4 Interpreting the Complex Epithelium

Complex, or multilayered, epithelia (squamous and urothelial) may progress through a spectrum of changes, from benign hyperplasia and/or metaplasia, to inflamed reactive atypia, to dysplasia, to carcinoma in situ (CIS), to invasive carcinoma (crossing the basement membrane). The progression is not inevitable or consistent, and some lesions will regress. However, true dysplasia is generally regarded as a premalignant condition. Carcinoma in situ is one step from invasive cancer and therefore treated aggressively. Some lesions are easily monitored clinically, such as those in the cervix and oral cavity, and therefore each phase of change can be seen, biopsied, and followed. Others, such as in the nasopharynx, are generally not noticed until they are fairly large and/or symptomatic. This chapter will touch on basic principles that these epithelial layers have in common and introduce some organ systems that are covered in greater detail later in the book.

Approach to the Epithelium: General Principles

On low power (4×), look for the following:

- Type of epithelium: Is it squamous, columnar, ciliated?
- Architecture: Is it an exophytic structure, such as a verrucous lesion or a papilloma? Is there downward growth, as in an inverted papilloma or invasive lesion?
- Keratinization: Is keratinization present or absent? Hyperkeratosis? Parakeratosis? Mounds or church spires of keratin (as in a wart)?
- Thickness of the epithelium: Is the epithelium thickened and irregular (acanthotic) or thin and flat (atrophic)? A markedly thickened epithelium may indicate irritation and hyperplasia but not necessarily dysplasia.
- Architectural orderliness: Is there a clear difference between the basal layer and the superficial layer? Are the rows of cells orderly (Figure 4.1)? Are the nuclei lined up, either parallel to the surface or perpendicular to it?
- General color: What color is it? Although it is hard to compare one slide to another, within a single slide differences in color can make a dysplastic or inflamed area stand out as dark or blue. Islands of bright pink, on the other hand, may indicate deep keratinization, which is a feature of invasion.

On high power, look for the following:

- Architectural orderliness and polarity: Try to find a well-oriented fragment, not a tangential cut. In a benign, even reactive epithelium, all of the nuclei should appear to "know which way is up."

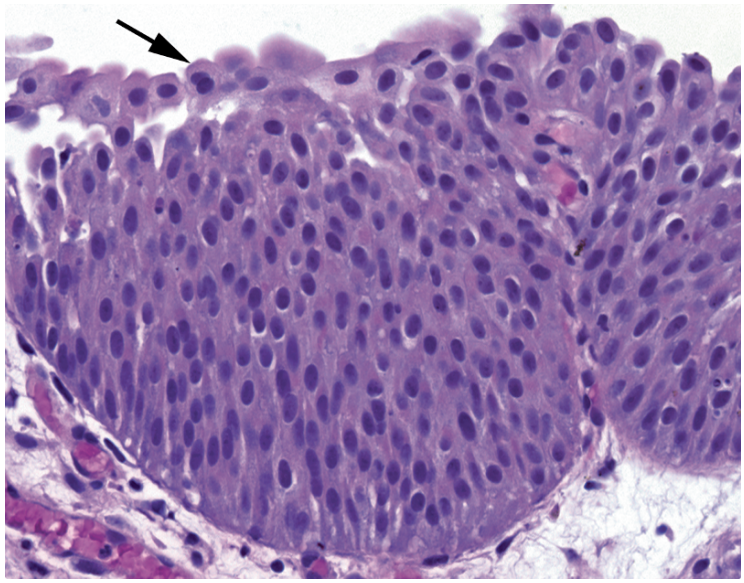

Figure 4.1. Polarity in an epithelium. In this section of urothelium, although it is thickened relative to normal, all of the nuclei can be seen to be roughly perpendicular to the surface; they "know which way is up." Plump umbrella cells are visible at the surface (arrow).

- Mitotic figures: Although a few mitoses near the basal layer are acceptable, mitoses higher in the epithelium are not. As above, a well-oriented fragment is very helpful.
- Dyskeratotic cells: Small, intensely pink, shriveled round cells that have detached from their neighbors (Figure 4.2) can be a feature of dysplasia.
- Inflammation: Look for polymorphonuclear leukocytes (polys), plasma cells, and lymphocytes. Keep a high threshold for dysplasia in the setting of intense acute inflammation (polys).
- Nuclei, eggs versus boulders (Figure 4.3): Reactive nuclei may enlarge but stay smooth and round to oval, and their chromatin condenses into several small nucleoli or speckles, like a bird's egg. The chromatin may have an overall grey-blue look, and the nuclear membrane is often indistinct. Dysplastic or immature nuclei, however, appear to have too much chromatin. They are large and tend to be angulated with irregular nuclear membranes (like boulders), and the chromatin is uniformly dense and dark, almost like it was drawn with charcoal. Nuclear membranes may also appear thicker and more prominent.
- Nucleoli: Prominent nucleoli are actually a feature more suggestive of reactive changes than of dysplasia. A prominent nucleolus in a background of fine pale chromatin, in a smoothly rounded nucleus, is likely benign. Carcinomas usually do not acquire large dark nucleoli until they become invasive.
- Nuclear to cytoplasmic (N/C) ratios: The N/C ratio is normally high in the basal layer but should fall off as the cells mature. A high N/C ratio at the surface, especially in the setting of "boulder" nuclei, is very worrisome. This creates the impression of blueness at low power.
- Invasion: Stromal invasion is a sure sign of cancer but is not always obvious. Pseudoepitheliomatous hyperplasia and tangential sectioning are the main mimickers. Features that suggest true invasion include deep aberrant keratinization (pinking up) and single infiltrating cells with atypical nuclei (Figure 4.4). The basement membrane border should appear ragged and discontinuous in invasion. Well-differentiated squamous cell carcinoma can acquire prominent nuclei (usually not seen in CIS) and mimic reactive nuclei, but it should have the architectural features of invasion.

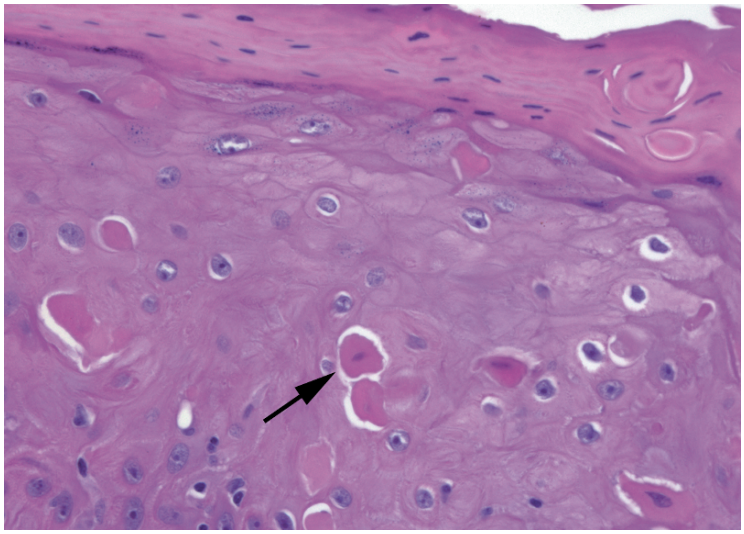

Figure 4.2. Dyskeratotic cells in the epidermis. These cells are essentially mummified; their nuclei are dying, and they have lost their connections to other cells. Their dense pink keratin stands out relative to the neighboring cells (arrow).

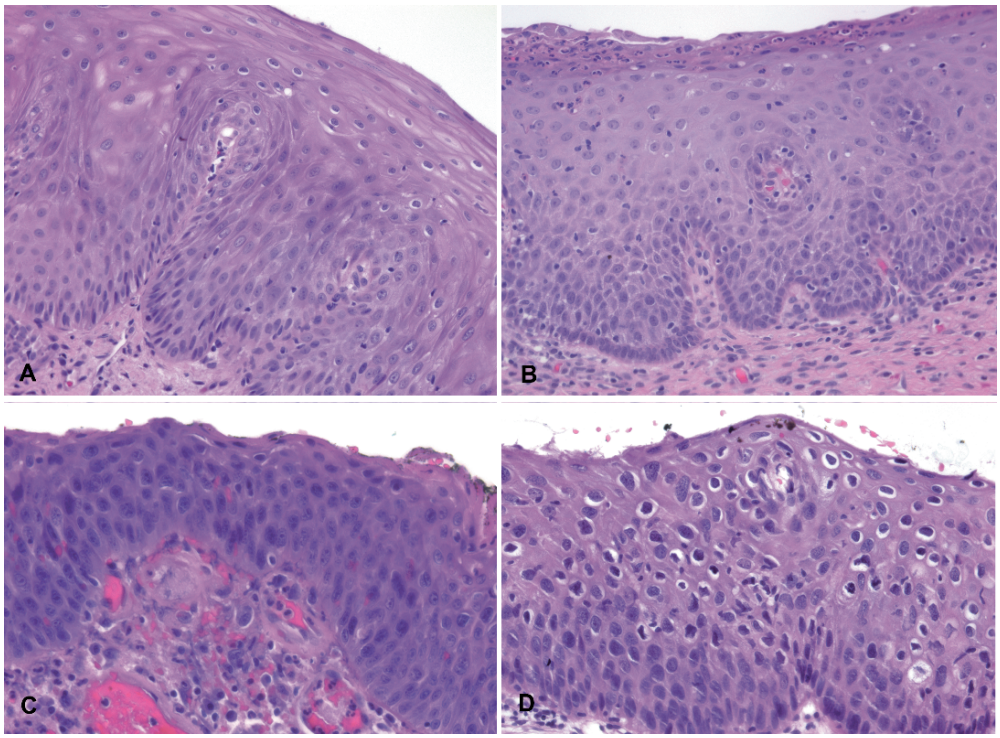

Figure 4.3. Examples of reactive, benign nuclei **(A, B)** and dysplastic nuclei **(C, D)**. In reactive conditions, the nuclei may be enlarged and have visible nucleoli, but the N/C ratios are still low (abundant cytoplasm), there is nuclear polarity relative to the surface, the chromatin is not too dark, and the nuclear membranes are smooth and oval. Maturation is visible in that as cells get closer to the surface the nuclei get smaller and the cytoplasm more abundant. In dysplasia, the nuclei are significantly darker, the N/C ratios are higher, there is more disorder to the epithelium, and the nuclei (being more closely packed) may take on irregular shapes to fit more closely together, similar to boulders in a rock wall.

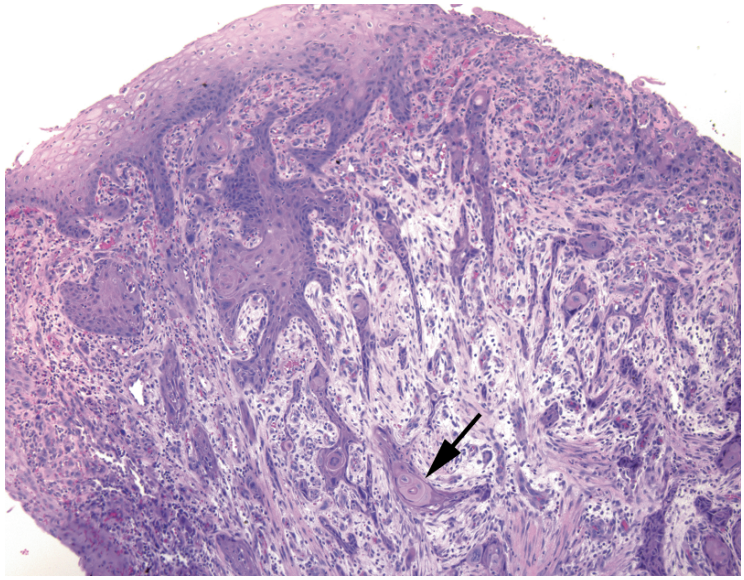

Figure 4.4. Invasive squamous cell carcinoma. Irregular nests and spicules of cells invade down into the stroma from the surface (top). Although single infiltrating cells are not visible at this magnification, the deep aberrant keratinization (arrow), in which a deep nest of cells takes on the color and texture of the normal surface keratin, is highly suspicious for invasion.

Definitions of Terms

Hyperkeratosis: too much keratin, which sits on the epithelial surface in a thick pink layer, often accompanied by parakeratosis

Inverted papilloma: endophytic growth of islands of benign squamous epithelium. The nests should be surrounded by stroma, and fibrovascular cores are not seen. Each nest is bordered by a smooth continuous basement membrane. It is essentially an inside-out papilloma.

Orthokeratosis: "normal" keratin, found on the skin, with a basket weave pattern; anucleate

Papilloma: exophytic growth of finger-like, arborizing projections with fibrovascular cores, lined by squamous epithelium (Figure 4.5)

Parakeratosis: the retention of small pyknotic nuclei in surface keratin

Pseudoepitheliomatous hyperplasia: a benign reactive condition that simulates invasive squamous cell carcinoma. It has a very characteristic look, as though someone dragged the epithelium down into the stroma with a toothpick, like marbling a cake (Figure 4.6). The individual nuclei should look reactive, not dysplastic. There should not be deep keratinization.

Verrucous: an exophytic growth pattern with prominent hyperkeratosis (Figure 4.7) and an appearance described as "church spire" (pointy projections) or "cauliflower" (rounded projections)

Cervix

The cervix (discussed in detail in Chapter 16) is sort of the prototypical mucosal squamous epithelium. It can be closely studied, and the changes of dysplasia are well documented and well-understood. Dysplastic changes in the cervix are nearly all HPV-related, whereas reactive changes and squamous metaplasia are so common that they are considered normal. Dysplastic changes are grouped into low- and high-grade, with high-grade encompassing cervical intraepithelial neoplasia (CIN) grades 2 and 3. The low-grade squamous intraepithelial lesions

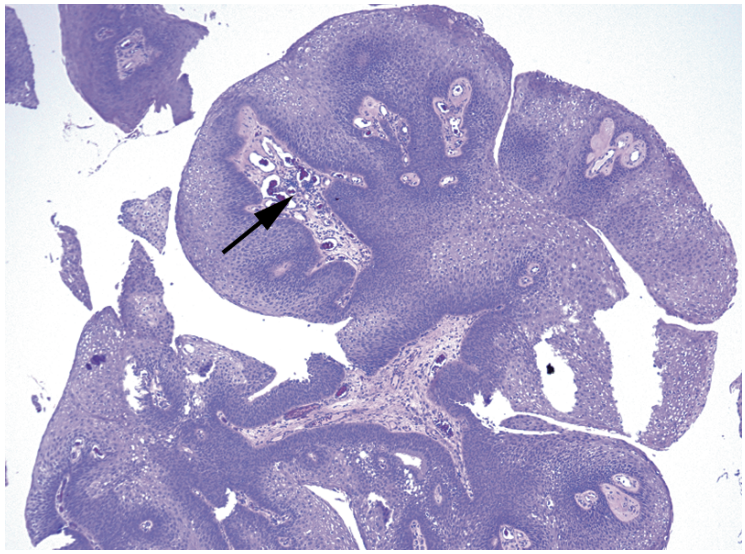

Figure 4.5. Papilloma. The squamous papilloma is defined by a squamous epithelium overlying branching fibrovascular cores (arrow).

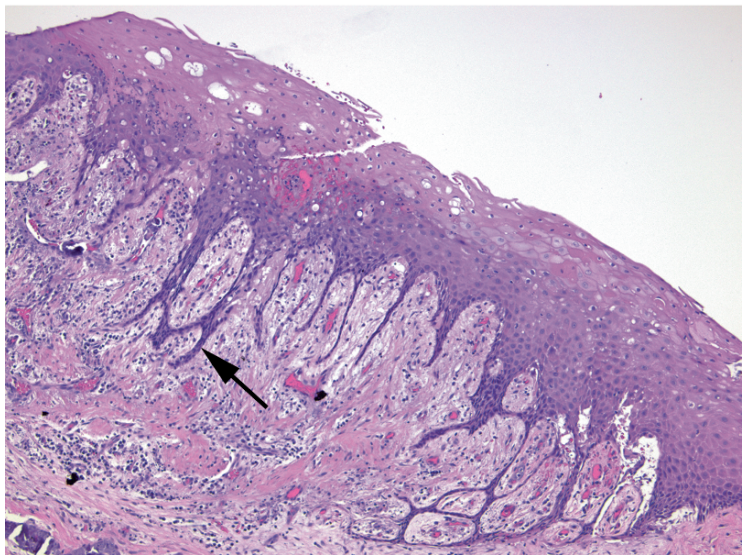

Figure 4.6. Pseudoepitheliomatous hyperplasia. In this reactive condition, thin strands of epithelium (arrow) are pulled down into the underlying dermis or lamina propria. However, the strands should not expand out into nests or show deep keratinization (compare to Figure 4.4).

(LSILs) show predominantly viral-type changes in the superficial epithelium and can regress. The high-grade lesions (HSILs) show significant dysplasia rising up from the basal layer and overtaking part or all of the epithelium. They are less likely to regress and are treated aggressively.

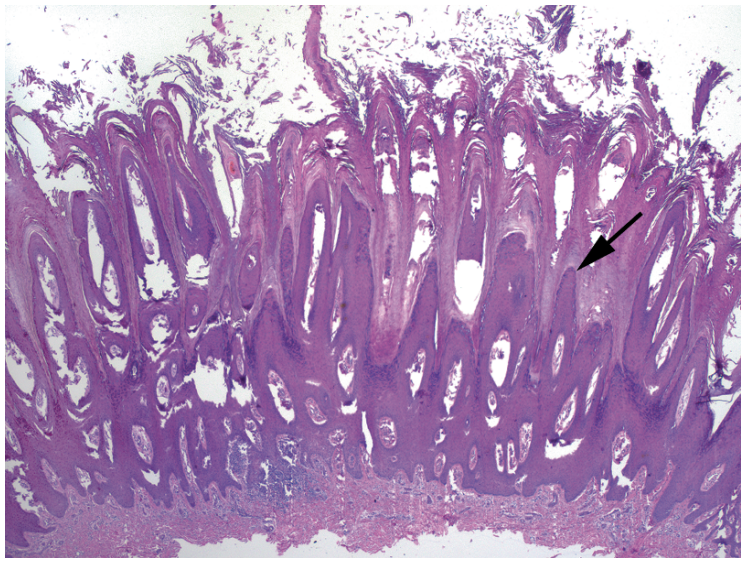

FIGURE 4.7. Verrucous pattern in a wart. Verruca vulgaris is characterized by prominent exophytic spires of the epidermis (arrow), with overlying hyperkeratosis and parakeratosis.

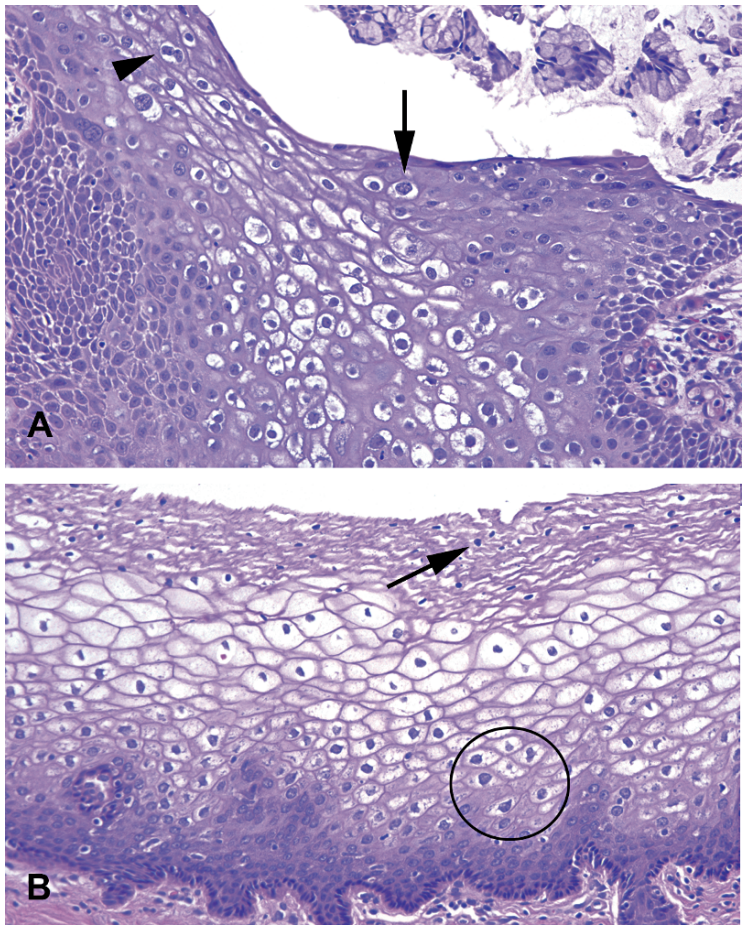

FIGURE 4.8. Viral or koilocytic atypia versus glycogen. **(A)** In this cervical lesion (low-grade squamous intraepithelial lesion [LSIL]), koilocytes are visible as large cells with prominent, crinkled, dark nuclei and perinuclear halos (arrow). Nuclei that get larger as you approach the surface are an indicator of dysplasia. Binucleate cells are suggestive of LSIL (arrowhead). **(B)** Normal glycogenated cervical epithelium can appear to have prominent nuclear halos, but the nuclei at the surface should be tiny and pyknotic (arrow). Larger cells may be seen near the basal layer (circle).

Low-Grade Squamous Intraepithelial Lesions (Cervical Intraepithelial Neoplasia Type1)

- Koilocytic (viral) changes, characterized by ballooned, cleared-out cells with enlarged, raisinoid nuclei, are present. Beware glycogenated normal cells, which are also ballooned but have small nuclei (Figure 4.8).
- The basal layer is disorganized, with mitoses in the lower one third of the epithelium.
- Condylomas have the same changes but a verrucous architecture.

High-Grade Squamous Intraepithelial Lesions (Cervical Intraepithelial Neoplasia Types 2 and 3)

- Undifferentiated, immature cells occupy >50% of the epithelium (Figure 4.9).
- Mitoses occur above the lower one third of the epithelium.
- Overlying koilocytes or adjacent LSIL may be present.
- Cells can be deceptively bland looking without prominent mitoses, but nuclei should still be enlarged with high N/C ratios.
- Beware immature squamous metaplasia, which can look like HSIL at low power.

Urothelium

The urothelium (discussed in detail in Chapter 12) when benign is five to seven cells thick, with an umbrella cell layer. Reactive changes look similar to those in other organs, and squamous metaplasia can also occur.

Carcinoma arising in the urothelium can follow two pathways: flat and papillary. Flat lesions are those that progress from dysplasia to CIS to invasive carcinoma, without making an exophytic lesion; these are similar to epithelia in other sites. Papillary lesions, however, are graded as benign (papilloma), borderline (papillary urothelial neoplasm of low malignant potential), or cancer (low- and high-grade papillary urothelial carcinoma) based on histologic features.

Although most papillary cancers are in situ, by convention papillary cancers are called invasive or noninvasive. *Carcinoma in situ* refers only to flat lesions. The term *dysplasia* is also not applied to papillary lesions, as there is a fairly low threshold for calling low-grade carcinoma.

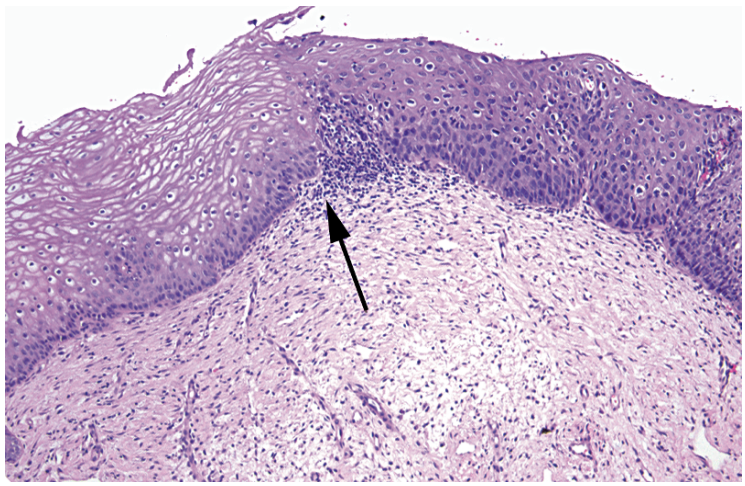

Figure 4.9. High-grade squamous intraepithelial lesion. An abrupt transition (arrow) is visible between normal (left) and dysplastic (right) epithelium. The epithelium at right shows a persistence of immature cells (large nuclei and high N/C ratios) up to the surface. Compare this to the clear distinction between basal cells and maturing cells seen at left.

Do not be fooled by the von Brunn's nests. These are invaginated folds of normal urothelium, which can simulate invasion.
Features of urothelial carcinoma include the following:

- Increased number of cell layers (mainly in papillary lesions)
- Loss of polarity (loss of parallel arrays of nuclei)
- Increased mitoses, above the basal layer
- Enlarged, irregular, or hyperchromatic nuclei
- Discohesive cells or partially denuded epithelium

Oropharynx, Larynx, Tongue

Squamous papillomas are relatively common in the larynx. Features of benign papillomas include hyperkeratosis (para or ortho), basal layer hyperplasia, abnormal mitoses, and koilocytic changes (HPV change). They should *not* have significant atypia, high-grade dysplasia, or warty architecture (church-spire keratosis).

The mouth and larynx are lined by a nonkeratinized squamous epithelium, like the cervix. Unlike the cervix, however, the oral mucosa tends to keratinize in dysplasia. This leads to a different pattern of dysplasia called *severe keratinizing dysplasia*. In severe keratinizing dysplasia, the dysplasia does not have to be full thickness to behave like CIS, so it is a more insidious lesion. The criteria for grading dysplasia are much more subjective than in the cervix.

Features of squamous dysplasia in the mouth include the following:

- Loss of polarity of basal layer and maturation arrest (basal-type cells above the basal layer)
- Dyskeratosis (abnormal keratinization), hyperkeratosis, and acanthosis

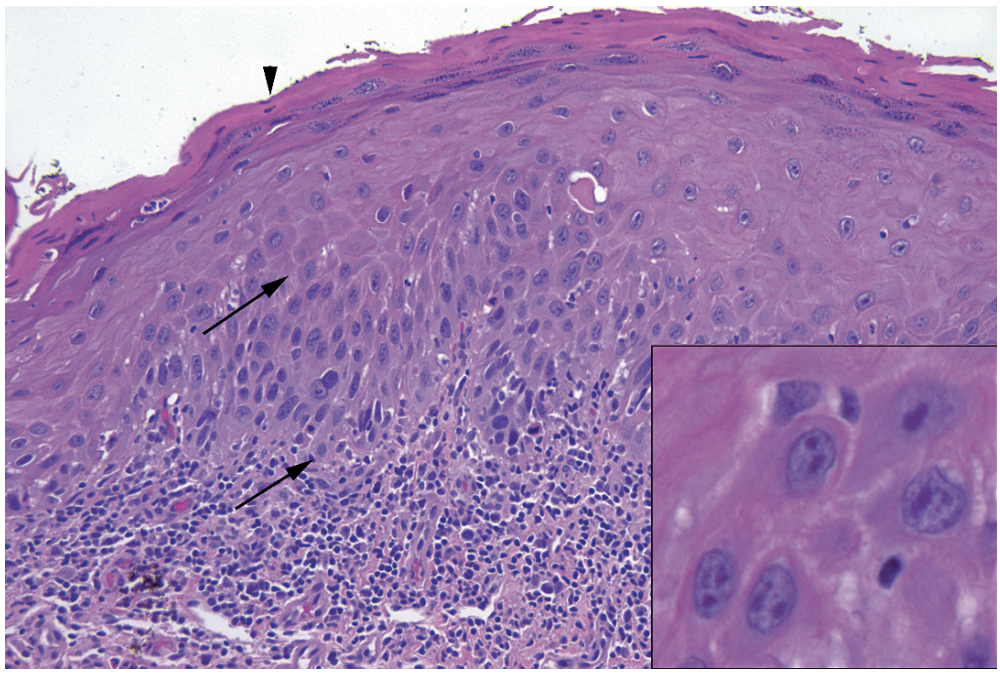

Figure 4.10. Squamous dysplasia in the mouth. In the area between the arrows, dysplastic cells with high N/C ratios and hyperchromatic, irregular nuclei can be seen occupying the lower half of the epithelium. The surface shows parakeratosis (arrowhead), which clinically will appear as a white plaque. **Inset**: Unlike in cervical dysplasia, prominent nucleoli are often seen in keratinizing dysplasia of the oral cavity. Notice the irregularly shaped nuclear membranes.

- Increased mitoses and/or mitoses above the basal layer
- Cellular and nuclear pleomorphism (unlike at many other sites, dysplastic nuclei tend to show prominent nucleoli and nuclear membranes, almost like an invasive carcinoma; Figure 4.10)
- Variable N/C ratios (in keratinizing dysplasia, there may be abundant pink cytoplasm)
- Not necessarily full-thickness involvement, even in severe dysplasia

Nasopharynx

Schneiderian (sinonasal) papillomas are characterized by the following:

- They are lined with a nonkeratinizing squamous or intermediate epithelium, 5–30 cells thick, and may have a ciliated or mucous lining (Figure 4.11). Neutrophils are common.
- They may be fungiform (exophytic, septal) or inverted (inward growing).
- They should have only mild atypia, orderly cells, and few mitoses.

The differential diagnosis for an inverted papilloma includes an invasive squamous carcinoma. Atypia and pleomorphism, increased mitotic activity, and cells invading as nests and cords should be present.

Trachea and Bronchi

Respiratory epithelium undergoes squamous metaplasia when irritated. Dysplastic lesions may then arise from the squamous epithelium.

Esophagus

In the esophagus (which is discussed in detail in Chapter 6), the squamous mucosa is not usually the bad actor; dysplasia is more often seen in the setting of Barrett's esophagus.

Mild reactive changes are very common, and correspond to reflux changes. More intense reactive changes can be seen in infection.

Squamous dysplasia is not often seen on biopsy, as it is asymptomatic. Squamous carcinoma looks similar to that found in other sites.

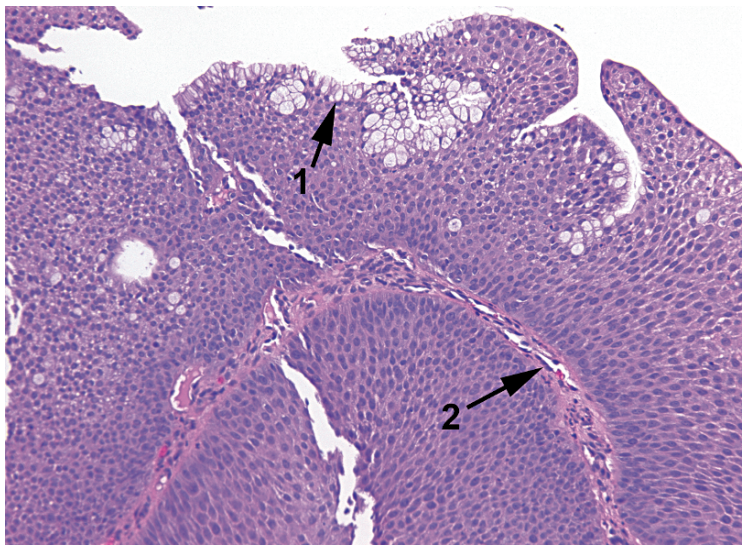

Figure 4.11. Schneiderian papilloma. The typical features are a squamous or respiratory epithelium with goblet cells (1) and neutrophils (not seen at this power). As in any papilloma, there are fibrovascular cores (2).