Paparella: Volume I: Basic Sciences and Related Disciplines

Section 3: Histology and Pathology:

Part 2: Head and Neck

Chapter 21: Pathology of the Upper Aerodigestive Tract Mucosa

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It is clear that it would be foolhardy to attempt to consolidate all available information on head and neck pathology in a single chapter. Invariably, such attempts result in a superficial overview of a multitude of complex topics, which leaves the reader with little new knowledge and no satisfaction. In consultation with the editors, the author has decided to narrow the scope of subject material in this chapter and to concentrate on general principles of surgical pathology as it is applied to head and neck surgical specimens and the pathology of squamous mucosa. In several of the controversial areas, the author has taken the liberty of simplifying complex topics and injecting the interpretations that have helped in actual practice over the years. Understandably, the authors' views are not infallible but should form a foundation from which the reader can subsequently agree (or disagree) after gaining additional experience and knowledge about diseases of the head and neck. In several complex areas of classification, the organization of complex and potentially confusing areas of pathology have been greatly simplified. The author recognizes that many colleagues competent in head and neck pathology may not agree with this approach, but the author feels strongly that the student of otolaryngology needs a simplified framework of basic knowledge on which to structure future knowledge.

Pathology of Squamous Mucosa of the Oral Cavity, Oropharynx, Pharynx, and Larynx

Head and neck pathology is not a recognized subspecialty. In institutions with extensive head and neck cancer programs, it is common for one or more pathologists to develop special interests in diseases of the head and neck region. One of the attractions of head and neck pathology is the great variety of diseases encountered. However, squamous cell carcinoma clearly represents the most common neoplasm encountered in the wide spectrum of head and neck tumor pathology. Although squamous carcinoma is exceedingly common, little clinicopathologic research on squamous carcinoma of the aerodigestive tract is reported in the literature. Areas of investigation that need to be pursued are the great variability of biologic behaviour, histologic expression, relationship to intraepithelial precursors, and growth patterns of squamous cell carcinoma that occur in different anatomic sites in the head and neck. The pathologist must have a comprehensive knowledge of the various appearances of squamous cell neoplasia, and an appreciation of the clinical presentation and biologic behavior of the disorder and how these variations in behavior relate to treatment in order to make a meaningful contribution to patient management. Without an understanding of the clinician's concerns in treating squamous cell carcinoma, the pathologist is not able to communicate the pertinent features that contribute to the

best clinical strategy.

Biopsy Technique

Tissue biopsy remains the time-proven technique for establishing the diagnosis of squamous cell carcinoma. Several types of biopsies are routinely performed. The most common type is performed with the cup forceps, which utilizes a pinching and shearing separation of the tissue sampled from adjacent tissue. Surprisingly, little tissue distortion results from these biopsies. Major pitfalls of this technique are use of too small cut forceps and superficial sampling of necrotic debris or keratin or only superficial portions of tumor. Many surgeons believe that superficial portions of tumor are sufficient to support the clinical impression of cancer and that the only role for the pathologist is to confirm the clinical impression of cancer. Important information regarding the growth pattern and differentiation of the neoplasm is not possible with superficial biopsies. Although cut forceps biopsy is a time-tested technique, problems may occur during the removal of tissue from the forceps. This is often performed by the other team members who may not understand the delicate nature of biopsy tissue. A fine needle is often helpful to "pluck" the tissue in order to avoid further crush and mechanical distortion. Shaking or rinsing the forceps in sterile saline also allows removal of the tissue from the biopsy forceps with minimal distortion. Adequate, carefully handled tissue samples with minimal distortion are more likely to allow proper orientation at the time of embedding. Although proper tissue orientation may not be of importance in many instances, the evaluation of intraepithelial neoplasia (dysplasia, carcinoma in situ) and the documentation of early or microinvasion requires a carefully embedded biopsy with orientation of a cross-section for proper evaluation.

Cup forceps biopsies are usually carefully removed and eventually placed directly into fixative. In most institutions, buffered formalin (10 per cent formaldehyde) remains the standard fixative but other special preparations are occasionally utilized. Placing the biopsy on filter paper, with the mucosa side up, is advocated by some pathologists as a mechanism to enhance proper embedding. Cold knife excisional biopsies, vocal cord strippings, and other relatively large fragments of mucosa must be oriented and mechanically stabilized prior to immersion in the fixative to avoid shrinkage artifact. Consultation with the pathologist to establish a mutually acceptable method of avoiding the shrinkage and distortion of these larger specimens should result in a more satisfactory examination of the tissue. The method that I have found the easiest to perform and that causes the least tissue distortion is to simply use straight pins to position the mucosa on a cork board, paraffin block, or some other soft material. Once the mucosa is secured and positioned as in situ, the specimen, pins, and supporting block can be immersed in fixative. This technique allows proper orientation of the specimen, identification of the anatomic boundaries, and appropriate evaluation of the margins of resection in excisional biopsies.

Considerable forethought and care must enter into choosing the site of biopsy. Biopsy and the patient with squamous cell carcinoma of the head and neck is performed for two major reasons. The first is to establish the diagnosis and to identify specific parameters about the neoplasm (tumor grade, degree of keratinization, and growth pattern). The second objective of the use of biopsy is to evaluate the extent of the growth of the neoplasm, including identification

of multicentric or simultaneous presenting second cancers. From the pathologist's point of view, the optimum site of biopsy is at the edge of an infiltrating carcinoma. In ulcerated cancers, biopsy from the ulcer crater or its edge reveals inflammation, necrotic debris, and partially necrotic tumor fragments. A biopsy from the edge of an indurated infiltrating carcinoma allows the pathologist to evaluate a viable neoplasm with optimum cytologic preservation. In addition to a more detailed evaluation of the tumor morphology, a biopsy from this site allows evaluation of the tumor growth pattern and other features of the host-tumor interface.

Neoplasms that grow in a superficial manner without significant induration or deep invasion also must be sampled with care. Squamous cell carcinomas with this growth pattern are commonly encountered in multiple sites in the upper aerodigestive tract. It is important to biopsy areas of induration that often are the sites of submucosal invasion by the tumor. It is extremely important to identify squamous cell carcinomas with a prominent superficial spreading or a horizontal growth pattern, since the approach to therapy can be significantly modified in selected patients with cancers of the floor of the mouth. T1 and T2 squamous carcinomas with early or microinvasion are amenable to local excision, whereas extensive and deeply invasive carcinomas require radical therapy (Crissman et al, 1980).

Biopsies performed for evaluating the extent of the cancer or for the diagnosis of a second neoplasm also have to be selected with care. The use of mucosal stains, such as toluidine blue, to identify abnormal epithelium has been advocated but appears to have limited use (Mashberg, 1980). Some surgeons believe that in vivo staining is helpful, whereas others believe that using careful visual examination to choose biopsy sites is just as effective. The mucosal changes associated with the advancing edge of a cancer lesion may be superficially invasive or entirely intraepithelial, as previously discussed. Palpable induration or bulging mucosa in non-ulcerated cancers are preferable sites for biopsy and establish the site of maximum invasion. The diagnosis of multiple synchronous primary cancers results from careful examination, and many surgeons routinely perform triple endoscopy in head and neck cancer patients (Gluckman et al, 1980). The procedures for establishing tissue diagnosis of an anatomically separate second primary cancer are essentially the same as for the presenting carcinoma. When the neoplasms are closely situated, multiple mucosal biopsies are often helpful to identify the extent of the suspected separate primary malignancies. Identification of normal or nonmalignant intervening mucosa between suspected multicentric adjacent primary cancers is mandatory to support such diagnoses (Warren and Gates, 1932). Identification of mucosal origin in second primary squamous cell carcinoma is also an indication of multicentric cancer spread. Although physicians believe that such a demonstration is of little clinical value, there is a growing body of evidence suggesting that patients with multiple primary carcinomas have a poorer prognosis. There is no question that therapy used in patients with multicentric cancers must be tailored to the individual patient and commonly results in more extensive use of chemotherapy and radiation with a lower incidence of radical surgery. In patients with "recurrent" cancer, the differentiation of persisting neoplasm from a second multicentric carcinoma would also be important in planning appropriate therapy. Obviously, a second primary neoplasm developing from a focus of carcinoma in situ with early submucosal invasion may be alleviated by local resection, whereas recurrent or persisting tumor from a previously treated cancer usually implies extensive submucosal and deep soft tissue

involvement that requires a much different therapeutic plan.

Precursors to Squamous Cell Carcinoma

Leukoplakia and Erythroplakia

The reaction of squamous mucosa to various forms of injury, including carcinogenic influences, depends in part on the anatomic site of injury. The common reaction of squamous mucosa to injury invariably includes cellular proliferation or hyperplasia, either reactive (reversible) or neoplastic (irreversible). The hyperplasia is reflected by epithelial thickening (acanthosis) and varying degrees of accumulation of surface keratin (hyperkeratosis), regardless of the type of injury. When the degree of epithelial thickening and accumulation of surface keratin is sufficient, the clinical appearance of these mucosal changes is referred to as leukoplakia, or white patch. In the majority of instances, when sufficient surface keratin is present to result in a white appearance, the histologic counterpart is usually an orderly progression or maturation of the cells from the basilar layer to the anucleated surface keratin. In spite of this factor, historically there has been some reference to leukoplakia as representing one of the "premalignant" or precursors to the development of squamous cell carcinoma. A number of patient series that entailed careful documentation of both the gross appearance and well-defined histologic criteria for epithelial abnormalities, including dysplasias and carcinoma in situ, do not appear to support this concept (Mashberg, 1977; Waldron and Shafer, 1975) (Table 1). It appears that the mucosal changes that appear to be homogeneously white or leukoplakic are only rarely associated with significant intraepithelial abnormality. Long-term follow-up of patients with leukoplakic changes also demonstrates an extremely low frequency of transformation of these changes to invasive squamous cell carcinomas (Einhorn and Wersall, 1967) (Table 2). The majority of homogeneous leukoplakic mucosal changes are usually found on the buccal mucosa, dorsal tongue, and alveolar ridges, anatomic sites in which invasive carcinomas are relatively infrequent. However, leukoplakic changes in the floor of the mouth, ventral tongue, soft palate, and oropharynx have a significant, but low, association with and subsequent transformation to invasive carcinoma.

As opposed to the relatively low frequency of association with invasive carcinoma in leukoplakia, it has become clear that erythroplakic or red mucosal changes are commonly associated with invasive carcinomas or have a high rate of transformation to malignant lesions (Mashberg, 1978; Shafer and Waldron, 1975) (Table 1). Erythroplakia is the most common physical finding in early asymptomatic squamous cell carcinoma and must always be viewed with suspicion. The most common sites of erythroplakia are the floor of the mouth, ventral tongue, and soft-palate tonsil complex. This statistic corresponds to the common sites of squamous cell carcinomas in the oral cavity. In contrast to the orderly progression or maturation of the thickened hyperkeratotic squamous mucosa in leukoplakia, erythroplakia seldom is a result of thickened mucosa, and abnormal maturation is invariably the most significant histologic feature. Erythroplakic mucosa is characterized by replacement of a normal to thin mucosa by abnormal or immature cells. The relatively thin mucosa with submucosal telangiectasis results in the red velvety appearance of the erythroplakia. There is little question that many of the mucosal changes

that appear red are associated with significant maturation abnormalities within the mucosa. Many of the erythroplakias are best categorized as dysplasia or keratosis with atypia; others fulfill the histologic criteria of carcinoma in situ or intraepithelial neoplasia. A sizable percentage of intraepithelial neoplasias are associated with infiltrative squamous cell carcinomas at the time of biopsy (Mashberg, 1978; Shafer and Waldron, 1975).

Although it is agreed that leukoplakic mucosal changes are seldom associated with serious epithelial abnormalities and erythroplakias have a high association or subsequent transformation to carcinoma (Table 3), the classification of the admixture of these two mucosal changes has for many years been considered a form of leukoplakia. However, it has become evident that this admixture of speckled mucosal changes should be considered a variant of erythroplakia, the most serious of mucosal changes. It is possible that the erroneously high association of leukoplakia with underlying epithelial abnormalities reported in the literature, in reality, represent leukoplakia with erythroplakia or speckled mucosal change and not pure leukoplakic mucosal change (Mashberg, 1978).

Dysplasia and Carcinoma in Situ

In biopsies from thin mucosa with little maturation or surface keratinization and mucosa composed of immature basaloid-appearing cells, the neoplastic nature of the intraepithelial change is apparent and is considered carcinoma in situ. When there are attempts at epithelial maturation, usually characterized by surface keratinization, a diagnosis of mild or moderate intraepithelial neoplasia or dysplasia is indicated. The current grading system, separating these four subgroups of epithelial change (mild, moderate, and severe dysplasia and carcinoma in situ), although somewhat subjective, is based on the combination of maturation abnormalities of the epithelium and the cytologic atypias of the individual cells.

In the author's experience, the most important criteria for diagnosing neoplastic transformation in upper aerodigestive tract mucosa are the loss of epithelial organization and abnormal maturation within the epithelium. Normally, the squamous mucosa matures in an organized fashion with a well-defined basal layer. Loss of this orderly maturation is characterized by a mosaic distribution of nuclei. Nuclear alterations as well as lack of cytoplasmic differentiation are important parameters in evaluating cytologic atypia. Altered cytologic characteristics (eg nuclear-to-cytoplasmic ratio and nuclear pleomorphism (as defined by variation in nuclear size and chromatin content in conjunction with the maturation abnormalities) result in mucosal alterations diagnostic of intraepithelial neoplasia or dysplasia. Changes in nuclear size, shape, and staining characteristics represent alterations in nuclear chromatin and other nuclear proteins. Using the Feulgen staining reaction specific for DNA, it has been demonstrated that increased DNA is present in intraepithelial neoplasia (Giarelli et al, 1977; Hellquist et al, 1981). The increase in DNA over normal diploid levels is referred to as hyperdiploid and represents an abnormal or aneuploid chromosomal population, a marker of neoplastic transformation.

The critical issue remains - at what point can we distinguish mild to moderate mucosal alterations that are truly neoplastic from those that represent a response to some other reversible

injury? The classic cytologic observation of nuclear pleomorphism does not readily differentiate neoplastic aneuploidy from reactive tetraploidy or an increased nonchromosomal nuclear protein. Most the intraepithelial neoplasias of the upper aerodigestive tract have surface keratinization, and the standardized histologic evaluation of these forms of dysplasia and carcinoma in situ is not well defined. As a result, the criteria for diagnosis and grading of intraepithelial neoplasia in the head and neck are not uniform. There is prominent surface keratinization with prominent maturation abnormalities in the lower portion of the mucosa. There is also has extensive surface keratin with prominent parakeratosis. The epithelium is thickened without normal maturation but with nuclear pleomorphism, crowding, and frequent mitoses. These changes are diagnostic of severe dysplasia and carcinoma in situ. The "classic" or nonkeratinizing form of intraepithelial neoplasia characteristic of carcinoma in situ found in the female reproductive organs is uncommon in upper aerodigestive tract mucosa. Proliferations of immature or basaloid-appearing cells are usually present in the lower portions of the epithelium and are often accompanied by "premature" expression of intracellular keratin (dyskeratosis). In addition, nuclear pleomorphism, which is reflected by variation in nuclear size, shape, and staining characteristics, is usually present along with increased numbers of mitotic figures.

An anatomic site that characteristically displays this form of thickened hyperkeratotic intraepithelial neoplasia is the laryngeal glottis. The glottis represents a special type of squamous mucosa that possesses a high propensity for the development of surface keratin. Instances of injury to the glottic mucosa usually cause marked proliferation of the epithelium, resulting in acanthosis and some accumulation of surface keratin. This may give the area a leukoplakic appearance. There is an example of thickened mucosa over both the true and false cords. There is a photomicrograph, produced with higher magnification, of the mucosa over the true cord, and the lack of maturation and severe cytologic change is diagnostic of severe dysplasia and carcinoma in situ. Erythroplakic changes, on the other hand, are seldom encountered in the glottis but do occur in other sites in the laryngeal mucosa.

In several series of carefully studied patients with laryngeal glottic dysplasias or keratoses, between 3.26 and 4.31 per cent of the patients developed subsequent invasive carcinomas (Table 4). Most epithelial proliferations of the laryngeal glottis are well differentiated and usually do not demonstrate appreciable cytologic atypia. This is reflected in the low frequency of progression to invasive cancers. Nevertheless, mucosal alterations that persist or recur after vocal cord stripping are indicative of intraepithelial neoplastic transformation and should be treated accordingly (Crissman, 1982).

Early Invasive or Microinvasive Carcinoma

The differentiation between benign papillary ingrowth of hyperplastic mucosa and early neoplastic invasion of submucosal tissue is often difficult. In many situations, irregular poorly organized projections of squamous cells originating from an overlying neoplastic epithelium are easily diagnosed as early or microscopic invasion. When the mucosa is hyperplastic, without significant atypia, and projections extending into the submucosa are well organized, a diagnosis of pseudoepitheliomatous hyperplasia is appropriate. It is the author's impression that the key

issues involved in this critical differential diagnosis are the status of the overlying epithelium (reactive versus neoplastic) and the pattern of the suspected foci of invasive epithelium. When a well-defined basal layer with evidence of maturation is present and the epithelium is sufficiently organized to appear capable of producing basement membrane at the epithelial-stromal interface, a diagnosis of papillary ingrowth or pseudoepitheliomatous change is indicated. When the suspected focus of invasion is poorly organized with appreciable cellular pleomorphism and irregular intercellular relationships, it is not reactive process and a diagnosis of invasion is indicated. The interface of the epithelial and stromal demarcation is an important parameter in differentiating these two diagnoses. When the epithelium is sufficiently organized to indicate that a basement membrane of normal thickness is present, the suspected epithelium is most likely to be reactive. These rules do not apply to verrucous neoplasms, which present a different spectrum of diagnostic problems.

Epithelial ingrowths arise in an area of intraepithelial carcinoma. When these ingrowths have poorly demarcated borders separating them from the host stroma, they represent invasive cancer. If the infiltration is in the form of single groups or cords of cells, the lesion is invasive. When a broad band of epithelium extends into the submucosa, the differentiation between reactive and invasive neoplasm becomes more difficult. If there is sufficient cytologic atypia and lack of organization or orderly maturation, the lesion is most likely invasive carcinoma. If there is a well-demarcated epithelial stromal interface and evidence of organization or maturation suggests that the epithelium is sufficiently organized to be producing basement membrane, the author generally takes a conservative posture and does not call it invasive carcinoma. It is hoped that the use of immunohistochemistry to demonstrate the presence or absence of basement membrane (laminin or type IV collagen) will be helpful in this critical differentiation (Barsky et al, 1983). Lack of immunologically detectable basement membrane may represent a major indicator for differentiating malignant invasion from reactive ingrowths. There is an illustration of a section of squamous epithelium stained immunohistochemically with antibodies to basement membrane. The normal mucosa on the right and the blood vessels have an easily identifiable basement membrane. However, on the left, the epithelium has developed changes indicating severe dysplasia and carcinoma in situ and the expanding epithelium has lost its capacity to maintain an identifiable basement membrane. This technique holds great promise to be a major tool for pathologists who are faced with this critical differential diagnosis.

The clinical significance of early or microinvasive carcinoma is not well documented. When the pathologist is confident that the focus of early invasion is the most severe change present, ie in excisional biopsies or biopsies of small erythroplakic mucosal changes, and the amount of invasive carcinoma is truly microscopic, a diagnosis of microinvasion is indicated. It appears that these foci of early invasion, or microinvasion, have a prognosis similar to carcinoma in situ and presumably allow a less radical procedure than that commonly elected when an unqualified diagnosis of invasive squamous cell carcinoma is rendered (Crissman et al, 1980).

Histologic Grading of Infiltrating Squamous Cell Carcinomas

Histologic grading of any neoplasm represents an estimation by the pathologist as to the anticipated biologic behavior of the neoplasm. Histologic grading was initiated by Broders in a study of squamous cell carcinoma of the lip and subsequently was extended to include other carcinomas (Broders, 1926). Broders' classification for grading of malignant neoplasms evolved from these early studies and divides neoplasms into four grades based on the proportion of the tumor that histologically recapitulates the tissue of origin. The greater number of cells that assume a similar structure and cytology of normal tissue, the greater the degree of differentiation. Conversely, the fewer cells resembling normal cells, the less well-differentiated or the higher the grade of neoplasm. There is general agreement by clinicians and pathologists that the degree of differentiation has some role in the management of head and neck cancers, but the reproducibility of histologic criteria is suspect. As a result, most pathologists have simplified the classification of lesions to three grades - poorly, moderately, and well differentiated. In most instances, the major histologic parameters used in determining these grades include cell cytology, number of mitoses, and production of intercellular or extracellular keratin pearls.

Jakobsson has suggested a more comprehensive grading scheme for squamous cell carcinomas (Jakobsson, 1976). Parameters for both the tumor cell population and what is referred to as host response are incorporated into the grading system. The observations dealing with the tumor cell population include the structure or growth pattern, the degree of keratinization, nuclear pleomorphism, and number of mitoses. The histologic parameters used in evaluating the tumor-host interface include the mode of invasion of the neoplasm, the degree or stage of invasion, the presence or absence of vascular invasion, and the plasma-lymphocyte cellular response. Although these observations are used by experienced pathologists in determining a grade of squamous cell carcinoma, the fact that they are given equal weight in the Jakobsson grading scheme may or may not be appropriate.

Evaluations using some of these parameters have been reported. In a study on pyriform sinus squamous cell carcinomas, increased numbers of distant metastases were noted with nonkeratinizing neoplasms (Martin et al, 1980). In addition, higher rates of local recurrence were observed with keratinizing tumors and neoplasms with infiltrating margins. In a study of laryngeal carcinomas, an infiltrating pattern of invasion and perineural invasion were of value in predicting lymph node metastasis (McGavran et al, 1961). The identification and importance of lymphatic invasion was associated with a higher frequency of metastasis in one study (Poleksic, 1978) but not in another (McGavran et al, 1961). In the Jakobsson study on laryngeal squamous carcinomas (1976), he noted that the pattern of invasion was important in the prognosis of T1 neoplasms and that increasing nuclear pleomorphism or polymorphism correlated with aggressive behavior in the larger T2 to T4 squamous cell carcinomas. This grading scheme was also adapted to a series of patients with carcinomas of the floor of the mouth, and only the level of invasion was found to be of importance in predicting tumor behavior and, then, only in T2 tumors (Crissman et al, 1980). Although Jakobsson's attempts at quantitating the differentiation of squamous cell carcinomas is admirable, it appears to be of limited use in most situations. Also, although quantitative approach to the grading of these neoplasms and the independent evaluation

of multiple parameters may be of some value, the summation or cumulative score used to develop a quantitative tumor grade is premature. In a study of squamous cell carcinomas of the oropharynx in which Jakobsson's criteria were applied, it was found that the pattern of invasion and frequency of mitoses were the only histologic parameters with significant prognostic values (Crissman et al, 1984). The degree of keratinization, inflammatory infiltrate, or other histologic parameters were not helpful in predicting patient outcome. It illustrates a poorly differentiated, nonkeratinizing carcinoma with small irregular cords of invading cells. Figure shows a moderately differentiated squamous cancer with well-defined borders of invading tumor and prominent keratinization. The neoplasms with well-defined borders of invasion appear to be less likely to successfully gain access to vascular spaces and are less likely to metastasize.

Intraoperative Evaluation of Surgical Resection Margins

(Frozen Section Examination)

The rapid, or frozen section, technique is used during surgical procedures for microscopic evaluation of selected portions of tissue. The most critical issue in the evaluation of resection margins is proper tissue sampling. Admittedly, frozen section histology is less optimal than paraffin-prepared permanent tissue sections, but the majority of errors in evaluation of resection margins are due to improper or inadvertent tissue sampling. In most instances, the author favors the surgeon choosing the tissue in which there is the greatest possibility of involvement by carcinoma. Because of the complexity of the anatomy of the head and neck region, and because the surgeon is essentially "tailoring" the surgical resection to the site and distribution of the neoplasm, the author believes that the surgeon is best aware of the tissue margins that have the greatest risk of containing infiltrating carcinoma.

In most instances, intraoperative surgical judgment is of great value in choosing the tissue for evaluation by frozen-section. In patients who have received either preoperative radiation or chemotherapy, commonly resulting in tissue fibrosis, the intraoperative evaluation of the surgical margins for completeness of resection is much more difficult. The tissue fibrosis often masks residual neoplasm and creates difficulty in the histologic interpretation of frozen tissue sections. Both radiation and chemotherapy cause tumor necrosis that results in inflammation and fibrosis. This tissue change complicates the clinical expertise required of the surgeon, since firm indurated tissue usually indicates previous neoplasm but may or may not contain viable neoplasm. In these cases, it is sometimes helpful for the pathologist to consult with the surgeon in the operating room. Visualizing the specimen in situ helps to pinpoint the anatomic orientation of the specimen and to hear the surgeon's concerns. Subsequently, a more complete frozen section analysis using mutually chosen tissue samples of the resected specimen can be evaluated. Figures demonstrate the two techniques commonly used to evaluate margins of resection. The small central tumor in Figure appears to be widely excised, and either the tangential (BA) or radial (A) cut sections adequately document the completeness of the resection. Figure illustrates the problem of a large poorly circumscribed neoplasm. In this example, the section BC was submitted by the surgeon for intraoperative (frozen section) diagnosis because of the proximity of the identifiable tumor to the planned line of resection. BA was free of tumor and properly identified on the main

resection specimen (point A). Although the radial section documents that the tumor is in close approximation to the apparent line of resection in the main specimen, the knowledge that the frozen section (BC) was properly identified allows a confident confirmation of the completeness of the resection.

The second problem encountered in the evaluation of resection margins after therapy is the proper identification of nonviable tumor, keratin granulomata, and other indicators of sterilized neoplasm. Although this evaluation is usually quite easy to conduct on permanent tissue section, it is occasionally difficult to perform on frozen sections. In most instances, the morphologic establishment of viability is extremely difficult. In selected instances, intact cells may be identified, but large, pleomorphic, hyperchromatic, often pyknotic nuclear features suggest severe injury. Nevertheless, in most instances, identification of persisting intact cells must be considered evidence of residual viable neoplasm. Usually, these unquestionable foci are confined to the main portion of the resected specimen. When they are identified in or near the margins of excision, determination of tumor viability is of extreme importance (Looser et al, 1978).

Selection of Margins for Evaluation

(Postoperative Specimen Evaluation)

The evaluation of resection margins in major head and neck cancer specimens is often difficult and cannot be contained within a simple set of guidelines. This is one of the areas of head and neck pathology in which the experience of the surgical pathologist is of the greatest help. The most important role, which is commonly ignored by inexperienced pathologists, is to clearly understand the orientation of the specimen. Proper specimen orientation also includes identification of the sites from which intraoperative frozen section specimens were sampled. This requires that either the pathologist visit the operating room during surgery or the surgeon help orient the specimen in the surgical specimen dissection area. In a busy service, good communication between surgeon and pathologist and use of appropriate diagrams are often sufficient for proper specimen orientation. The diagrams can be drawn freehand or printed forms, similar to those utilized for clinical staging, can be used. Strategic use of markers (eg sutures) by the surgeon are helpful in identifying important anatomic landmarks. More will be said about the use of diagrams in the section on head and neck surgical pathology reports.

There are essentially two methods for sampling resection margins. The first is to select the tissue sections parallel to and including the margin of resection. Identification of the resection margin surface (or nonmargin surface) can be done with the application of india ink. Many pathologists apply the ink to the surface opposite the surgical resection margin surface with instruction to the histotechnologist to embed the tissue so the microscopic tissue sections are preferentially cut nearest the resection margin surface. The second method of examining resection margins is to select tissue sections for embedding that are perpendicular to the resection surface. This technique allows a better evaluation of the growth pattern of the neoplasm and its approximation to the margin of resection. One of the drawbacks of this method is that only a

small proportion of the actual resection margin is examined microscopically. In practice, a combination of both techniques provides the optimum examination of resection margins and its use is dictated by the type of surgery performed. Mucosal lines of excision are readily amenable to one of these methods of embedding to determine completeness of excision. However, soft tissue margins are much more difficult to evaluate. In many instances, the tissue is severely fragmented and the technical difficulty in sharp dissection of tissue parallel to the excision margin often results in multiple disorganized tissue fragments. As a result, it is often difficult to evaluate these sections microscopically. Full thickness sections that are perpendicular to soft tissue resection margins are technically more feasible and are the type most commonly used.

The proper histologic evaluation of resection margins entails more feedback than whether it is positive or negative for tumor. Obviously, when infiltrating squamous cell carcinoma is found in the submucosa or soft tissue of a resection margin, it should be reported as such. However, other clinically significant observations can result from such histologic evaluations. One is identification of intraepithelial neoplasia, severe dysplasia or carcinoma in situ. In such cases, it appears that there is a greater risk for recurrent neoplasm in the form of invasive squamous cell carcinoma (Looser et al, 1978). It is presumed that the recurrence of invasive carcinoma in this situation develops from the nonresected intraepithelial neoplasia. The second important histologic observation is when infiltrating carcinoma is identified "near" a resection margin. Although there is no clear definition of a "close" or near margin, some authors have considered cancers infiltrating with 0.5 cm of the excision margin is fulfilling this definition. Although the author does not totally agree, this definition has some practical value. When tissue can be dissected, embedded, and sectioned with the confidence that it is truly representative of the margin of resection in question, the author would not use the term close or near margins. However, in evaluating a deep soft tissue margin of excision, especially with large bulky cancers, when there is some question about the confidence of the pathologic evaluation, the term close or 0.5-cm margin may be appropriate. This diagnosis often results in postoperative radiation therapy or, if radiation has already been used, a local boost to the area in question may be instituted. However, in the majority of surgical resections in which the possibility of close margins is raised, the primary neoplasm has an extensive amount of infiltration and radiation therapy is already planned.

Pathology Examination of Radical Neck Dissections

The techniques used by pathologists vary only slightly in the examination of neck dissections. In most instances, the orientation of the specimen is easily discerned and the anatomic subdivision of the lymph node chain can be determined without difficulty. Most pathologists prefer to dissect the specimen while it is fresh, which allows the identification of smaller lymph nodes in the specimen. Serial sectioning at 0.3 to 0.5 cm and careful palpation allow identification of most lymph nodes 0.3 cm in diameter. Separate labeling of lymph nodes located in different portions of the resection is mandatory.

The anatomic subdivisions of the lymph nodes from radical (or modified) neck dissections varies from institution to institution. In general, the lymph node distribution in neck dissections

can be divided into the following categories:

1. anterior triangle, which includes submental and submandibular regions;

2. digastric or upper jugular chains;

3. omohyoid or middle jugular region;

4. lower jugular region; and

5. posterior triangle, which includes the occipital and subclavian triangles.

The anatomic details of neck dissections are not as clearly demarcated as the diagrams indicate. The anterior triangle may be extensive, as in floor of the mouth resections, or modified significantly, as in laryngeal cancers. It is appropriate to divide the anterior triangle contents into submental and submaxillary portions whenever an extensive dissection is done. The jugular or cervical chain of lymph nodes is usually divided into three levels. This is done arbitrarily and not by identifiable anatomic landmarks. The posterior triangle is usually scanty and includes the soft tissue posterior and inferior to the sternocleidomastoid muscle. The number of lymph nodes identifiable in radical neck dissections is quite variable but usually exceeds 20. Many surgeons have a strong opinion regarding the number of lymph nodes in each neck dissection. However, Fisher and co-workers (1978), in a study of breast carcinomas, documented that identification of minute lymph nodes does not contribute to staging. However, preoperative radiation or chemotherapy markedly shrinks lymphoid tissue and, as a result, only 6 to 10 lymph nodes are identifiable in some of these previously treated specimens.

Other approaches to the sampling of lymph nodes in the neck need to be mentioned. In selected patients, a neck mass may develop, either prior to or after definitive therapy. Therapeutic decisions may be determined depending on whether or not the mass consists of metastatic carcinoma. In this instance, the use of fine needle aspiration biopsy, a procedure with low morbidity, can be performed rapidly at the bedside (Frable, 1979). Although this procedure has a great potential for false negative results due to sampling errors, when cells derived from squamous cell carcinoma are identified, the patient can often be spared the morbidity and potential seeding of the wound that are inherent in an incisional biopsy.

Confident histologic assessment of lymph nodes is dependent on tissue sampling. Adequate embedding and multiple sections increase the probability of an acceptable yield in pathologic examination of radical neck dissections (Wilkinson and Hause, 1974). In most instances, histological identification of lymph node metastases is not a problem. However, the differentiation of a viable neoplasm versus sterilized metastatic deposits can occasionally be a problem. The same guidelines for determining viability of residual tumor apply as those used for determining viability of primary resections. In metastases sterilized by chemotherapy or radiation, residual inflammation, keratin granulomas, or fibrosis is often evident. Identification of residual keratin is diagnostic of sterilized tumor, and finding scar or resolving inflammation is, at best,

only suggestive of a previous neoplasm. Obviously, large deposits of metastatic tumor are not a problem in diagnosis. Identification of microscopic metastases is dependent on the size of the tumor, size of the lymph node, and the volume of tissue examined histologically. These relationships have been calculated, and predictions of the amount of sampling to the probability of identifying variable-sized micrometastases have been determined (Wilkinson and Hause, 1974).

Identification of micrometastases becomes a problem only in neck dissections without obvious gross identification of tumor. Careful gross examination of the lymph nodes allows the identification of many "micrometastases" greater than 2.0 mm in size. Identification of small metastases when other adjacent lymph nodes contain large volumes of tumor is not of clinical value. It is evident that tissue sampling becomes critical in the grossly negative neck dissection. It has been recommended that large lymph nodes be divided into a minimum of four sections for embedding (Wilkinson and Hause, 1974). This results in an improved yield of diagnosis of micrometastases. It has been my policy to embed the majority of identifiable lymph node tissue in grossly negative dissections. This results in relatively few instances requiring embedding of large number of tissue cassettes. In neck dissections in which the tissue has undergone previous chemical or radiation therapy, embedding all lymphoid tissue is recommended. The volume extent of the replacement by metastatic neoplasm in the lymph node is also important. Micrometastases should be indicated in order for the clinician to understand the "negative" clinical examination of the neck. Conversely, massive metastases with lymph node capsule invasion should be documented because of the low survival "rate" in patients with this condition (Johnson et al, 1981). Another histologic feature of lymph nodes that may be of interest to oncologists is an assessment of the various lymph node compartments, ie follicular, paracortical, and reticuloendothelial. It has been proposed that hyperplasia of the follicular and paracortical compartments reflects immunologic reactivity by the host to the neoplasm and is associated with increased survival (Berlinger et al, 1976). The same study demonstrated that patients with lymph node atrophy (in tissue that was not previously treated) had decreased survival rates (Berlinger et al, 1976). Only in this latter circumstance would the author recommend upgrading an assessment of lymph node histology in the surgical pathology report. The value of such assessments has been questioned and therefore they are not commonly used clinically.

Surgical Pathology Reports

The evaluation of margins in head and neck cancer resections, which was discussed in a previous section, is an important component of the surgical pathology report. In addition to the evaluation of completeness of excision of the neoplasm, the surgeon should be apprised of the extent of tumor, its growth pattern, and its distribution in relation to certain anatomic landmarks. The first requirement in determining the growth pattern of squamous cell carcinoma is the correct anatomic orientation of the specimen. In laryngectomies and other major resections with characteristic anatomy, specimen orientation is not a problem. However, many other head and neck surgery specimens need to be oriented, and consultation with the surgeon during or after surgery may be required.

It is the author's firm impression that the most helpful adjunct to communicating the pathology of head and neck specimens is the use of graphic or pictorial data. No matter how articulate the pathologist or how careful and detailed the description, the author is convinced that a proper diagram (or gross photograph) is of greater value in communicating the distribution and growth pattern of the neoplasm. In addition, use of a diagram allows the pathologist to specify where the specimen has been sampled for histologic study and how the samples are labeled. The labeling of the sample is extremely helpful in reconstructing the pathologic examination after the fact. The type of diagrams available for head and neck specimens is variable. The majority are similar to the clinical staging forms used by most head and neck surgeons. Some institutions photograph the specimen with instant processed prints. These prints are then incorporated into the surgical pathology report.

The other features that should be incorporated in the surgical pathology report include the following:

1. Histologic evaluation, including grade of differentiation, degree of keratinization, and growth pattern.

2. Extent or growth pattern of the neoplasm. Features such as the presence of intraepithelial changes, microinvasion, and massive tumor should be reported. In summary, pathologic observations sufficient to stage the cancer should be included in the report. Although determination of the pathologic stage is valuable, the pathologic features used in determining the stage of disease should also be described.

3. Evaluation of the margins of surgical excision with an assessment of the completeness of excision. This evaluation should include confirmation of the frozen section specimens in relation to the permanent sections and how their interpretation is integrated into the final assessment of the completeness of the resection.

4. Description of the distribution and size of the regional lymph node metastases, if present. Although a diagram is not a necessity for lymph node evaluation, many institutions have found them helpful.

It cannot be emphasized enough that results of careful pathology examination are of little value unless the appropriate information is included in the surgical pathology report. In addition, the pertinent information should be in a concise form (such as in diagrams) and not buried in a lengthy description. Although a description may be of value in reconstructing the details of a specimen, it may also dilute the essence of information needed for patient management.