including cellular swelling. The changes on microvilli, which are clearly discernible by TEM, are more difficult to observe by SEM. From the foregoing statements, it can be concluded that it is indeed possible to construct a numerical index which would morphometrically quantify the extent of injury (e.g., percent of surface area showing cavitation) observed on a single epithelial cell, and a mean index could be computed for a population of damaged individual cells. However, in the authors' opinion such an index based on individual cell responses would not be meaningful because of the known dynamics of this cell type. That is, the progression through such stages is very rapid, and the initial SEM detectable cavitation is not the earliest evidence of cell injury, as proven by TEM analysis. Cavitation is unlikely to be a reversible type of cell injury, and it will inevitably be followed by cell death, although ultrastructural techniques do

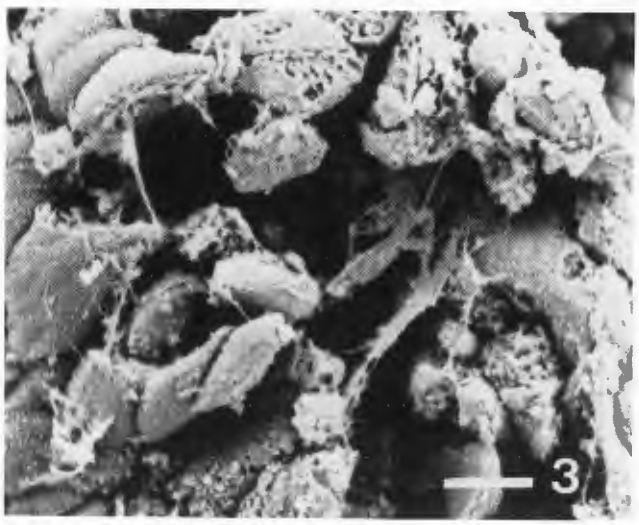

2A


B


D


E


F



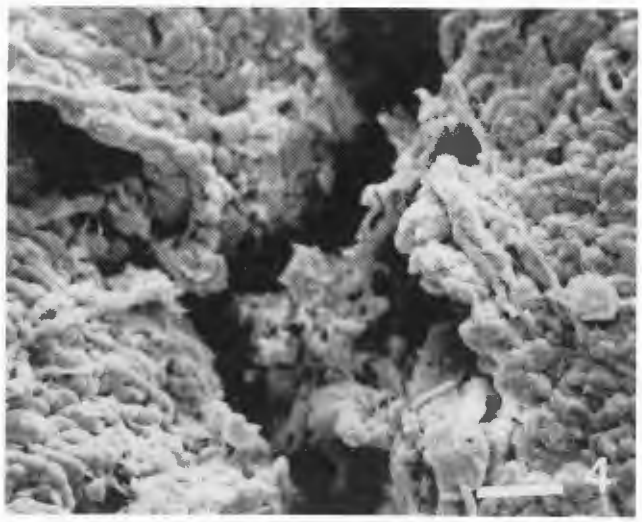


Fig. 3. Compared to Fig 2F, the surface damage evident by SEM (in this case on the duodenum in a cysteamine-treated rat) is more advanced, making estimation of damaged cell number impossible. Bar = 10 μm.

Fig. 4. A still more advanced ulcerative lesion is shown here for the cold water-stressed mouse. This lesion is on the stomach, and clearly is so large as to make SEM quantification difficult. It would be grossly visible. Bar = 40 μm.

not allow this to be proven. Furthermore, at any given point of time, cells in all stages of SEM detectable injury may be observed, all likely passing rapidly through the progression. In conclusion, at the level of individual cell injury, SEM permits useful staging of pathology from a limited perspective, but does not lend itself to development of a meaningful quantitative index.

Pathologic Changes to Fields of Cells

It is in the quantitative analysis of fields of surface epithelial cells that an SEM lesion index can be developed that is biologically meaningful. Thus, at the SEM perspective, actual tissue damage is appraised but it is based upon injury of cell surfaces. Several criteria can be studied in this respect, including *in situ* degeneration and sloughing (which may be increased above levels for normal tissue), and changes in cell surface configurations such as swelling or blebbing. Following per os exposure of experimental animals to irritants such as acetylsalicylic acid or ethanol, or to non-irritant ulcerogens such as cysteamine, enlarging fields of injured surface epithelial cells (Figs. 2E, 2F, 3, and 4) can be readily observed by SEM (Pfeiffer and Weibel, 1973, Pfeiffer et al., 1987b, c). In these instances clusters of *in situ*

⇦Fig. 2A. SEM perspective of normal rat gastric antrum. Note individual surface epithelial cells, and openings (arrow) to pyloric glands. Bar = 20 μm.
Fig. 2B. SEM perspective of normal mouse gastric antrum. Individual epithelial cells are clustered in groups separated by depressions (arrow) on the gastric surface, known as mucosal surface convolutions (Pfeiffer, 1971b). Although most cells in this view appear normal in this untreated animal, note that the magnification would be too low to assess accurately damaged epithelial cells for an SEM lesion index. Bar = 20 μm.
Fig. 2C. One of the inconsistent and early changes that can be observed by SEM on the duodenal mucosal surface, perhaps due to drug-induced osmotic changes, is pleomorphic distortion of epithelial cells. In this case it was induced in the rat by per os administration of the duodenal ulcerogen cysteamine. This type of pathologic change, also seen during carcinogenesis, does not lend itself to quantitative indexing. Bar = 10 μm.
Fig. 2D. One of the earliest pathologic changes seen by SEM on mucosal surface epithelial cells is cavitation (arrow) which usually begins as a central cavity smaller than the one shown here. The small nodules are surface microvilli, and the indentations delineate cell boundaries. This *in situ* degeneration is in human antral mucosa. Bar = 1.5 μm.
Fig. 2E. A few areas of localized cellular degeneration (arrow) can be observed here on a duodenal villus of a rat treated with the ulcerogen, cysteamine. In this case, numbers of damaged cells, or relative areas of damage can be measured and converted to an SEM lesion index. This represents early stage damage. Bar = 20 μm.
Fig. 2F. This cellular injury was induced by per os administration of acetylsalicylic acid to the laboratory ferret. Note that a cluster of epithelial cells shows significant early damage, but damaged cell number can be roughly counted. Bar = 2.5 μm.

damaged cells can be seen as a consequence of earlier identified single cell injury which is initiated (at SEM level) by minute surface cavitations. Although pleomorphism (Fig. 2C) can sometimes be observed following cysteamine treatment, it is obviously difficult to morphometrically analyze. However, the quantification of early injury, which includes enlarging areas of damaged cells, can be quantitatively assessed by determining the relative numbers of injured cells or areas of cell injury. Computerized planimetry of photo enlargements, and statistical analyses can be undertaken on cell number of area data. This method is best restricted to early tissue lesions, since if surface erosion is sufficiently high, boundaries of damaged cells cannot be determined, making cell counting impossible. Control treatments, in which the tissues may show some normal level of damaged cells, should be studied for comparison. An example of a calculated SEM lesion index for one particular field of cells can be shown for Figure 2E, a duodenal villus. In this case 18 of 376 cells demonstrate injury (4.8 percent) and computerized planimetry shows that 15 percent of the total field area is injured. The discrepancy between these two assessments illustrates the hazards of taking area measurements if the surface is not flat, with resultant distortions in cell surface area. If a flat surface is assessed both ways (cell number and area), the results should be comparable. This can usually be done with the gastric surface, but with intestinal villi lesions usually appear near the apex, where curvature is greatest.

The use of such an SEM lesion index, based on fields of cells, is not without other inherent problems, including possible differences of opinions of various investigators on a) which criteria best reflect injury, b) the subjective discrimination during analysis of boundaries of those criteria, c) the great amount of time needed for final morphometric analysis, and d) the risk of false quantitation, i.e., assigning numbers to inappropriate criteria. In addition, the investigator must be experienced enough not to be misled by other variables or artifacts, such as exuded mucus, curvature of the surface which distorts cell size and area determinations, inadvertent mechanical disruption of the surface epithelium, goblet cell openings, etc. Further, it should be mentioned that utilization of an SEM index to assess gastrointestinal mucosal pathology is not apt to become a routine procedure since the procedure requires electron microscopic equipment and expertise which is not universally available. In conclusion, and in spite of the hazards and limitations listed above, an SEM lesion index can provide a useful semi-quantitative measure of early damage to the gastrointestinal mucosa. Based upon our extensive experience with gastrointestinal pathology, but only preliminary experience with SEM lesion index development, we offer the following guidelines.

Recommended Guidelines for SEM Lesion Index

Restrict use to early tissue damage, i.e., damage of multiple cells when individual cells remain distinguishable.

If damage is unicellular, progression or stage of cytopathology can be judged, but indexing is meaningless because a) response of one cell not biologically important, and b) probable great rapidity of transit through pathologic stages in single cell.

If damage is too advanced, area and/or number of cells lost cannot be discerned. Such extensive damage can be assessed alternatively by conventional Ulcer Index.

Undertake morphometric analysis of number of cells damaged per standardized field, or of relative area damaged. S.E.M. L.I. = Average Percent Area or Average Percent of Cells Damaged. Assess multiple fields of multiple samples taken from standardized areas.

Assess flat surface. If curvature of villus must be assessed, use S.E.M. L.I. based on Average Percent of Cells, not on Average Percent Area.

Assess SEM Lesion Index at optimal magnification, i.e., 800-1,200 x.

Definition of cellular damage less accurate at lower magnifications and field size and cell number too small at higher magnifications.

Due to complications arising from variation in degree of damage and types of damage to different cells, simplify by assessing "damaged" or "normal."

Be aware of complicating factors, including: a) mucus; b) debris; c) dehydration artifacts; d) mucosal surface convolutions and other normal structures; e) normal degree of in situ degeneration and desquamation; and f) damaged cells due to inadvertent mechanical effects during tissue processing.

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Discussion with Reviewer

B. R. McPherson: The use of the cell number and area index on flat surfaces only is somewhat limiting when you consider the topography of many epithelial sheets, particularly that of the GIT. Wouldn't montage/mapping topographical photography of the epithelial sheet be a more effective method of analysing a greater area and number of cells?

Authors: The reviewer correctly mentions that all number and area indices are somewhat limiting. He mentions the interesting idea of montage mapping, and we do agree that this would add greater area or cell numbers. As this would add one more significant labor intensive step to the indexing process, which already is quite laborious, we do not include it here as a recommended step. As a matter of fact, the entire question of SEM indexing (even by the simple method we describe) can be challenged as non-practical (as one other reviewer alluded). We, as authors of this topic, simply discuss this novel process in the perspective of a "possibility", rather than an adamant promotion.